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Contribuições da Bioacústica ao estudo do comportamento e suas aplicações tecnológicas

Texto apresentado à Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo, como parte dos requisitos para obtenção do título de Livre-Docente em Psicologia.
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"*Esta é uma história digna de ser contada, de como ele pulou de um viveiro no PET e quebrou o pé, pois estava fazendo um focal de um* (macaco) *prego; o focal já estava comprido o bastante para valer a pena fazer qualquer coisa para terminá-lo, e ainda não tinha alcançado o número de minutos - acho que eram oito - para ser considerado na análise. Como ele estava observando os pregos do telhado, acho, de um viveiro no PET e o prego estava querendo sumir de vista, ele pulou do viveiro para não perder o focal... esta é a história mais ilustrativa de como o Mássimo trabalhava*." (Beatriz Beisiegel, grande amiga, contando como o Max quebrou o pé, coletando dados durante o mestrado, no Parque Ecológico do Tietê, SP).

Resumo

Monticelli, P. F. (2019). *Contribuições da Bioacústica ao estudo do comportamento e suas aplicações*. Texto de Livre Docência, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Ribeirão Preto, São Paulo.

As vocalizações são comportamentos e, como tal, tem um conjunto de causas proximais e distais. Em estudos comparativos de espécies filogeneticamente próximas, a estrutura e o uso das vocalizações como sinal de comunicação e a composição dos repertórios acústicos fomentam discussões acerca da evolução do comportamento, das espécies, do ambiente, e sobre efeitos de experiência e das estratégias comportamentais. Há um mundo de caminhos de investigação que se pode tomar a partir da bioacústica nas ciências comportamentais, com aplicações em educação, saúde e conservação de biodiversidade. Em um primeiro capítulo, eu tomo o assobio emitido pelo filhote de cobaia como ponto de partida para uma longa linha de investigação, que segui desde minha iniciação científica, caminhando por temas como origem dos sinais, efeitos do desenvolvimento e motivacionais, domesticação e comportamento, apego e apoio social. No segundo, meu ponto de análise não é um sinal em particular, mas uma medida de complexidade dos sistemas de comunicação acústica de roedores caviomorfos (preá, mocó, capivara e ratos-de-espinho) e carnívoros (lontra, lobo-guará e quati). Quais as possibilidades e os *constraints* desse sistema em relação à vida social? Sociedades animais de maior complexidade (i.e., nas quais os indivíduos interagem com frequência, com muitos outros, em diferentes contextos, e repetidamente ao longo do tempo) devem contar com um sistema de comunicação amplo o suficiente para fomentar essas interações de caráter tão diferentes (apaziguadoras, competitivas, convencedoras, cooperativas, enganadoras, etc.). Minha sugestão é que em quatis e capivaras, as espécies de maior nível de socialidade que estudei, a complexidade acústica foi surgindo a partir da combinação de unidades acústicas básicas e da percepção diferencial dos efeitos não-lineares que surgem naturalmente em alguns sinais, em decorrência de características do sistema fonador. Por fim, no capítulo três, falo da bioacústica como ferramenta tecnológica para estudo, monitoramento e melhoria de qualidade de vida de populações animais, de vida livre ou cativeiro, para fins de enriquecimento ambiental, práticas de conservação, manejo ou redução de conflitos humano-fauna. Espero, ao final dos três capítulos, mostrar que os estudos da comunicação acústica animal fazem parte da Psicologia, à medida que acrescentam conhecimento generalizável sobre mecanismos proximais e distais do comportamento.

Palavras-chave: Carnívoros. Comportamento de apego. Comunicação animal. Domesticação de animais. Etologia. Mamíferos terrestres. Psicologia evolucionista.

Abstract

Monticelli, P. F. (2019). *Bioacoustic contributions to the study of behavior and its applications*. Livre Docencia document, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Ribeirão Preto, São Paulo.

Vocalizations are behaviors and as such have a set of proximal and distal causes. In comparative studies of phylogenetically related species, the structure and use of vocalizations as a sign of communication and the composition of the acoustic repertoires foster discussions about the evolution of behavior, species, biomes, and effects of experience and behavioral strategies. Different research paths may be taken from bioacoustics in the behavioral sciences with applications in education, health, and biodiversity conservation. In a first chapter, I take the infant guinea-pig whistle as a starting point for a long line of research, which I have followed since my scientific initiation, by tracing themes such as the origin of signs, developmental and motivational effects, domestication and behavior, attachment and social support. In the second chapter, my point is not a particular signal, but a measure of the complexity of the acoustic communication systems of caviomorph rodents (cavies, the capybara and a spiny-rat) and carnivorous (otter, maned wolf, and coati). What are the possibilities and constraints of this system to evolve with a social life? More complex animal societies (ie, in which individuals frequently interact and with many others in different contexts, and repeatedly over time) requires a communication system large enough to foster convincing, cooperative and/or deceitful interactions, among other variants. I suggest that in coatis and capybaras, the species with the highest level of sociality that I had studied, the acoustic complexity arose from the combination of basic acoustic units and from the differential perception of nonlinear effects that naturally arise in some signals as a result of the vocal system. Finally, in chapter three bioacoustics in presented as a technological tool for studying, monitoring and improving the quality of free-living or captivity animals, for environmental enrichment or conservation purposes or reduction of human-fauna conflict. I hope to show by the end of the three chapters that animal acoustic communication studies are part of Psychology, as they add generalizable knowledge about proximal and distal mechanisms of behavior.

Keywords: Animal communication. Attachment behavior. Carnivores. Domestication. Evolutionary psychology. Ethology. Terrestrial mammals.

Sumário

Prefácio

O interesse humano pelo som produzido pelos animais deve ser tão antigo quanto nossa espécie. Cigarras, pererecas e aves já deviam cantar com frequência aos ouvidos daqueles caçadores-coletores pleistocênicos. E, se eles tiverem prestado atenção a padrões e correlacionado sons e comportamentos, podem ter feito uso do som para caça e pesca (Busnel, 1956).

Quando nos preocupamos, no passado, em entender o comportamento, enveredamos por explicações subjetivas e exotéricas, e à visão cartesiana, que separava as mentes de “bestas” e “humanos”, e tratava o comportamento das bestas (inclusive seus sons) como respostas “autômatas” ou impulsos (Rocha, 2004). Não é possível explicar o comportamento olhando só para uma espécie, a nossa, por exemplo, e com viés antropomórfico. A tal “natureza humana”, i.e., aquele suposto rol de comportamentos exclusivos da nossa espécie (e.g., Man the Toolmaker de Oakley, 1949), fica cada vez mais reduzida quando a Biologia Evolutiva, a Psicologia Comparativa e a Etologia tiram o foco do ser humano. Alguns paradigmas sobre essa natureza humana foram quebrados com estudos de vocalizações animais (e.g.: Marler, 1970: aprendizagem vocal/ontogenia do canto em aves; Seyfarth, Cheney & Marler, 1980a,b: semântica na comunicação de macacos vervet; Cheney & Seyfarth, 1980: reconhecimento pela mãe da voz de seu filhote). E esse já é um exemplo de como a Bioacústica vem servindo à Psicologia, tema central deste texto.

O professor Jacques Vielliard, criador do primeiro laboratório de bioacústica, e nele a primeira biblioteca sonora do Brasil, gostava de começar a contar a história da bioacústica a partir de Hercules Florence, curiosamente, o inventor da fotografia (Kossoy, 2006). Entre 1825 e 1829, Florence teria acompanhado a expedição de um barão, Langsdorff, pelo Planalto Paulista, Pantanal e Amazônia, como desenhista (Kossoy, 2006; Vielliard & Silva, 2010). Durante a viagem, fez registros escritos de “vozes” de animais não-humanos, ao que chamou de Zoophonia (Vielliard, 1993). Seus registros não tinham, contudo, o objetivo de compreensão do comportamento. Eram “partituras” (ou apenas uma representação visual de timbres e tempos) que serviriam tão somente para a identificação visual dos diferentes sons (Catunda, 1998). Para Vielliard, Florence “enunciou a primeira regra que iria nortear, um século e meio mais tarde, muitos estudos sobre a comunicação sonora animal” (Vielliard & Silva, 2010, p. 2), que diz respeito ao seu carácter espécie-específico. Essa noção nos permite identificar e diferenciar espécies acústicas pelo seu canto e fazer inferências sobre a origem evolutiva dos sinais de comunicação animal.

Foi depois da Segunda Guerra que a tecnologia para registro acústico do som avançou (Vielliard & Silva, 2010). Os equipamentos eram muito diferentes dos que são usados hoje, analógicos e enormes, mas foi com eles que os amantes dos cantos das aves começaram a montar seus acervos. Na década de 1960, a Bioacústica se desenvolveu rapidamente, com esses gravadores "portáteis", o trabalho de campo dos "recordistas" e o uso da análise sonográfica para estudo objetivo dos sons (Vielliard & Silva, 2010). Uma busca por publicações com o termo "Bioacoustic AND animal", entre 1950 e 1960, me remeteu a 77 trabalhos, apenas um deles sobre a bioacústica da qual trata este texto. Na maior parte dos outros, o termo se refere aos sons produzidos pelo corpo humano, com aplicação médica. O "Some New Aspects of the Acoustical Behavior of Animals", de 1956, fala de "progressos recentes em técnicas acústicas" que permitiam, já naquela época, que biólogos estudassem os sons bio-acústicos (e., produzido por seres biológicos, animais vivos) e os correlacionassem a "formas específicas de comportamento" (Busnel, 1956). Essas correlações levariam, segundo o autor, à descrição de uma "*pseudo- language*" (as aspas aqui são do autor) e o estabelecimento do vocabulário animal. Nos dois primeiros capítulos deste texto, vou tratar disso, do que se descobriu sobre o comportamento animal e suas habilidades cognitivas, a partir do estudo de vocalizações, repertórios e reações a *playback* de vocalizações (reprodução de som à um ou mais animais) para se testar hipóteses sobre a função comunicativa dos sinais de comunicação. Publiquei a maioria dos artigos nestes temas e vou apresentar nossa contribuição e a da literatura.

Interessava a Busnel, particularmente, o estudo das reações dos animais a sons produzidos por seres humanos, que poderiam tanto colocá-los em movimento ("*phonokinesis*") ou atraí-los ("*phonotaxis*"), como repeli-los. Esta seria uma segunda forma de contribuição da Bioacústica para a Psicologia: as vocalizações podem ser usadas para provocar uma resposta comportamental, assim como podem, os ruídos produzidos pelo ser humano, alterar o comportamento das espécies de vida-livre. Esse pode ter sido o primórdio do conceito da poluição sonora (os efeitos nocivos do som resultante de atividades humanas, sobre as outras espécies animais e a nossa), e o vislumbre da aplicação de som na conservação e resolução de conflito humano-fauna. O projeto Elephant listening do laboratório de Ornitologia da Cornell (http://www.birds.cornell.edu/brp/elephant/), usa gravadores automáticos para monitorar populações naturais em áreas de conflito com humanos (áreas rurais, de plantações). Vou tratar também disso, de uma Etologia da Conservação com base na tecnologia bioacústica, no capítulo 3, novamente, apresentando minhas contribuições de forma contextualizada, no estado da arte, a partir da literatura.

Ainda no capítulo 3, das aplicações tecnológicas da Bioacústica, vou apresentar modos de se acessar a emoção através do estudo estrutural das vocalizações, usando principalmente dados da literatura e de colaborações estabelecidas com colegas que estudam bem-estar de animais de criação. Eu, particularmente, tenho apenas um trabalho concluído na área de bem-estar animal, a dissertação de mestrado de Débora Silveira Martins, na qual inovamos usando o *playback* de uma vocalização espécie-específica (bugios vermelhos, *Alouatta* *caraya*) como item de enriquecimento ambiental.

O texto que apresento está dividido por temas nos quais trabalhei e produzi os artigos que compõe parte da minha atuação em pesquisa até aqui. Ao final de cada capítulo, encontram-se os artigos que sustentam e ampliam o que foi apresentado. Em sua maioria, são os artigos em revistas científicas, mas também foram utilizadas monografias, dissertações e teses que orientei. Esse material é apresentado em apêndices ao final de cada capítulo, e referenciado no texto (também inseri links para acesso virtual às teses e artigos). Os artigos são apresentados na ordem em que foram usados na construção dos capítulos, independente da data de publicação.

Capítulo 1

O chamamento de filhotes: origem evolutiva, desenvolvimento ontogenético e mecanismos proximais

> "*Crying is the most conspicuous of early attachment behaviors. Unlike smiling, which gratifies a caretaker, crying arouses displeasure or alarm and elicits interventions aimed at terminating it and discouraging its recurrence. Herein lies the power of crying to promote proximity more effectively than other early signaling behavior.* " (Bell e Ainsworth, 1972, p. 1172).

Os estudos sobre comunicação animal são relevantes para Psicologia do ponto de vista da compreensão do comportamento enquanto um mecanismo genético-ontogenético com uma história evolutiva. Em estudos comparativos de espécies filogeneticamente relacionadas, as vocalizações e a composição dos repertórios acústicos fomentam discussões acerca da evolução do comportamento e das espécies (e.g., McCracken e Sheldon, 1997), do ambiente (e.g., Mares, Willig, Lacher Jr, 1985), e sobre efeitos de experiência e das estratégias comportamentais de indivíduos com diferentes papéis sociais (e.g., Oliveira-Ueno, 2017).

O porquinho-da-índia (*Cavia porcellus*), ou cobaia, é um modelo experimental que há tempos se adota em laboratórios de diferentes áreas de pesquisa, entre a Medicina, a Biologia, as Neurociências e a Psicologia Experimental (e.g., Burnstein e Wolff, 1967). Para a Etologia, a espécie oferece a possibilidade de se estudar mecanismos e processos de desenvolvimento em animais que apresentam estratégias reprodutivas bem particulares. Como um roedor histricomorfo, as cobaias produzem filhotes precoces, que aos primeiros dias de vida já se alimentam sozinhos (embora ainda dependam de leite), e o macho permanece/faz parte no grupo social, independentemente do estado estral das fêmeas (Kleiman, 1974; Weir, 1974).

A riqueza do comportamento acústico da cobaia chama a atenção dos pesquisadores pelo menos desde Louttit (1929). Na década de 70, Julia Berryman e John Arvola publicaram, independentemente, descrições muito parecidas do repertório de sinais acústicos da cobaia (Arvola, 1974; Berryman, 1976). Pelo menos 11 categorias estruturalmente distintas, agrupadas por Arvola segundo a forma de produção (nasal, naso-oral ou oral) e por Berryman pela forma e contexto de emissão, foram reconhecidas. São chamados de coesão e de contato (*subsounds* para Arvola e as onomatopeias chut e chutters para Berryman); choros melódicos de interações entre machos e fêmeas ou entre adultos e filhotes (*squeak-squeak* e *sociable squeal* ou *whine*) ou em exibições agonísticas (*after shriek* e *distress squeal* ou *squeal* e *scream*); chamados de alarme de curto (drrr) e longo alcance (*song* ou chirrup, que soa como um trinado rápido de um pardal); um chamado de corte (purr), também usado na demonstração de dominância; gritos (*distress squeal* ou *scream*) e assobios (*shriek of hunger* e *agitating squeal* ou *low/high whistles*) usados em contextos de maior urgência de resposta ou de maior excitação (*arousal*), e a batida de dentes, uma produção que não envolve as cordas vocais e é usado em situações de enfrentamento (Arvola, 1974). Entre essas formas, ambos os autores, mais Coulon (1982) e Eisenberg (1974, para os histricomorfos de forma ampla), notaram a ocorrência comum de formas transitórias, que relacionaram a variações nos estados internos do emissor. Variações em intensidade (decibéis, dB) e em ritmo de emissão (ou velocidade) também foram relacionadas a estados internos do emissor e à qualidade da interação (Eisenberg, 1974; Coulon, 1982).

Diante de tal repertório comportamental, a Etologia pergunta: para que essa espécie desenvolveu (no sentido evolutivo-filogenético) essa variedade de sinais de comunicação?; de onde ou quando surgiu? (ou, em outras palavras, é compartilhado por outras espécies? quais espécies? quais sinais são mais derivados e quais poderiam ter sido as forças seletivas de cada um deles?); o que controla cada um desses comportamentos acústicos? Qual a origem ontogenética de cada um, pensando em influências genético-epigenéticas e efeitos de aprendizagem ou do amadurecimento? As respostas à cada uma dessas perguntas podem revelar mecanismos e processos evolutivos e desenvolvimentais de um grupo taxonômico maior, inclusive com aplicação à nossa espécie.

Uma análise evo-devo dos assobios da cobaia

Os assobios da cobaia renderam muitos estudos na Psicologia, inclusive ao nosso grupo de pesquisa (Apêndices A-I deste capítulo). Notamos que eram individualmente distintos (Tokumaru, Ades e Monticelli, 2004; Apêndice A: Individual differences in infant guinea pig pups isolation whistles) e estruturalmente variáveis durante o desenvolvimento ontogenético (Corat, Tarallo, Savalli, Tokumaru, Monticelli e Ades, 2012; Apêndice B: The whistles of the guinea pig: an evo-devo proposal; Monticelli, 2013; Apêndice C: Getting older, being more unstable: nonlinear effects on guinea-pigs isolation calls); e que também estavam sujeitos a mudanças estruturais ao longo do tempo de separação, que poderiam resultar de um mecanismo motivacional de controle endócrino (Monticelli, Tokumaru e Ades, 2004; Apêndice D: Isolation induced changes in guinea-pig *Cavia porcellus* pup distress whistles).

Embora pareçam ser de um só tipo ao ouvido humano, os assobios das cobaias são emitidos em duas situações. O "assobio de separação" (*isolation* ou *distress whistle*; Pettijohn, 1979a) emitido em uma situação forçada de isolamento, parece ser "uma reação que surge pronta, desde o nascimento" (Ades, Tokumaru e Beisiegel, 1994, pg. 81). É usado há décadas como indicador comportamental dos mecanismos fisiológicos do estresse (uma revisão história é oferecida por Hennessy, 2003), da ansiedade e depressão (e.g., Molewijk, Hartog, Van der Poel, Mos e Olivier, 1996), das relações de apego entre mãe-filhote (Pettijonh, 1979 a,b) e da relação entre mecanismos neuro-imunológicos e a psicopatologia da depressão (Hennessy, Deak, Sensenbaugh, Gallimore, Garybush, Mondello e Schiml, 2019).

O "assobio ao tratador", como foi chamado por Ades *et al.* (1994), é uma resposta aprendida, em contextos ambientais regulares e específicos, de natureza antecipatória, adquirida de forma "análoga à formação de um condicionamento pavloviano ou respondente" (pg., 81-82). Cobaias aprendem rapidamente a associar os estímulos da rotina de um tratador, que precedem a entrega de alimento (frutas ou verduras), e assobiam repetidamente frente a esses estímulos. Os autores estudaram a frequência e o padrão temporal de emissão de assobios por um porquinho-da-índia mantido como animal de estimação. O porquinho foi submetido a uma série de 82 episódios de alimentação, ora provida por um tratador humano, ora por outro. Os dois seguiam a mesma rotina: entrar na cozinha, abrir a geladeira, pegar um saco plástico com cenoura ou couve. A partir daí o tratador 1 fechava a geladeira e entregava a verdura na caixa do animal, enquanto o outro apenas largava o saco e fechava a geladeira. Em poucas sessões, o porquinho passou a vocalizar (*subsounds* ou chut e chutters, que passavam a choros, que culminavam em uma sequência de assobios), desde a abertura da geladeira até o fechamento; mas só para o tratador 1. Essa capacidade de discriminação foi entendida pelos autores como um processo de aprendizagem social.

A literatura não oferecia uma explicação sobre a relação entre esses assobios, se existiam como sinais distintos (em forma e função) ou se eram uma variação (ontogenética?) um do outro. Foram apontadas diferenças entre eles (1) estruturais: eram mais longos quando emitidos por adultos (que só assobiavam em antecipação ao alimento, nunca quando sozinhos) e de velocidade de emissão (ritmo mais lento em adultos; Coulon, 1982) e (2) em forma de produção: o assobio de separação (obtidos só de filhotes) tinha uma fase naso-oral seguida de oral (boca aberta), enquanto o *shriek of hunger* era só oral (Arvola, 1974). Mas, por outro lado, talvez tivessem uma mesma função, de atrair a atenção de coespecíficos (ou humanos provedores de alimento) para si (Coulon, 1982).

Começamos a buscar respostas para esse quebra-cabeça estudando a espécie selvagem, o preá. A comparação entre cobaias e preás permite discussões interessantes sobre o processo de domesticação de animais (e.g., Sachser, 1986; Monticelli, Tokumaru e Ades, 2017), sobre herança genética do comportamento (e.g., Rood, 1972), e sobre aspectos do comportamento acústico (Monticelli, 2000, 2005). O assobio de separação também é emitido por filhotes de espécies selvagens de preás (Monticelli, 2005; Monticelli e Ades, 2013; Apêndice E: The rich acoustic repertoire of a precocious rodent, the wild cavy *Cavia aperea*; Verzola-Olivio e Monticelli, 2017; Apêndice F _ The acoustic repertoire of *Cavia intermedia* as a contribution to the understanding of the Caviidae communication system; Verzola-Olivio, 2014; Apêndice G: Descrição comparativa dos repertórios acústicos dos preás *Cavia magna* e *Cavia intermedia*). Em um cercado onde eu observava grupos de preás recém-capturados, certo dia houve um estrondo de um trovão enquanto os animais de um grupo forrageavam. Todos eles correram para a direção dos abrigos que havia dentro do cercado. Passados alguns segundos, um filhote, que havia ido sozinho para um dos abrigos, assobiou intensamente, apenas uma sequência de poucas notas, enquanto movia-se rapidamente. Depois, ficou em silêncio, movendo-se lentamente, até que a mãe veio em sua direção. Quando tentei, contudo, obter mais registros desse assobio para comparar, em termos de estrutura, com os de cobaia, não tive sucesso: fiz cerca de 12 tentativas, usei duplas de filhotes ao invés de um só, e consegui que apenas um dos filhotes de preás, testado em dupla com o irmão de ninhada, assobiasse. Preás adultos nunca foram vistos assobiando, nem quando um deles foi criado com cobaias, na residência da secretária do Prof. Norbert Sachser, da Universidade de Münster (comunicação pessoal de Sabine Krüse, observada pessoalmente por mim, em 1999). Esse preá criado com cobaias tinha coloração branca misturada à aguti (Figura 1.1). Esse fenômeno havia sido observado algumas vezes, segundo me relataram, no criadouro de preás descendentes de uma população parental, capturada ao redor de Buenos Aires. Há cerca de 20 anos, os animais vinham sendo reproduzidos em 6 linhagens reprodutivas (Linhagem A1 a A6) e pelo menos uma vez, quando estive lá em 1999, uma nova amostra de animais capturados (linhagem B) havia sido levada. O interessante dessa história é que o surgimento da cor branca nos pelos é um efeito comum do processo de domesticação, como discutirei a seguir.

Figura 1.1. Preás descendentes de uma população capturada na região de Buenos Aires, mantidos na Universidade de Münster (em 1999), sob responsabilidade do Prof. Norbert Sachser e identificados como *Cavia aperea pamparum*. Eu os fotografei durante minha visita para gravação de vocalizações para o projeto de mestrado. À esquerda está um macho de pelagem aguti, em um cercado do biotério. À direita, um macho descendente de uma das linhagens, que nasceu com os pelos predominantemente brancos, criado com cobaias no quintal de uma casa. Acima da casinha de madeira, há uma cobaia da raça roseta, que tem os pelos voltados para diferentes direções, virada para a esquerda e com a cabeça abaixada. Fotografados em setembro de 1999, em Münster, Alemanha.

O que era, então, esse "assobio ao tratador", emitido apenas por cobaias? Tínhamos duas hipóteses:

(1) Hipótese da novidade evolutiva: o "assobio ao tratador" seria um chamado novo no repertório de *Cavia*, que desempenhava uma função comunicativa diferente daquela do assobio de separação. Podia ser, por exemplo, um sinal de comunicação interespecífica entre humano e cobaia. Hoje parece claro que cães e humanos desenvolveram um sistema de comunicação interespecífica que não é compartilhado entre lobos (*Canis lupus*) e cães (*Canis lupus familiaris*), e nem parece se desenvolver ao longo da história de vida cão-tutor (Hare & Tomasello 2005). Cães de diferentes raças e com diferentes histórias de vida compreendem dicas humanas (apontar) para encontrar um objeto escondido (e.g., Hare, Call e Tomasello, 1998; Miklósi e Sophroni, 2006); e tutores, de sua parte, são capazes de avaliar o estado de desconforto de seus cães a partir do comportamento e das expressões visuais (Mariti, Gazzano, Moore, Baragli, Chelli e Sighieri, 2012).

O processo evolutivo que teria selecionado essa habilidade de compreensão interespecífica poderia ser o de domesticação (Hare & Tomasello 2005). A favor desse argumento, está o achado de que cães são melhores do que chimpanzés, filogeneticamente mais próximos à nossa espécie, na tarefa de encontrar comida usando sinais humanos como dica (Hare *et al.*, 2002; Braüer *et al.*, 2006). O "assobio ao tratador" teria sido modificado por um processo de ritualização (Cullen, 1966; Eibl-eibesfeldt, 1979), a partir do assobio do filhote ou do grito da fêmea durante o cortejo, promovendo uma compreensão entre humano e animal. Essa hipótese depende da premissa de que essa comunicação com os humanos seja, ou tenha sido, vantajosa às cobaias (ao emissor do sinal, como propõe Fitch e Hauser, 2003).

(2) Hipótese da variante ontogenética: o "assobio ao tratador", alternativamente, poderia não ser um chamado novo do repertório de *Cavia*. Essa hipótese nos parece mais plausível: não encontramos diferenças estatísticas entre os assobios (Apêndice B) e a variação estrutural entre eles é condizente com uma variação pela ação do desenvolvimento ontogenético, como relatamos em Corat et al. (2012). Gravamos os assobios de cinco filhotes separados da mãe por alguns minutos, em uma sala isolada, aos 7, 14 e 21 dias de vida (a partir dos 14 dias de vida, os filhotes começaram a assobiar em antecipação à chegada de alimento); depois levamos esses animais para uma situação de criação como pet, isolados uns dos outros, para que não houvesse sobreposição entre assobios de diferentes animais. E comparamos a estrutura acústica de assobios ao tratador (AT) e de separação (AS) em diferentes idades, dentro de um mesmo sujeito (Figura 1.2; Fig. 1.3 para preás). O AT tem notas mais longas (mediana 0,34 contra 0,24 de AS, p=0,063), frequência mínima mais alta (mediana 676,26 Hz, contra 595,88, p=0,063), e contém saltos de frequência (frequency jumps, e outros efeitos não-lineares descritos por exemplo em Volodina, Volodin e Filatova, 2006) e ritmo mais lento (2,74 contra 3,15 notas/s, p=0,125). Mas essas diferenças não foram significativas em um teste de Wilcoxon, talvez pela pequena amostra.

Uma análise subsequente de tendências ao longo do tempo, foi feita com os parâmetros para os quais a suposição de normalidade havia sido satisfeita. Os resultados mostraram uma tendência significativa de aumento na duração da nota e na altura da frequência mínima, e de diminuição no ritmo, ao longo do desenvolvimento. Não havia sido possível comparar o assobio AT antes dos 30 dias com os obtidos mais tarde, para cada filhote (os adultos vocalizam mais e mais alto, sobrepondo-se com os assobios dos filhotes). Como não encontramos suporte estatístico para as diferenças entre AT e AS, e a análise de tendência mostrou ser contínua a variação ao longo do tempo de desenvolvimento do filhote, do primeiro AS ao AT, concluímos que o assobio ao tratador era uma variante ontogenética do assobio de separação. Mudanças anatômicas no trato vocal durante o crescimento, como o aumento do comprimento da laringe e o espessamento das cordas vocais, e o aumento da capacidade pulmonar ao longo do desenvolvimento do animal, podem explicar as diferenças entre as vocalizações (Corat *et al.*, 2012).

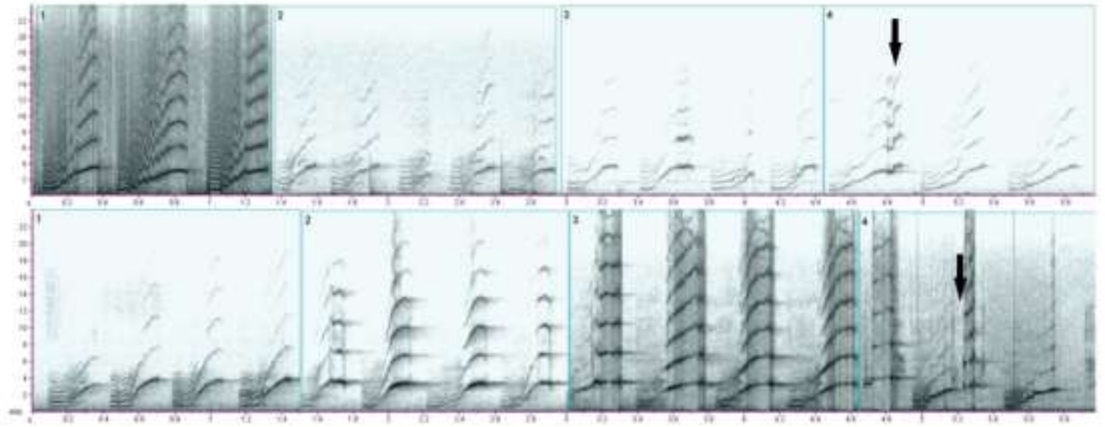

Figura 1.2. Sequências de notas de assobio emitidas pelos filhotes Marronzinho (acima) e George (abaixo). De 1 a 3 estão representados trechos de assobio de separação, aos 7, 14 e 21 dias de vida, nessa ordem. O último trecho de assobio de cada filhote é de assobios ao tratador (AT), obtidos a partir dos 30 dias de vida, em resposta a estímulos que antecedem a entrega do alimento. O AT tem notas mais longas, saltos de frequência (seta) e ritmo mais lento. Extraído de Corat et al., 2012.

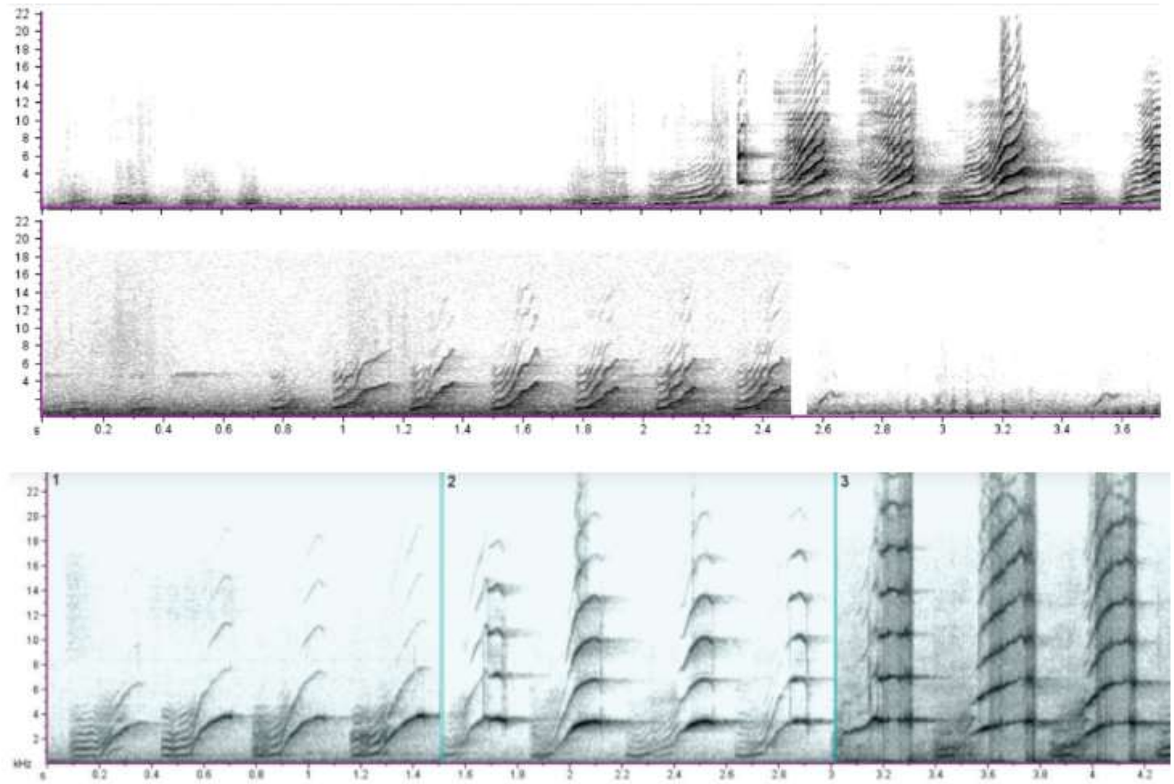

Figura 1.3. Sequências de notas de assobio emitidas por um filhote de preá (duas linhas acima) sobrepostos aos assobios do filhote de cobaia George (abaixo), para comparação. De 1 a 3 estão representados trechos de assobio de separação aos 7, 14 e 21 dias de vida (vide Fig. 1.2). Sonogramas de preás extraídos de Monticelli e Ades, 2013.

Por que, então, o assobio (agora, um só) era emitido em contextos diferentes: filhotes separados e adultos (também filhotes?) em antecipação à entrega de alimento? Que mecanismo modula a emissão do assobio, que o dispara em momentos, aparentemente, tão distintos?

Uma possibilidade é que essa regulação seja feita pelo sistema pituitário–adrenocortical (PAC: Sachser, 1998) ou hipotalâmico-hipofisário-adrenal, responsável pela resposta fisiológica de estresse. Ao longo do tempo de separação, o nível de cortisol plasmático sobe linearmente, ao passo que os assobios diminuem (Pettijohn, 1979a; McInturf & Henessy, 1996). Essa relação inversa sugere que a resposta de assobiar só seja disparada por níveis baixos de cortisol (estresse). Conforme esse nível aumenta ao longo do tempo de separação, segue-se um bloqueio, como propusemos em Corat *et al.*, 2012, das respostas de assobiar e locomover-se (Pettijohn, 1979a). Henessy, Ptstick, O'Leary, Maken & Farré (2001) também relataram diminuição na taxa de assobios de forma concomitante ao aumento dos níveis de cortisol no sangue. Nossa conclusão, então, foi que o estresse moderado (mas não intenso) era o que disparava os assobios e que esse é o nível de estresse que acompanha tanto os episódios breves de separação como a percepção dos estímulos que antecedem a entrega de alimento (e.g., Mistlberger, 1993). Em modelos animais não-humanos, a antecipação da ingestão de comida provoca mudanças endócrinas que incluem um aumento na atividade do eixo hipofisário-pituitário-adrenocortical HPA (Ott, Friedrich, Prilop, Lehnert, Chara, Born e Hallschmid, 2011). Em humanos, o anúncio de uma refeição, com a apresentação visual e olfativa de itens alimentares, duas horas depois seguido do anúncio de que a refeição não virá, produz um aumento do nível de cortisol (Ott *et al.*, 2011).

Voltamos à proposta de Coulon (1982) de que haveria um contexto comum aos dois assobios: chamar a atenção de coespecíficos (ou simplesmente a mãe). Esse sistema integrado de disparo quando o nível de cortisol é moderado e supressão a níveis mais altos, poderia ter sido selecionado pela vantagem conferida ao filhote, justamente na situação de forrageamento, quando a separação entre ele e a mãe deve ser mais provável. Sob condições de biotério ou de criação como *pet*, esse sistema será disparado apenas na situação de antecipação de estímulos relacionados à entrega de alimento (Corato *et al.*, 2012).

Também essa hipótese, como a anterior, encontra apoio nos estudos que compararam espécies domesticadas com seus representantes selvagens atuais, ou linhagens com maior ou menor nível de seleção genética (dito "aprimoramento genético", à gosto do ser humano; Hale, 1962). Os processos de domesticação de animais, aves ou mamíferos, muitas vezes produziram um fenômeno de "neotenia" (Price, 1984), i.e., a retenção de características de filhotes (no representante selvagem) até a fase adulta (na variante domesticada), causada por um atraso na taxa de desenvolvimento de traços reprodutivos e somáticos (Trut, Plyusnina e

Oskina, 2004). Aqui, é necessário lançar mão do incrível estudo longitudinal do geneticista russo Dmitry Belyaev (Laboratório *Fur-bearing animal breeding*, depto de Genética e Seleção da Universidade Estatal de Moscou). Ele esteve por anos interessado nos processos de desenvolvimento e de regulação do desenvolvimento em animais que passaram por processos de domesticação. Para ele, a domesticação começa com um momento no qual os processos que afetam os sistemas de desenvolvimento estão sujeitos primordialmente à seleção natural (Belyaev, 1979). O ser humano, o "novo fator ambiental" desse estágio inicial de evolução por domesticação, age como um direcionador do comportamento e da capacidade do animal de "existir no novo ambiente antropogênico", mas não é a força seletiva mais forte. Gradualmente, então, a seleção artificial exercida pelo criador ganha força, a princípio praticada de forma inconsciente, e mais tarde, de forma sistemática (Trut, Plyusnina e Oskina, 2004, pg. 794).

Ao selecionar por 20 anos raposas para mansidão, dentre tantos comportamentos parecidos com os cães (e.g., buscar proximidade com humanos e atender a chamamentos através de apelidos individualmente atribuídos), Belyaev notou que os animais exibiam também vocalizações afiliativas e apaziguadoras, dirigidas a humanos. Nas palavras dele: "*There is something moving in the emotions of these foxes, that as the sight of even a strange person, they try actively to attract attention with their whining, wagging of tails, and specific movements.* " (Belyaev, 1979, pg. 303). As mudanças no comportamento resultantes da seleção por mansidão, foram acompanhadas de mudanças em funções reprodutivas, produzindo o que ele chamou de "seleção desestabilizadora" (Belyaev, 1979). Esse termo, tal como o autor justifica, se refere ao efeito observado sobre o sistema neuroendócrino do desenvolvimento; a domesticação quebrava sistemas ontogenéticos antes integrados, sistemas esses que antes garantiam a normalidade, ou estabilidade, do desenvolvimento e do fenótipo, e descartavam o que não se encaixava a isso. Como consequência, permitiam o aparecimento de "efeitos múltiplos no fenótipo" que não estariam geneticamente relacionados ao traço selecionado (no caso, a mansidão); ao contrário, relacionar-se-iam a mudanças na regulação gênica (i.e, no momento e na quantidade de genes expressos, ao invés de mudanças em genes estruturais individuais; Belyaev, 1979).

Mudanças morfo-fisiológicas (e.g., encurtamento do focinho, levantamento do rabo e caimento das orelhas, surgimento da coloração branca no pêlo e adiantamento da fase anual de troca de pêlo) também foram registradas por ele (Belyaev, 1979). Ora, se os sistemas endócrino e nervoso estão inter-relacionados, discorre o autor, a seleção por mansidão deve ter alterado o quadro hormonal das raposas e se refletido no desenvolvimento: hormônios regulam genes que sintetizam ou bloqueiam a síntese de proteínas e regulam atividades bioquímicas. No estudo de Belyaev, em ambos os sexos, os animais mansos diferiram significativamente dos não-selecionados (os que viviam em fazendas de criação para extração de pêlo) tanto no nível de cortisol plasmático (*11-oxycorticosteroids*) como na atividade secretora e na morfologia da glândula adrenal (Belyaev, 1979).

Além de alterar o sistema hipotalâmico-hipofisário-adrenal das raposas, o autor também notou alteração para cima dos níveis de hormônios esteroides (estradiol e progesterona) e de serotonina e seu metabólito (ácido 5-hidroxindolaecetico), o que explica a redução da agressividade dos animais selecionados (Belyaev, 1979). No início do desenvolvimento, o filhote mamífero passa por um período de socialização, cuja janela temporal se fecha com a maturação de sistemas sensoriais e locomotores (Bornstein, 1979; Belyaev, Plyusnina e Trut, 1985). Essa maturação sensório-motora, acontece em filhotes de lobo e de raposas *Vulpes vulpes* aos 45 dias de vida (Trut *et al.*, 2004), em cães, isso só acontece entre 4 e 6 meses (em alguns deles, 8 a 10 meses); como os cães, os filhotes *V. vulpes* selecionados por Belyaev (gerações 28-30 da seleção de 1988-90) levavam mais de 3 meses até que a janela de socialização se fechasse (momento indicado pelo surgimento das respostas de medo, *fearful response,* quando os filhotes passam a se afastar de estranhos). Ou seja, as mudanças nas taxas de desenvolvimento sensório-motora descritas por Belyaev, estenderam o período sensível e a eficácia da adaptação social, inclusive à humanos (Trut *et al.*, 2004). Mais tarde, as pesquisas continuadas pelo grupo de Belyaev sugeriram a relação da expressão de QTLs (*quantitative loci genes*) com as respostas de medo e com os níveis plasmáticos de hormônios adrenocorticais (Trut et al., 2004). O surgimento da resposta de medo nas raposas não-selecionadas de 45 dias foi acompanhado de um aumento abrupto no nível de glicocorticóides plasmáticos; em filhotes domesticados da mesma idade, nem a resposta de medo está presente, nem se nota diminuição da atividade exploratória, e também não há variação do nível plasmático de glicocorticoides (Trut *et al.*, 2004). Os glicocorticoides devem, então, estar envolvidos na determinação das taxas de desenvolvimento e nas mudanças dessas taxas durante a domesticação (Trut et al., 2004).

Voltando às cobaias e preás, também em cobaias a atividade funcional dos sistemas SAM (simpático-adrenomedular: relacionado à produção de adrenalina e noradrenalina) e HPA (relacionado à produção de cortisol, antes atribuído ao que Belyaev chamou de sistema hipotalâmico-hipofisário-adrenal) é significativamente menor do que em preás (Künzl e Sachser, 1999), mesmo depois de gerações em cativeiro (Künzl, Kaiser, Meier e Sachser, 2003). Os estímulos que evocam em preás uma rápida resposta fisiológica e comportamental de estresse, precisam de maior intensidade (ou um “limiar mais alto de estimulação”: Ratner e Boice, 1975) para produzir respostas semelhantes em cobaias (Sachser, 1999). Por exemplo, preás emitem com frequência um trinado alto (o chirrup de Berryman, 1976), após um evento repentino no ambiente físico ou social (Monticelli, 2005). Nunca nenhum de nós, que trabalhamos com cobaias no laboratório de Psicoetologia do Instituto de Psicologia da USP, desde 1990 (quando a Dra Beatriz Beisiegel iniciou a colônia, para seu projeto de mestrado), ouvimos o chirrup. Diversas vezes, adotamos cobaias como animal de estimação, e foi em uma dessas situações que alguns de nós notaram o “pi pi pi pi pi”, sempre à noite, mas ainda assim raramente (Monticelli e Ades, 2011; Apêndice H: *Bioacoustics of domestication: the alarm and the courtship calls of* Cavia; Monticelli, 2005). Portanto, esse sinal faz parte do repertório das duas espécies, mas deve ser desencadeado pelos sistemas SAM e HPA, que atuam em menor atividade na espécie domesticada. Quando o nível de estresse que leva preás a emitirem o chirrup é atingido em cobaias, a resposta seria desencadeada.

É provável que preás também aprendam a identificar estímulos associados ao fornecimento de alimento, mas o medo da presença humana bloquearia a resposta vocal. Quando eu observei uma colônia de preás que eu havia capturado em Itu, eu notei que os animais se aproximavam da borda da caixa onde eram mantidos, cheiravam o ar e olhavam na direção do tratador no início da rotina de alimentação, mas não assobiavam (Monticelli, 2005; Monticelli e Ades 2013, Apêndice E). Novamente, esse resultado pode ser entendido dentro do cenário de Belyaev (1979): ao agir em sistemas reguladores do desenvolvimento, a domesticação promove uma ampliação da janela temporal de socialização na cobaia que já estaria fechada nesses filhotes de preás. Também em ovelhas, filhotes de uma linhagem altamente selecionada apresentam maior taxa de vocalização de separação (*distress call*) do que filhotes de linhagens menos selecionadas (Dwyer, McLean, Deans, Chirnside, Calvert e Lawrence, 1998).

O estudo sobre os assobios de *Cavia*, que começou em 1996, com minha iniciação científica, só chegou à uma conclusão sobre o que era o assobio ao tratador na publicação de 2012. O assobio ao tratador é, a nosso ver, uma forma amadurecida do assobio do filhote, e não uma vocalização que surgiu durante o processo de domesticação; e o mecanismo de disparo das duas emissões deve ser o mesmo, mas em limiares diferentes de ansiedade (Corat *et al.*, 2012).

O papel das vocalizações nas relações de apego

A amamentação é uma etapa necessária do desenvolvimento psicossocial em mamíferos. Na nossa espécie, o contato corpo-a-corpo durante a amamentação, a estimulação do mamilo pelo sugar do bebê, e quiçá outras tantas formas de trocas interacionais, (1) favorecem o reconhecimento do filhote pela mãe através do odor do leite (Porter, 2004); (2) ativam a sequência inicial de eventos em cascata que desencadeiam e regulam o comportamento materno (Numan, 2006); (3) tornam mais efetiva a conservação de energia corpórea nas primeiras horas após o nascimento (Porter, 2004); (4) promovem a contração uterina necessária para a expulsão da placenta, prevenindo o acúmulo de sangue no útero (Porter, 2004), dentre tantos outros efeitos já conhecidos, ou que ainda virão a ser. O reconhecimento entre mãe-filhote, por sua vez, torna possível a formação de vínculo afetivo (apego), que é uma peça-chave na garantia de cuidado: um mecanismo proximal vantajoso para o sucesso reprodutivo da mãe e do filhote.

O apego ainda hoje é entendido tal como descrito por Bowlby (1958) e mais tarde ampliado por Ainsworth (1979). Constitui-se em um repertório de comportamentos ("*attachment behaviors*"), presente em muitas espécies de mamíferos e de aves (Bell e Ainsworth, 1972), que levam à proximidade entre mãe e filhote. Em <u>espécies altriciais</u>, que produzem filhotes incapazes de ver e aproximar-se da mãe, o principal comportamento de apego ao nascer é a vocalização (Bell e Ainsworth, 1972; Muller & Shair, 2016). Essa vocalização, que é o choro do bebê humano e de outras espécies altriciais (Figura 1.4), é irritante e promove ações que o interrompam. O custo energético da produção é compensado pela chance de se restabelecer o contato e promover o cuidado (Christensson, Cabrera, Christensson, Uvnas-Moberg, Winberg, 1996; Rao, Blass, Brignol, Marino e Glass, 1997). Como nos assobios da cobaia, o choro cessa quando os bebês são reunidos à mãe, e diminui mesmo pela simples exposição do bebê ao odor do líquido amniótico da mãe (Varendi, Christensson, Porter, Winberg, 1998). O reconhecimento do líquido amniótico, inferido pelo efeito calmante, é um indício de aprendizagem <u>pré-natal</u> de pistas da mãe em humanos (Porter, 2004) e, provavelmente, em outros mamíferos; essa aprendizagem é um pré-requisito para o estabelecimento da relação tão particular entre infante e cuidador, que envolve vinculação afetiva (Porter, 2004).

O choro dos filhotes altriciais é, contudo, diferente do assobio da cobaia e de outros filhotes precoces, em forma e provavelmente em função. O incômodo causado pelo choro do neonato altricial vem do timbre alto e da irregularidade; além disso, é um som facilmente localizável e estruturalmente muito variável. A variabilidade (compare as notas de cada linha da Figura 1.4: foram emitidas em sequência por um mesmo filhote, urso ou humano, e cada uma é diferente da outra) reside também na presença de efeitos não lineares (dos quais falarei no capítulo 2), que talvez sejam indicadores honestos do nível de urgência ou excitação do emissor (Zeskind & Marshall, 1988; Owren and Rendall 2001).

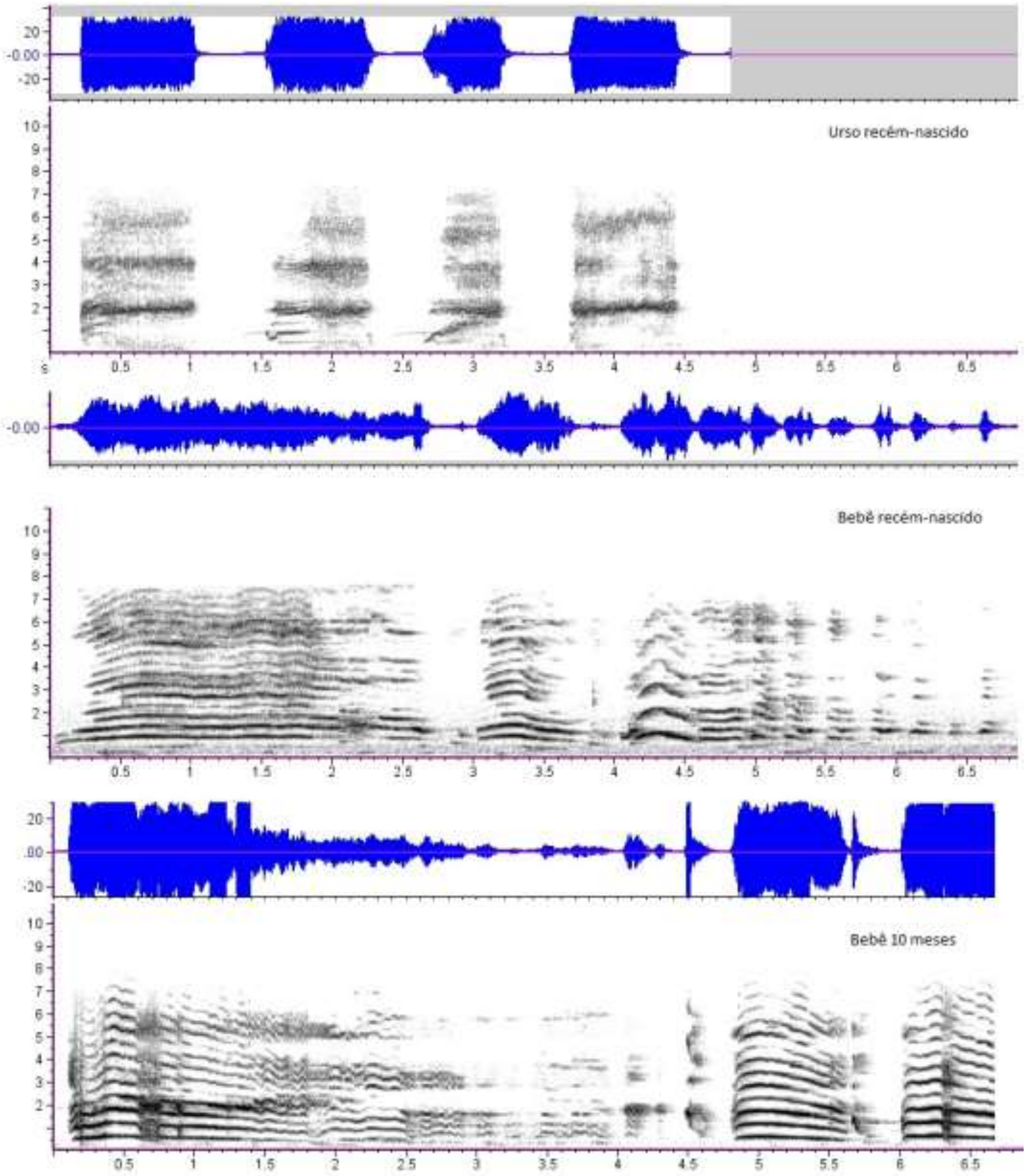

Figura 1.4: O choro é um comportamento compartilhado por mamíferos que produzem filhotes altriciais, para quem essa é a única forma de defesa (os olhos estão fechados, não se alimentam sozinhos e não podem mover-se bem). Acima, mostro um trecho de choro de um filhote urso de poucos dias de vida (obtido de https://www.youtube.com/watch?v=Msbmi5EwGkA); abaixo, um trecho de choro de um recém-nascido humano, obtido de uma biblioteca sonora digital (https://freesound.org/people/the_yura/sounds/211528/); na parte inferior, o choro de um bebê de 10 meses, já na hora de mamar (gravado em celular pela profa. Annie Hsiou). Os três registros foram tocados a partir de suas fontes e gravados no programa Avisoft SAS Pró 2.0. As imagens (espectogramas: frequência em kHz por tempo em segundos; envelope de som acima de cada um: amplitude em kU por tempo) foram gerados no programa Raven Pró 1.5.

O assobio da cobaia, ao contrário, é uma nota curta, tem o timbre mais baixo (que poderia facilitar a transmissão à média distância), mas a curta duração não facilita a rápida localização do emissor, por exemplo, um predador. São estratégias comportamentais distintas, originadas de formas de vida distintas. No preá, o filhote que se separa do grupo, como observei uma vez em Itu, assobia um pouco e então se cala, mas continua a se mover apressadamente; a mãe pára e move-se para fora do abrigo, talvez vocalize (*subsounds*, Monticelli & Ades, 2013), e o filhote a encontra (Monticelli, 2005).

O apego em filhotes precoces, i.e., capazes de mover-se em direção ou em busca da mãe, se desenvolve dentro de um processo de "estampagem" (do termo inglês *imprinting*; Hess, 1959). Esse processo envolve um período de aprendizagem independente de condicionamento (Sluckin, 1968). Tal como descrita por Lorenz (1995, pp. 343-368), a estampagem à uma figura de apego se dá em um período crítico de desenvolvimento, em um nível supra individual (o vínculo é generalizado para toda a espécie, na fase adulta), é duradoura e irreversível (talvez não seja: Salzen e Meyer, 1967), e influencia padrões comportamentais futuros (não para todos os sistemas comportamentais: Immelman, 1975; Rantala e Marcinkowska, 2011).

Houve uma leva de estudos feitos nos anos 60 e 70, sobre o mecanismo de estampagem usando porquinhos-da-índia como modelo experimental. Filhotes foram testados em diferentes aparatos, sob o paradigma experimental da escolha entre duas possibilidades: objeto familiar x outro objeto (e.g., Gaston, Stout e Tom, 1969; Sluckin, 1968), aproximar-se de um coespecífico desconhecido no alto de uma caixa inclinada ou permanecer sozinho na parte baixa (Nagy e Misanin, 1970), manter-se perto da mãe de criação (da mesma espécie ou de outra) x outra fêmea (outra espécie, própria espécie lactante ou não; Beauchamp e Hess, 1971; Porter, Fullerton e Berryman, 1973).

Nos anos 70, de um lado da literatura, a estampagem não se diferenciava, em termos de mecanismo, de um processo de aprendizagem condicionada. Para Cairns (1966), por exemplo, a causa das relações de apego entre animais de um grupo social (e.g., entre mãe-filhote) era o efeito reforçador da satisfação do relacionamento próximo e continuado entre os sujeitos, da conspicuidade e relevância da figura de apego às atividades do "sujeito apegado". A retirada da figura de apego seria um estressor psicogênico, que provoca uma ruptura no comportamento do sujeito apegado, mas que pode ser reparada com a volta do objeto de apego (Cairns, 1966). Na prática, filhotes de uma hora de vida treinados a responder com seguimento a um objeto (bloco de madeira, cachorro de pelúcia, bolinha com guizo, entre outros), seguem e/ou passam mais tempo com esse objeto quando, em sessões de escolha subsequentes (até o 17 dia de vida), ele é apresentado junto com objetos novos (Gaston *et al.,* 1969). Para os autores, a escolha indicava que o filhote havia se apegado e de forma surpreendente (algumas horas após nascer), duradoura (todos os 16 filhotes que sobreviveram à falta de amamentação e socialização, mantiveram a escolha até o 17o dia de vida) e específica ao objeto durante o treino (sessões de 10 minutos, a cada duas horas da 2a à 26a horas de vida). De outro lado, a estampagem era vista como um processo que não envolvia apenas respostas de aproximação e seguimento: envolvia apego à uma figura específica (*attachment to a given figure*; Sluckin, 1968).

Pettijohn (1979b) acompanhou a relação entre filhotes de cobaias até os 2 meses de vida, olhando para a quantidade de tempo que gastavam com a mãe e o pai (objetos sociais familiares), eliminando efeitos de confusão que pudessem decorrer da apresentação de objetos não-familiares, dos paradigmas da década de 60 (e.g., Gaston, Stout e Tom, 1969). Ele usou 24 animais de 8 ninhadas, metade de cada sexo, nascidos e mantidos com os pais em uma mesma caixa. Uma vez por semana, nas semanas 2, 4, 6 e 8 de vida, um filhote por vez foi testado em um aparato semelhante à caixa-moradia, mas que dividia os pais em compartimentos diferentes. Quando as escolhas disponíveis são dois estímulos sociais, familiares e seguros, havendo preferência por um deles, essa diferença dever-se-ia à um vínculo diferencial do filhote com a figura escolhida. Nas semanas 2 e 4 (i.e., até os 30 dias de vida), os filhotes passaram significativamente mais tempo no compartimento próximo à mãe. Nas outras semanas, a escolha entre mãe e pai não foi maior do que o acaso[1]. Essa mudança ao longo do tempo pela preferência pela mãe também havia sido notada por Beauchamp e Hess (1971) e coincide exatamente com o desmame (na 4a semana de vida filhotes raramente mamam). O vínculo com a mãe se dava em decorrência do provimento do leite, então? O casal Freud e John Bowlby e Melanie Klein discordaram sobre isso (Bowlby, 1958; Klein, 1975; Fonagy, Luyten, Allison e Campbell, 2018). A preferência pela mãe independia do fornecimento de leite, no clássico (e cruel) experimento de Harry Harlow, usando mães de arame (que fornece leite) e de pano: "*We were not surprised to discover that contact comfort was an important basic affectional or love variable, but we did not expect it to overshadow so completely the variable of nursing; indeed, the disparity is so great as to suggest that the primary function of nursing as an affectional variable is that of insuring frequent and intimate body contact of the infant with the mother.*" (Harlow, 1958, p. 677)

Cobaias neonatas criadas com uma galinha, uma cobaia fêmea ou sua mãe biológica, quando são depois submetidas a testes de preferência entre "sua figura de mãe" e "outra figura de mãe", seguem suas mães de criação (i.e., galinha, cobaia fêmea ou mãe biológica), nas três primeiras semanas de vida (Beauchamp e Hess, 1971). Depois disso, as jovens fêmeas não mostram mais preferência entre seu modelo de mãe e outro modelo, e os machinhos,

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[1] O autor descarta um efeito de habituação do filhote com o aparato e a situação de teste ao longo das semanas, com base na percepção de que filhotes não diminuem a taxa de assobio de separação ao longo da repetição de sessões em um mesmo aparato (Pettijohn, 1979a)

independente de terem sido criados por galinha ou cobaia, escolhem as cobaias.

Os estudos de Pettijohn indicam que haja, também em *Cavia porcellus*, uma preferência dos filhotes pela mãe, mesmo quando não é possível mamar (Pettijohn, 1979b). A discriminação entre mãe e pai pode se dar por pistas olfativas e visuais ou pelo comportamento da mãe que, nas primeiras sessões buscava ativamente contato com o filhote, através da tela. Ao longo do desenvolvimento dos filhotes, o vínculo com a mãe ou (1) se enfraquece ou, (2) se expressa em segurança para explorar o ambiente, como propõe alternativamente o autor: estando a mãe ao alcance, o filhote já mais hábil em termos sensório-motores, investiria em exploração do ambiente do aparato, sem perder o contato com ela (o cheiro dela estaria presente em toda a caixa-teste, mesmo quando ela não estivesse à vista). Em crianças, chamamos essa segurança advinda da presença da mãe, de estilo de apego seguro com o cuidador (Ainsworth, 1979). Em humanos, o estilo de apego desenvolvido na infância, tem um grande poder de previsibilidade do estilo de apego do adulto, com impacto nas futuras relações amorosas (Kirkpatrick e Hazan, 1994).

O assobio como indicador de estresse e sua associação a processos neurobiológicos

O assobio de separação do filhote da cobaia é um indicador confiável não só para o estudo do mecanismo de apego (Pettijohn, 1979a; Hennessy, 1997). Tem servido à compreensão dos mecanismos neuroendócrinos e imunológicos de ação central, relacionados ao desenvolvimento de transtornos que acometem humanos, como ansiedade e depressão (Hennessy, 2003; Hennessy, Deak, Sensenbaugh, Gallimore, Garybush, Mondello e Schiml, 2019).

Quando separado da mãe e do ambiente social, o filhote cobaia assobia à uma taxa que permanece constante da 1a para a 4a semana de vida, e gradualmente cai a zero até a 12a semana (Pettijohn, 1979a). Independente do ambiente, se isolado em sua caixa-moradia (pela retirada dos outros animais) ou em caixa-teste (levado sozinho para uma caixa vazia), a introdução da mãe acalma o filhote, que assobia e locomove-se menos quando ela está presente (Pettijohn, 1979a).

Os assobios de separação de filhotes de cobaia não parecem ser explicados simplesmente pela necessidade de termorregulação, dada a precocidade dos neonatos (Hennessy, 1997; Hennessy & Ritchey, 1987; Pettijohn, 1979a). Têm sido, ao contrário, interpretados como resposta comportamental de estresse pela correlação entre a taxa de assobios e os níveis plasmáticos de cortisol e ACTH (*Adrenocorticotropic hormone*) (Hennessy, 2003). Como saber se a resposta do sistema hipotalâmico-pituitário-adrenal (HPA) não é efeito da falta de um coespecífico familiar, qualquer que seja, e não especificamente da mãe?

Ritchey & Hennessy (1987) comparam as respostas fisiológicas de filhotes isolados com a de filhotes colocados na mesma situação experimental, mas com um irmão de ninhada (lembro o leitor que essa também foi a única forma que eu encontrei para registrar o assobio de separação de preás, Apêndice E). A presença do irmão não diminuiu as respostas de estresse da separação, que foram tão intensas quanto a do filhote separado. A separação involuntária do objeto de apego, mas não de um parceiro com quem se tem apenas relações afiliativas, é o que leva, como constatado por Hennessy (1997), à pronta e duradoura ativação do sistema de resposta ao estresse, o HPA. Esse resultado levou Hennessy (1997) a distinguir relações de apego e relações afiliativas. O ser apegado tem uma forte relação emocional com a figura de apego, que é vista como "base segura para a exploração"; relações entre juvenis e adultos podem ser pacíficas ou até afiliativas, mas não necessariamente constituem-se em uma vinculação afetiva de apego.

Wewers, Kaiser e Sachser (2003) gravaram o comportamento de 32 filhotes, metade de cada sexo, em suas colônias (cercados estabelecidos dentro de salas de um biotério), em duas situações: na presença e na ausência da mãe, mas sempre na companhia dos outros membros do grupo social (eram dois grupos, cada um com 9 machos adultos, 13 fêmeas e 5 a 23 filhotes). Filhotes de ambos os sexos locomoveram-se e assobiaram por mais tempo na ausência do que na presença da mãe, mesmo quando mantidos "em casa", com outros membros do grupo; a diferença no tempo que os filhotes machos passaram assobiando não foi significativa (ainda assim, é considerável: na ausência da mãe assobiaram em mediana, por 1,08 min, e algum deles assobio por 4,47 min; na presença da mãe, assobiaram em mediana por 0,08 min, e o máximo de tempo assobiando não chegou a meio minuto; Wewers et. al, 2003). Em relação aos parâmetros fisiológicos, quando os filhotes foram mantidos em seu cercado, não houve variação significativa no nível de cortisol plasmático entre o início e aos 120 minutos de sessão, nem na presença da mãe, nem na ausência. Quando o isolamento foi feito em ambiente não familiar, ao contrário, o aumento foi significativo, do início aos 120 minutos. Os autores interpretam, com base nesses e em resultados anteriores (Sachser, 1986, 1998), que a vinculação social é tão importante como o estabelecimento de relações de dominância no controle das respostas fisiológicas de estresse. A vinculação a um parceiro social, segundo eles, acontece em filhotes em relação à mãe, e em adultos em relação a outro coespecífico que, como a mãe para o filhote, amenizará com sua presença as respostas agudas de estresse no isolamento social (i.e., oferece "apoio social" ou, em inglês, "*social support*"; Sachser, 1998). Só parceiros com quem se tem vínculo, poderiam servir como apoiadores sociais, reduzindo as atividades dos sistemas pituitário-adrenocortical e simpático-adrenomedular (Sachser, 1998).

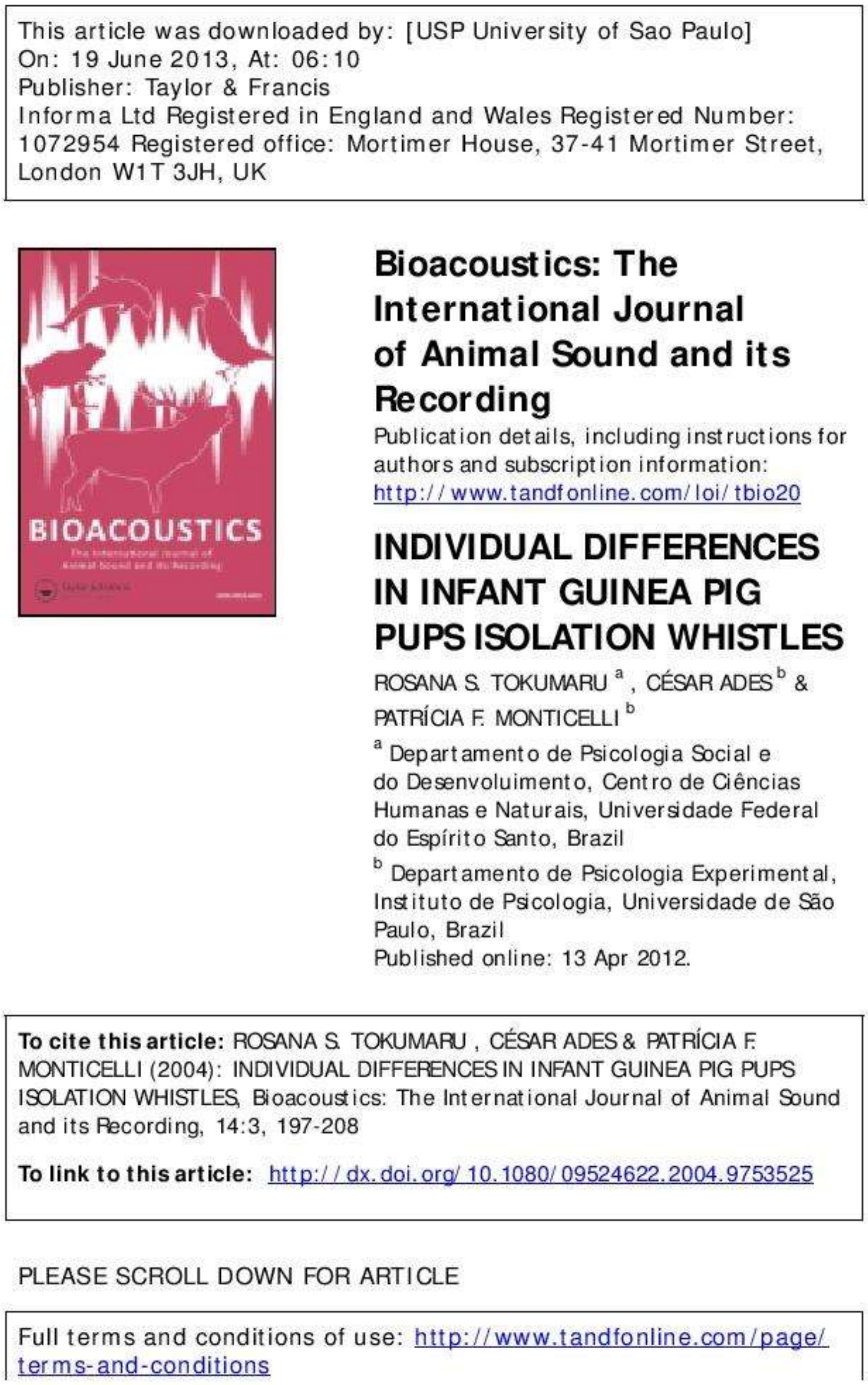
Depois desse resultado, o grupo liderado por Hennessy testou as respostas fisiológicas de filhotes isolados e depois de adultos, em comparação com toda a sorte de companheiros que eles puderam pensar. Para nós, poderia haver uma explicação alternativa, um efeito de tranquilização via apoio social ("*social support*"), que independia da vinculação afetiva, como sugerido em Sachser 1998 e Wewers at al., 2003). Hennessy e seus colaboradores haviam visto que a frequência dos assobios é diminuída ou abolida quando filhotes entram em contato com a mãe e, em menor grau, com um irmão ou mesmo com uma fêmea desconhecida. E que animais associados também diminuem as respostas de estresse um do outro, em situação de separação. Todos esses companheiros não são agressivos e foram considerados provedores de apoio social, por reduzir as respostas de estresse e a taxa de assobios em um ambiente não familiar. Mas, o que aconteceria se o filhote isolado em uma caixa-teste, se visse em contato com um macho adulto desconhecido? Nossa perguntava era: estar na companhia de um macho desconhecido, potencialmente agressivo, produz um efeito de apoio social que reduz respostas de estresse? Se sim, esse efeito calmante não poderia ser atribuído a um laço de apego.

Usando o assobio como indicador do estado interno do filhote, concluímos que o apoio social não requer apego: qualquer coespecífico, até um macho estranho adulto, parece "tranquilizar" filhotes isolados de porquinhos-da-índia (Tokumaru, Ades e Monticelli, 2015; Apêndice I: Social support does not require attachment: any conspecific tranquilizes isolated guinea-pig pups). De uma forma muito interessante, prezado leitor, noto que quando se separam cães criados em duplas em um canil (em cercados de 3,7 x 1,8 m), sob o mesmo paradigma de separação (por 4 horas em local familiar ou não, com e sem o companheiro de canil), não há nenhuma diferença no nível de cortisol em relação a presença ou ausência do parceiro social. Mas, quando ao estímulo estressor (ambiente novo) se inseria o tutor, os cães latiam e moviam-se menos, e os níveis de glicocorticoides mantinham-se, sugerindo que o tutor, mas não o companheiro de canil, promovia apoio social ao cão (Tuber, Hennessy, Sanders e Miller, 1996).

Quando o assobio deixa de ser apenas um indicador comportamental do estado interno do emissor e passa a ser um sinal variável, passível de ser medido, outras informações podem ser obtidas. A estrutura física do assobio de cobaia se modifica com o tempo de sessão: ao longo de 15 minutos de separação, meus colegas e eu registramos uma queda significativa na duração da nota de assobio ($Z = -2.232$, $p < 0.05$), um aumento na frequência média da nota (*mean frequency*, no programa Avisoft Bioacoustics@) e, em 4 de 6 filhotes, um aumento do intervalo entre notas (Monticelli, Tokumaru e Ades, 2004; Apêndice D).

Para Müller e Shair (2016), as vocalizações induzidas por separação em mamíferos estão associadas ao núcleo accumbens (Müller e Shair, 2016), como uma parte em uma "constelação" de efeitos comportamentais (locomoção e vocalização), endócrinos (as respostas de estresse) e de respostas autonômicas, que compõem um sistema integrado de estados afetivos e respostas de enfrentamento. Qual ou em que ordem elas serão desencadeadas, dependerá da que melhor se adequaria à situação ou ao momento específico de cada episódio de separação - correr, parar, assobiar ou calar; a coordenação dessa resposta complexa seria feita pelo núcleo accumbens em ratos e supostamente em humanos (Müller e Shair, 2016). De fato, filhotes de *Cavia* assobiando sozinhos em dado ambiente, invariavelmente param de assobiar se um humano se aproxima. Isso nós vimos diversas vezes e também foi relatado por Hennessy (2003). Ele pára porque não está mais com medo? Não, no "sistema integrado de estados afetivos e respostas de enfrentamento" descrito por Müller e Shair, assobiar deixa de ser a melhor resposta para o momento. Niko Tinbergen explicaria isso em termos de hierarquização organizada de centros de controle do comportamento inato (1996). Ao contrário daquela época, hoje esses centros podem até ser elencados, ao que indica a literatura: o núcleo accumbens, que aparentemente inicia e controla o chamado à mãe, tornar-se-ia secundário à ação dos núcleos basolaterais da amígdala (*basolateral nucleus of the amygdala*), responsáveis por outra resposta comportamental mais urgente e extrema, a imobilidade tônica (Leite-Panissi, Ferrarese, Terzian, & Menescal-de-Oliveira, 2006; Donatti & Leite-Panissi, 2011). Ambos os centros de controle estão interligados no processo de consolidação da memória por via dopaminérgica, que, por sua vez, tem influência sobre qualquer uma das respostas de autoproteção (LaLumiere, Nawar & McGaugh, 2005). A partir de modelos animais como o rato e a cobaia, muito se caminhou na área da neurobiologia do comportamento. E em parte deles, as vocalizações, audíveis ou ultrassônicas, serviram de indicadores comportamentais importantes.

Apêndice A – *Individual differences in infant guinea pig pups isolation whistles*



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INDIVIDUAL DIFFERENCES IN INFANT GUINEA PIG PUPS ISOLATION WHISTLES

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ABSTRACT

When separated from their mother and other group members, guinea pig *Cavia porcellus* pups emit distinctive high pitched whistles. To determine if these vocalisations are individually distinctive, we recorded the whistles of isolated guinea pig pups, 8 to 10 days old, and subjected their acoustical parameters to discriminant analysis. The results of the reclassification accuracy were higher than random assignment, indicating the existence of individual differences. Individual pup vocalisations did not differ from one another by any single acoustic parameter, but by a set of parameters. Individual recognition of such isolation calls by mothers could play an important role in facilitating reestablishment of contact.

Keywords: distress vocalisation, individual characteristics, pup recognition, guinea-pigs, *Cavia porcellus*.

INTRODUCTION

Individual differences in calls and its use in parent-offspring or mate recognition have been demonstrated in a wide diversity of animals: parrotlets (Wanker et al. 1998), swallows (Beecher et al. 1981), gulls (Charrier et al. 2001), penguins (Jouventin et al 1999), seals (Petrinovich 1974; Renouf 1985), dolphins (Sayigh et al. 1998), bats (Balcombe 1990), domestic pigs (Ilmann et al. 2002), primates (Marler & Hobbett 1975; Lillehei & Snowdon 1978; Snowdon & Cleveland 1980; Smith et al. 1982; Newman & Goedking 1992). In many species, costs of misdirected parental care are high and the mutual parent/offspring recognition of vocalisations has been selected for (Geiss & Schrader 1996; González-Mariscal & Poindron 2002). Infant

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*Author for correspondence. Address: Rua C, n. 29, Curva da Baleia, Manguinhos, Serra, Espírito Santo, Brazil. CEP 29.173-420. E-mail: tokumaru@usp.br recognition may be especially relevant when infants are mobile and different females with their offspring coexist within a group (Beecher 1991) as occurs in domestic guinea pigs *Cavia porcellus*.

The social group of guinea pigs includes a dominant male, one or more females and their pups and one or more subdominant males (Jacobs 1976; Sachser 1986, 1998). From their very first day of life, pups are mobile, following their mothers and other conspecifics while they forage in grassy environments (King 1956; Rood 1972). Maternal care, in the form of grooming and suckling, is given for about three weeks (Künkele & Trillmich 1997; Künkele 2000). The dominant male, probably the sire of the pups, is tolerant of offspring but does not provide any care (Beisiegel 1993).

There is evidence that both domestic (*C. porcellus*) and wild (*C. aperea*) adult cavies are able to recognise familiar conspecifics (Beauchamp 1973; Beauchamp et al 1979; Ruddy 1980; Martin & Beauchamp 1982; Beauchamp & Wellington 1984; Drickamer & Martan 1992, Cohn et al 2004). Individual recognition, probably of a visual and olfactory kind, plays an important role in the maintenance of mother and pup spatial proximity. Guinea pig pups discriminate their mother from other lactating females (Pettijohn 1979a; Fullerton et al. 1974; Berryman & Fullerton 1976; Niciporciukas et al. 1999, Jackël & Trillmich 2003) and mothers recognise their pups at a close distance (Porter et al. 1973; Tokumaru 2000).

When mothers lose visual and olfactory contact with their pups, proximity may still be regained through the pup's production of isolation or distress vocalisations. Mothers may recognise their pup's isolation calls through individual acoustic features of such vocalisations. Approach responses to playbacks of a combination of pups' vocalisations were observed by Berryman (1981) who did not find any preference of females towards their own pups' playback vocalisations. Berryman (1981) did not, however, contain information about individual differences or signatures in pup calling.

The present study addresses the question of acoustic individuality in the most frequent and specific vocalisation pups emit when separated from their mother and from other members of the group: the isolation whistle. Chut, chutters and whines occur during isolation but are also recorded in other behavioural contexts (Berryman, 1976; Monticelli 2000). The isolation whistle is a distinctive high-pitched whistle, composed of repeated harmonic notes with marked frequency modulation (King 1956; Arvola 1974; Berryman 1976; Pettijohn 1977, 1979b; Coulon 1982; Ades et al. 1994; Hennessy & Richey 1987; Hennessy 1988). Coulon (1973, 1982) reported that separation whistles exert a potent attraction effect over other pups and adult guinea pigs and that they elicit vocal responses ("social cohesion calls") and approach from the mothers.

METHODS

Berryman (1976) distinguished two whistle variants which may be emitted in the same vocalisation bout: low whistles which last 0.05 to 0.15 seconds and contain up to seven harmonics distributed in a frequency band from 0.5 to 4 kHz; and whistles which last 0.15 to 0.55 seconds and contain 3 to 14 harmonics distributed in a frequency band of 0.5 to 30 kHz. We choose here to evaluate individual differences in the high whistles.

Subjects. We studied six 8 day-old guinea pig pups born from four litters of the colony at the Experimental Psychology Department in São Paulo University, descendants of a heterogeneous stock. Each litter was housed with the mother and the father in 90x60x30 cm white polypropylene boxes. Water, rabbit chow and fresh vegetables were offered once a day.

Procedure. Each pup was submitted to three 15-minute isolation sessions, one per day, from the 8^{rh} to the 10th day of life. In each session the pup was transported from the colony to the test room, put in a 49x47x27 cm wooden box and left in isolation without auditory contact with other animals. Recordings of the vocalisations emitted were made using a Sennheiser ME88 unidirectional microphone connected to a Sony TCD-D8 DAT recorder (sampling frequency: 48 kHz, frequency response: 20 Hz to 22,000 Hz ± 1.0 dB; dynamic range > 87 dB). The microphone was 50 cm above the box.

Sound analysis. Selected parts of the tape containing whistles were digitised with a 8-bit acquisition card using Avisoft SASLab Pro 3.0 (Raimund Specht, Berlin, Germany). This version generates sonograms with frequencies between 0 and 24 kHz (sampling frequency, 48 kHz). Settings used for the generation of the sonograms were FFT size: 512; bandwidth: 111 Hz; weighting function: Hamming; time resolution: 0.73 ms. Forty-five whistles notes were randomly selected from the 8th to the 10th day of life from each of the 6 pups, totalling 270 notes.

Whistle notes were divided into 3 segments (Figure 1): A, a segment with little or no frequency modulation; B, a segment with marked ascending frequency modulation, and C, a segment with varied frequency modulation, generally a descending one.

The following acoustic parameters were measured: (1) note duration, in seconds; (2) mean intensity of the note [(intensity at the beginning of A + intensity at the beginning of B + intensity at the beginning of C + intensity at the end of C)/4], in dB, (3) frequency modulation of A (difference in Hz between the starting and ending points of the segment), (4) frequency modulation of BC (difference in Hz between the initial point of B and the ending point of C), (5) intensity (dB) and (6) frequency (Hz) of the dominant frequency (frequency with the highest intensity), (7) maximum frequency (last

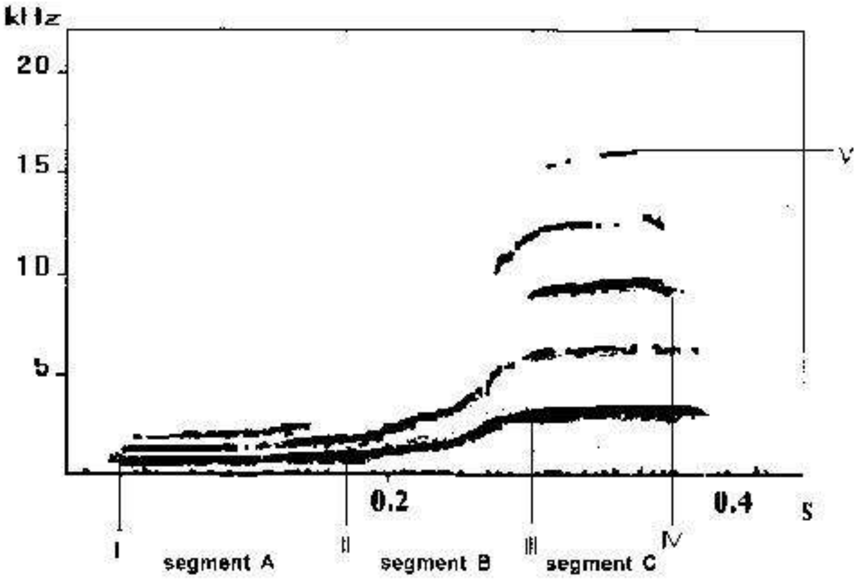

Figure 1: Sonogram of a whistle note. The note is divided into three segments: A, from I to II; B, from II to III; C, from III to IV. The end of the third harmonic (IV) was used as the end of the note. Some acoustic parameters measured were: note duration (from I to IV); maximum frequency (V), mean intensity of the note (average of intensity of fundamental frequency at I, II, III and IV), frequency modulation of fundamental frequency, segment A (frequency at II – frequency at I) and segment B + C (frequency at IV - frequency at II).

harmonic visible in the spectrogram). Except for the maximum and dominant frequencies, all other measures were taken from the fundamental frequency (1st harmonic) (Figure 1).

Statistical analysis. A one-way analysis of variance was performed to test for the significance of differences between individual pups' vocal features (*Statistical Package for Social Science 8.0, SPSS*). A stepwise, cross-validated discriminant analysis was performed to determine the probability of correctly assigning a vocalisation to a specific pup.

RESULTS

Table 1 presents the average values, standard deviations and range of the acoustic parameters measured in each pup vocalisation. Note duration varied from 0.14 to 0.35 seconds and maximum frequency from 6.72 to 21.96 kHz. Dominant frequency occurred, in most notes, in the first harmonic of C (± 2.8 kHz), with mean intensity of -21.04 dB. The marked difference between the minimum and maximum values of the dominant frequency (Table 1) is due to its occurrence either in the first (fundamental frequency) or in the third harmonic of

TABLE 1

Mean values, standard deviation and range of the acoustic parameters measured in whistles notes (45 notes per animal) of six guinea pig pups. Time is expressed in seconds, frequency in kHz and intensity in dB.

Parameters	Pups					
	8a	8b	9a	9c	10	12b
Note duration	0.24±0.04 0.16–0.35	0.21±0.03 0.16–0.33	0.20±0.03 0.15–0.27	0.18±0.03 0.14–0.24	0.20±0.01 0.17–0.23	0.24±0.02 0.19–0.29
Dominant frequency	4.50±2.76 0.86–10.77	2.51±1.73 0.60–9.73	2.63±2.26 0.60–11.63	2.46±3.24 0.60–11.03	2.52±1.65 0.52–10.16	2.21±1.36 0.52–6.12
Maximum frequency	15.60±2.20 10.25–19.98	15.20±4.27 9.56–21.62	13.76±3.39 6.72–20.50	14.90±3.28 9.82–21.71	14.13±3.22 8.27–19.81	14.34±3.49 8.44–21.96
Dominant Intensity	(–14.70)±8.86 (–30.91)–(–0.01)	(–17.00)±6.85 (–29.51)–(–0.02)	(–26.50)±6.37 (–38.76)–(–14.42)	(–15.41)±4.95 (–26.77)–(–1.80)	(–31.74)±5.67 (–38.94)–(11.92)	(–20.89)±7.95 (–38.60)–(–4.71)
Average intensity	(–40.83)±13.47 (–72.37)–(–20.52)	(-46.66)±9.05 (–73.97)–(25.96)	(–55.05)±14.78 (–94.03)-(–33.35)	(–37.50)±4.30 (–46.97)–(–29.20)	(–56.64)±9.98 (–90.48)–(–42.22)	(–47.34)±11.45 (–79.70)–(–28.58)
Frequency modulation of A	0.51±0.16 0.17–0.95	0.36±0.13 0.00–0.60	0.36±0.25 (–0.26)–0.86	0.28±0.10 0.00–0.43	0.30±0.12 0.09–0.60	0.35±0.11 0.17–0.69
Frequency modulation of BC	1.39±0.32 0.43–1.89	1.43±0.25 0.95–1.89	0.41±0.47 (–0.60)–1.29	1.64±0.33 0.95–2.41	1.70±0.46 0.60–2.41	1.58±0.31 0.78–2.24

C. Pup 1, for instance, emitted notes with the dominant frequency located mainly in the third harmonic of C.

Average values correspond approximately to those given by Berryman (1976). The author, however, recorded whistles with a maximum of 30 kHz while the maximum frequency presently obtained was about 22 kHz. This difference is due to differences in the frequency range of the equipment used and is not of major importance to test for individual differences as both fundamental frequency and dominant frequency are under those values.

One-way analysis of variance revealed significant differences between pups in all parameters except maximum frequency: *note duration* ($F_{5,264}$=13.683, p<0.001); *mean note intensity* ($F_{5,264}$=9.477, p<0.001; *A modulation*: $F_{5,264}$=4.325, p<0.001); *BC modulation*: $F_{5,264}$=423.525, p<0.001; *dominant frequency*: $F_{5,264}$=3.994, p<0.01; *dominant frequency intensity*: $F_{5,264}$=18.061, p<0.001; *maximum frequency*: $F_{5,264}$=1.496, p=0.196). The results of *post hoc* analysis showed that significant differences between a given pair of pups are different from those that differentiate any other pair (Table 2). Sonograms of the whistles of two different pups are shown in Figure 2.

Discriminant analysis was performed to estimate individual differences in whistle structure using a multivariate approach (Smith et al. 1982; Newman & Goedeking 1992; Fischer et al. 1995; McCulloch et al. 1999; Ilmann et al. 2002). Table 3 shows the percentage of notes correctly attributed to the sender in the cross-validated discriminant analysis. The sample (n = 270 notes) was randomly divided into two sub-samples. The first column of Table 3 shows the results obtained with one of the samples (analysis sample). The discriminant functions generated with this sample were used to analyse the second one (holdout sample). Results are shown in the second column of Table 3.

All note parameters, except mean intensity, contributed to the differentiation between pups in the cross-validation discriminant

TABLE 2

Significant differences (Bonferroni *post hoc* test, p < 0.05) between individual pups in note duration (1); mean intensity (2); frequency modulation of A (3); frequency modulation of B+C (4); intensity of the dominant frequency (5); dominant frequency (6).

	Pup 12b	Pup 10	Pup 9c	Pup 9a	Pup 8b
Pup 8a	3,5,6	1,2,3,4,5,6	1,3,4,6	1,2,3,4,5,6	1,3,6
Pup 8b	1	2,4,5	1,2	1,2,4,5	
Pup 9a	1,2,4,5	4,5	1,2,4,5		
Pup 9c	1,2,5	1,2,5			
Pup 10	1,2,5				

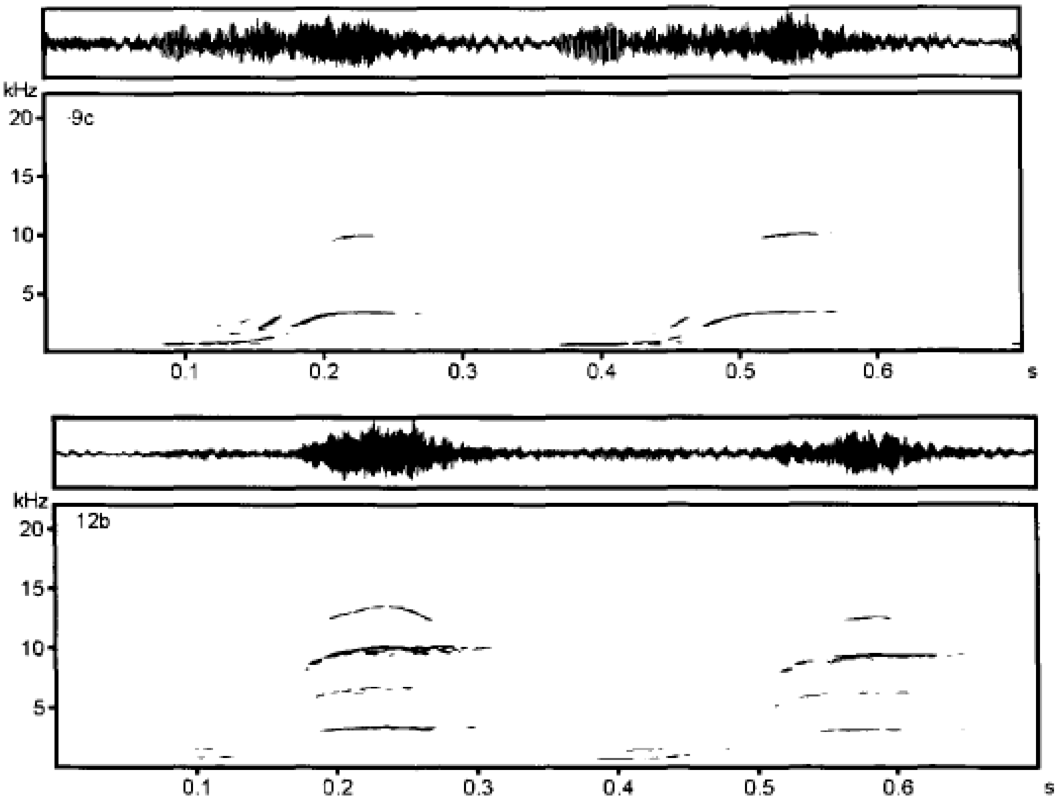

Figure 2: Sonograms and envelope curves of whistle notes of pups 9c and 12b, showing differences in acoustic parameters. Pup 9c: shortest notes (average: 0.18s), energy distributed in all segments, first and third harmonics most intense (darkest colour). Pup 12b: longest note (average: 0.24s), energy concentrated in segment B+C of the third harmonic.

TABLE 3

Percentage of notes correctly attributed to sender in the cross validation discriminant analysis (analysis sample, n=131; holdout sample, n=139)

Pups	Analysis sample	Holdout sample
8a	55.6	48.1
8b	81.0	75.0
9a	78.6	88.2
9c	66.7	58.3
10	61.9	37.5
12b	72.7	78.3
Total	70.2	62.6

analysis. The results of the reclassification accuracy was higher than random assignment (16%; Hair et al. 1995; Geiss & Schrader 1996). The holdout sample produced similar results to the analysis sample indicating that the profiles derived from this data set are the same as those produced in the original analysis, and thus can be seen as stable and reliable markers of individual differences (Smith et al. 1982) in whistle calls in this six pups sample.

DISCUSSION

Our results indicate that guinea pig pup whistles are individually distinctive and so may play a role in mother-infant recognition at a distance, when visual or olfactory information is lacking. It is not likely that the individual differences found were due to accidental environmental factors, or to momentarily fluctuations in motivational state. Environmental context of recording and the isolation procedure were made as similar as possible for different pups. Moreover, there were no marked differences between successive sonographic records of the same animals, an indication of the constancy of their vocalisation features.

As with other distress calls (Maestrepieri & Call 1996), the acoustic structure of guinea pig pup isolation calls is characterised by short, high-pitched pulses, with the broadband frequency spectrum uttered in fast repetitive sequences. These features are particularly suited for accurate localisation of the caller and have been found in the isolation calls of individuals of several species (Smith 1977; Bradbury & Vehrencamp 1998). According to the motivation-structural rules of Morton (1977), such acoustic features indicate that the sender is fearful and will not be hostile if approached or approaching. Stereotypy in general structure is expected in calls emitted under increased predation risk (Newman & Goedeking 1992).

Discriminant analysis and *post hoc* analysis showed that individual pup vocalisations did not differ from one another by any single acoustic parameter, but by a *set* of parameters. The multi-dimensional characteristic of call differences similarly have been reported in grey seal pup vocalisations (McCulloch et al. 1999), in contact calls of pygmy marmosets (Snowdon & Cleveland 1980) and squirrel monkeys (Smith et al. 1982), and in the parental calls of king penguins (Jouventin et al. 1999).

According to Berryman (1976, p. 102), whistles "may serve as proximity-regaining calls, emitted by individuals that are physically separated … acting as a long-distance auditory indicator of the individual's presence and location". As pups are primarily responsible for the proximity-regaining performance, whistles may have the double function of evoking whistles or other maternal vocalisations and of eliciting in the mother, and possibly other individuals of the group, immobilisation and vigilance behaviour that may facilitate the pups' recovering relevant social contact.

The acoustic features of the whistles, while appropriate for signalling a state of arousal or fear (Eisenberg 1974; Monticelli et al.

2004), may also contain individual information about the caller and may elicit differential approach or alertness responses from the mother. The mother and potential helpers (such as other lactating females) are probably able to use individual information contained in pups' calls to distinguish the differences in signal structure between their own and alien pups. Some of these calls were even discernible to the human ear.

Guinea pigs have a robust pup/mother system of non-vocal tracking and recognition. Pups follow their mother and remain in close proximity to her most of the time (King 1956; Fullerton et al. 1974; Herman & Panksepp 1978). Pups are able to recognise their mothers at close distances (Niciporciukas et al. 1999), and suck preferentially on her, even when other lactating females are available for alloparental suckling (Takamatsu *et. al.* 2003). This system of proximity maintenance reduces the risk of pups getting lost and increases the probability that the mother will be close when whistles and other isolation calls are produced. Recognition could thus depend, not only on a discrimination of *specific features* of whistles, but on the perception of the *nearness* of whistles.

The present findings about the individuality of isolation whistles in guinea pig pups afford the opportunity for an experimental examination of whether mothers actually recognise individual vocalisations of their offspring. Unpublished work on this issue, done in our laboratory, has yielded negative results. Thus it is possible that guinea pig mothers, rely on other information for regaining contact with their own pups even when a vocal signature is available.

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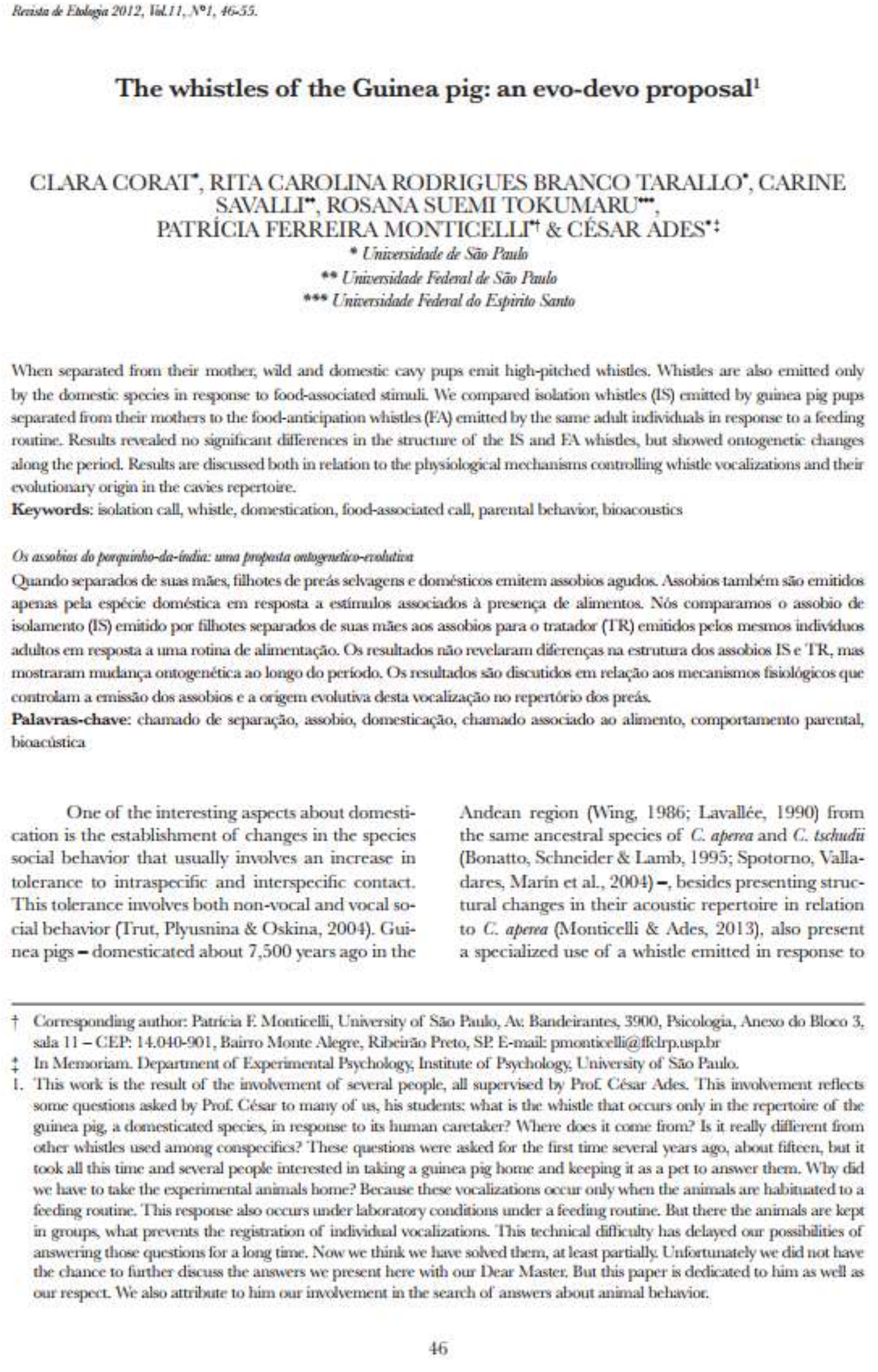
Apêndice B – *The whistles of the guinea pig: an evo-devo proposal*



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The whistles of the Guinea pig: an evo-devo proposal[1]

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When separated from their mother, wild and domestic cavy pups emit high-pitched whistles. Whistles are also emitted only by the domestic species in response to food-associated stimuli. We compared isolation whistles (IS) emitted by guinea pig pups separated from their mothers to the food-anticipation whistles (FA) emitted by the same adult individuals in response to a feeding routine. Results revealed no significant differences in the structure of the IS and FA whistles, but showed ontogenetic changes along the period. Results are discussed both in relation to the physiological mechanisms controlling whistle vocalizations and their evolutionary origin in the cavies repertoire.

Keywords: isolation call, whistle, domestication, food-associated call, parental behavior, bioacoustics

Os assobios do porquinho-da-índia: uma proposta ontogenetico-evolutiva

Quando separados de suas mães, filhotes de preás selvagens e domésticos emitem assobios agudos. Assobios também são emitidos apenas pela espécie doméstica em resposta a estímulos associados à presença de alimentos. Nós comparamos o assobio de isolamento (IS) emitido por filhotes separados de suas mães aos assobios para o tratador (TR) emitidos pelos mesmos indivíduos adultos em resposta a uma rotina de alimentação. Os resultados não revelaram diferenças na estrutura dos assobios IS e TR, mas mostraram mudança ontogenética ao longo do período. Os resultados são discutidos em relação aos mecanismos fisiológicos que controlam a emissão dos assobios e a origem evolutiva desta vocalização no repertório dos preás.

Palavras-chave: chamado de separação, assobio, domesticação, chamado associado ao alimento, comportamento parental, bioacústica

One of the interesting aspects about domestication is the establishment of changes in the species social behavior that usually involves an increase in tolerance to intraspecific and interspecific contact. This tolerance involves both non-vocal and vocal social behavior (Trut, Plyusnina & Oskina, 2004). Guinea pigs – domesticated about 7,500 years ago in the Andean region (Wing, 1986; Lavallée, 1990) from the same ancestral species of *C. aperea* and *C. tschudii* (Bonatto, Schneider & Lamb, 1995; Spotorno, Valladares, Marín et al., 2004) –, besides presenting structural changes in their acoustic repertoire in relation to *C. aperea* (Monticelli & Ades, 2013), also present a specialized use of a whistle emitted in response to

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[1] This work is the result of the involvement of several people, all supervised by Prof. César Ades. This involvement reflects some questions asked by Prof. César to many of us, his students: what is the whistle that occurs only in the repertoire of the guinea pig, a domesticated species, in response to its human caretaker? Where does it come from? Is it really different from other whistles used among conspecifics? These questions were asked for the first time several years ago, about fifteen, but it took all this time and several people interested in taking a guinea pig home and keeping it as a pet to answer them. Why did we have to take the experimental animals home? Because these vocalizations occur only when the animals are habituated to a feeding routine. This response also occurs under laboratory conditions under a feeding routine. But there the animals are kept in groups, what prevents the registration of individual vocalizations. This technical difficulty has delayed our possibilities of answering those questions for a long time. Now we think we have solved them, at least partially. Unfortunately we did not have the chance to further discuss the answers we present here with our Dear Master. But this paper is dedicated to him as well as our respect. We also attribute to him our involvement in the search of answers about animal behavior.

stimuli produced by the caretaker at the time of feeding. What is the evolutionary origin of this signal?

In both species, *C. porcellus* and *C. aperea*, as in most mammals, mothers are the main caregivers, but allosuckling also occurs among females from the same social group (Takamatsu, Tokumaru & Ades, 2003). Males are very tolerant towards pups and eventually display frisk hops in interaction with them, but do not provide direct care (Beisiegel, 1993; Adrian, Brockmann, Hohoff et al., 2005). King (1956), observing guinea pigs in a "semi-natural" environment, reported that the precocious pups were born in natural shelters on the ground. They remained there, hidden, until three or four days old. When they left, they preferentially followed their mothers. Pups require care only for their first four weeks of life (Rood, 1972). During this period, when visually separated from their mothers they readily emit whistles (Berryman, 1976; Monticelli & Ades, 2013), especially in the first two weeks of life and progressively less until the fourth (Pettijohn, 1979a).

The isolation-whistle (described by Berryman, 1976, as whistle) is emitted by other species of *Cavia* (*C. intermedia* and *C. magna*; unpublished data). In guinea pigs, isolation-whistles present individual characteristics (Tokumaru, Ades & Monticelli, 2004) and can be used by mothers to recognize pups, especially in large clutches (Kober, Trillmich & Naguib, 2008). This signal varies throughout development (Monticelli, Ades, Tokumaru et al., 2003; Monticelli , Tarallo & Ades, 2009) and along a 15 min isolation period, which indicates variation in the motivational state of the pups (Monticelli, Tokumaru & Ades, 2004). Besides the variation in the acoustic structure over time described in the papers cited above, the whistles also present a decrease (Pettijohn, 1979a) in the emission rate. These changes are accompanied by increases in plasma cortisol levels and immobility during a 30 min isolation period (Ritchey & Hennessy 1987; Hennessy 1988; Hennessy & Sharp 1990; Sachser, 1998; Hennessy, Deak & Schmil-Webb, 2001).

The food-anticipation whistle – in Portuguese called *assobio-ao-tratador* (Ades, Tokumaru & Beisiegel, 1994) – is auditorily very similar to the isolation-whistle. It is emitted only by the domestic species in response to the arrival of the caretaker and other stimuli related to food delivery. This vocalization differs from those emitted by other species during foraging. The food-associated vocalizations are emitted by foragers that first encounter a food item and can serve the function of attracting conspecifics to join the caller. Although the caller has some cost in sharing the food, a number of benefits have been demonstrated for the caller in different species (Cly, Smith & Blumstein, 2012). These vocalizations differ from the food-anticipation whistles of guinea pigs because these are emitted before food is encountered; it is emitted in the presence of a stimulus that precedes food. Clay et al. (2012) argue that although some authors claimed a referential function to the food-associated vocalizations due to the variation in call rate in the presence of different food items, this variation can also reflect the caller´s internal state.

Berryman (1976) does not distinguish between the isolation and the food-anticipation whistles. Coulon (1982) places them into two distinct categories – *cri de quetē* (isolation-whistle) and *sifflement d'appel* (food-anticipation whistle) – and points out differences in the rhythm of these whistles. According to Coulon (1982), although in different categories, both vocalizations share the same functional class: both serve the function of attracting attention, first of conspecifics and later of the human caretaker. The first response would be reinforced by the mother (or other conspecifics) – although pups emit this vocalization without training when isolated for the first time – and second, by the appearance of the food. Arvola (1974) also differentiated between the two whistles. While the food-anticipation whistle, which he called shrieks of hunger and thirst, was described as an oral emission (emitted with the mouth wide opened), the isolation-whistle, which he called agitating squeal, was described as a naso-oral emission.

The comparison of the two whistles in the literature is, however, restricted to the presentation and description of typical sonograms obtained from recorders and sonographic analyses softwares very different from what we have today (Arvola, 1974; Berryman, 1976; Coulon, 1982). There are insufficient systematic observations about the structure and variability of the acoustic signals that could clarify the relationship between the two whistles: are they really different in structure or is the food-anticipation whistle the result of ontogenetic changes in the isolation-whistle?

Our goal in this paper is to investigate these questions by comparing the food-anticipation whistles to the isolation-whistles emitted by the same guinea pig individuals. The results will be discussed in relation to the question about the origins of the food-anticipation whistle in the guinea pigs' communication repertoire.

Material and Methods

Study area and subjects

In this study we used one randomly selected individual from each of 8 different litters of 3 to 5 pups obtained from commercial or personal breedings (4 females and 4 males). The study was composed of two different phases. On Phase 1 the subjects were maintained in the animal husbandry facility of the Psychology Institute, University of São Paulo, São Paulo, Brazil, with their native colonies until they were 30 days old. On Phase 2 the subjects were adopted and kept as pet by two experimenters (RCRBT e CC). There was always only one animal per time kept as pet on phase two (each new subject was adopted just when data with the previous one had been concluded). In both phases the subjects were kept in 60x60x100 cm plastic or polypropylene cages, received water and guinea pig or rabbit chow *ad libitum* and fresh vegetables once a day, except on the weekends.

Data Collection

<u>Phase 1 – The isolation-whistle (IS) recording</u>: We performed 3 recording sessions of the isolation-whistle for each pup, around days 7, 14 and 21 of their lives. On the sessions we transported the pups individually to a test room and placed them in a wooden box (50x50x30 cm) for 5 minutes. No food or water was available. After that the animals returned to their living cages.

<u>Phase 2 – The food-anticipation whistle (FA) recording</u>: The subjects were taken to the researcher's homes when they were 30 days old, and they started living as pets, interacting and submitted to the family routine. Every day, a food supplement, preferred by the guinea pigs (fruits, carrots or cabbage) was offered, always at the same time and on the same routine, following Ades, Beisiegel and Tokumaru (1992): the experimenter went to the refrigerator, opened the door, shook a plastic bag producing a characteristic noise, got the food from the bag, closed the door, approached the subject and put the food in its living box. After subjects started whistling to the feeding routine, a recording day was scheduled. The equipment was prepared before the experimenter started the routine and was turned off about 5 minutes after the food delivery All vocalizations emitted during the routine were recorded.

Equipment

Sound recordings were made using a Sony professional portable digital audio tape recorder (DAT – TCD-D8; sampling rate: 48 kHz, frequency response: 20–24,000 Hz ± 1.0 dB; dynamic range >87 dB) and a hypercardioid directional microphone supported by a tripod and situated 30 cm above the floor (Sennheiser ME 67 with K6 powering module and bass roll-off, with the filter switch on).

Sound Analysis

All of the acoustic signals produced were digitized with 8-bit accuracy from DAT to a personal computer using Raven 1.4 (Cornell Lab of Ornithology: http://www.birds.cornell.edu/brp/raven/AwardsProgram.html) via a Sound Blaster Audigy A/D converter at a sampling rate of 48 kHz. Spectrograms were generated and analyzed using the following settings: Hamming window; 1,024 point fast Fourier transform (FFT); 100% screen size; 93.75% overlap; 47Hz frequency resolution; 0 to 24Hz frequency band; 1.33ms time resolution.

We defined as measurable notes of whistle (IS and FA) only those that had at list three harmonics and the three parts – A, B and C described in Tokumaru, Monticelli & Ades (2004). We selected the first 10 consecutive notes of each recording period (IS7, IS14, IS21 and FA) to be measured.

The acoustic parameters measured in all the calls were: the number of visible harmonics on initial part of the note – A, following Tokumaru et al. (2004) –, low frequency, higher frequency, dominant frequency, note duration, inter-note interval, rhythm (number of notes/second) and average power (the value of the spectrogram's power spectral density, as defined by Raven software). We used the first harmonic of each element of each note to delimit and measure the parameters.

Data Analysis

We first described the acoustic parameters of the whistles in the four recording periods (IS7, IS14, IS21 and FA) using average (standard deviation), minimum and maximum values. Previously to all analyses, the 10 notes of each individual and each recording period were summarized by the mean.

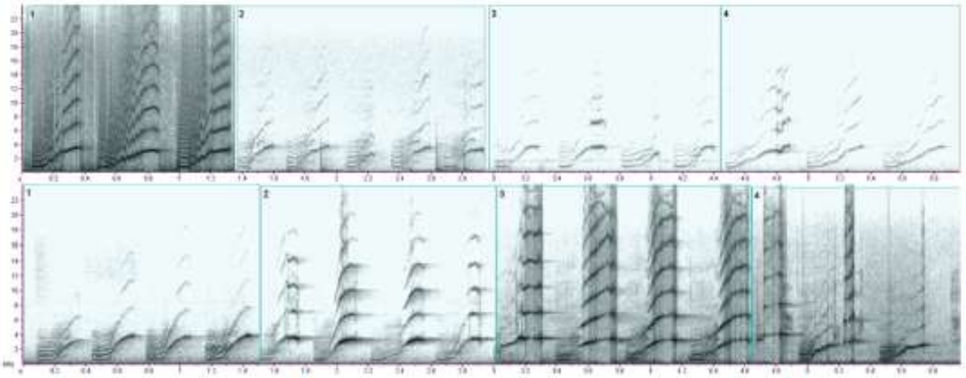

Figure 1 – Sonograms of sequence of notes of whistles emitted by 2 pups (above: Marronzinho; bellow: George) in each of the four moments: (1) IS7, (2) IS14, (3) IS21 and (4) FA (after 30 days of life). In (4) the notes are longer, which can be seen already in (3), and the rhythm is slow (in the same time window, less notes are seen).

Table 1 - Results of the descriptive and inferential analyses of the acoustic parameters of the whistles emitted by five guinea pigs in the isolation (mean values of recording periods IS7, IS14 and IS21) and in the food-anticipation context.

Acoustic parameters	Whistle	Minimum	Median	Maximum	p value
Duration of the notes (s)	Isolation	0.18	0.24	0.30	0.063
	Food-anticipation	0.24	0.34	0.45	
Interval between notes (s)	Isolation	0.10	0.15	0.29	0.813
	Food-anticipation	0.10	0.16	0.22	
Low Frequency (Hz)	Isolation	490.61	595.88	612.02	0.063
	Food-anticipation	613.48	676.26	1056.30	
High Frequency (Hz)	Isolation	13893.41	18391.12	21369.40	0.625
	Food-anticipation	13938.96	17843.24	21557.13	
Peak Frequency (Hz)	Isolation	1756.24	2278.16	3264.05	0.625
	Food-anticipation	1307.06	2526.57	3585.93	
Average Power	Isolation	67.73	98.57	101.17	0.813
	Food-anticipation	61.20	92.35	110.78	
Number of Harmonics in A	Isolation	4.15	6.06	7.44	1.000
	Food-anticipation	3.00	5.11	8.40	
Rhythm	Isolation	2.75	3.15	3.69	0.125
	Food-anticipation	1.83	2.74	3.32	

To all statistical analyses, we considered the data from five individuals. Three of the individuals could not be included due to different reasons (a female got sick when it was 14 days old; the recordings of one female were damaged and one male never emitted FA on phase 2).

To compare isolation (IS) and food-anticipation (FA) whistles, we summarized the three first recording periods IS7, IS14 and IS21 by the means and compared the data to those from the recording period FA using the Wilcoxon signed rank test (Hollander & Wolfe, 1998). The use of a non-parametrical test was due to the low number of subjects.

We also evaluated whether there were tendencies for each acoustic parameter across the recording periods indexed by time using the longitudinal growth curve data methodology (Winer, 1971; Singer & Andrade, 1986). This technique allows incorporating the statistical dependence between observations in the same individual in the estimation of the tendency.

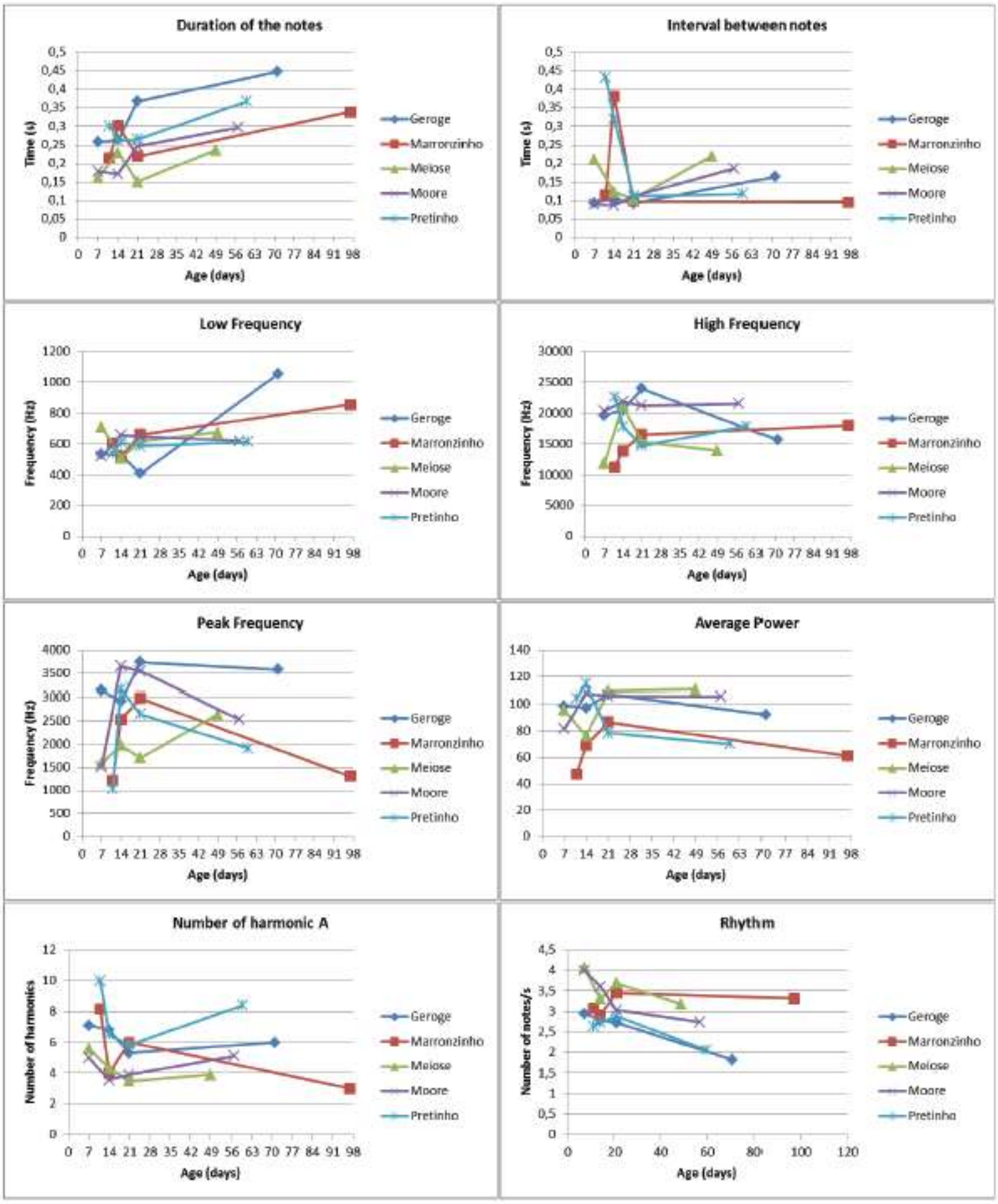

Figure 2 – Individual mean values of the acoustic parameters of whistles emitted by 5 guinea pigs in an isolation context (around 7, 14 and 21 days of life) and in a food-anticipation context (after 30 days of life).

Table 2 – Results of the inferential analysis of the acoustic parameters of the whistles emitted by five guinea pigs in isolation (IS7, IS14 and IS21) and in food-anticipation (FA) context. *The inferential analysis was performed only for the parameters that presented normal distribution.

Acoustic parameters	**Parameter***	**Estimate**	**Standard error**	**p value**
Duration of the notes (s)	Intercept	0.2134	0.0259	0.0012
	Slope	0.0018	0.0004	0.0003
Low Frequency (Hz)	Intercept	522.01	27.79	<0.0001
	Slope	3.69	0.95	0.0016
High Frequency (Hz)	Intercept	18071.00	1464.85	0.0002
	Slope	-2.97	29.40	0.9209
Peak Frequency (Hz)	Intercept	2497.72	335.59	0.0017
	Slope	-1.17	7.40	0.8775
Average Power	Intercept	93.57	7.40	0.0002
	Slope	-0.09	0.15	0.5429
Number of Harmonics in A	Intercept	6098	0.738	0.0012
	Slope	-0.018	0.013	0.1929
Rhythm	Intercept	3293	0.220	0.0001
	Slope	-0.009	0.003	0.0212

We used the compound symmetry structure for the variance-covariance matrix that assumes constant correlation between any two measures of the same animal in a line regression model. The assumption of normality was evaluated on the residuals of the model by Shapiro-Wilk and Kolmogorov-Smirnov. Only variables for which the normality assumption was satisfied were analyzed using this methodology. For the other variables, only a descriptive analysis of the individual profiles was considered.

Results

In the isolation context, all pups presented vocalizations in the first recording period, at 7 days old and one pup had not whistled when it was 21 days old. The analyses of the recording periods in this context (and in phase 2) revealed the presence of the five categories of vocalizations described by Berryman (1976) in the same context, occurring concomitantly to the whistles: chut and chutter – described as contact calls by Monticelli & Ades (2013) –, whine, low whistle (less than three harmonics) and the tweet.

Pups started to emit the food-anticipation whistle when they were still living in the colony, at about 14 days of life, in response to the feeding routine. After being taken home as pets, the individuals started responding to the feeding routine, in average, after 10 days in the new environment.

We noticed a positive relation between the whistle emission rate and the proximity of the food delivery: the rate of vocalization was accelerated as the experimenter approached the animal.

Comparison between isolation and food-anticipation whistles

The modulated frequency of the whistle notes allow us to divide them in three parts, as described in Tokumaru et al. (2004). Element A presents a smooth ascending modulation (0.36 kHz in average), element B presents a marked ascending modulation (from 1.03 kHz to 3.42 kHz, in average) and element C presents a varied modulation, generally descendant (until 2.64 kHz, in average). Figure 1 presents sonograms of whistles emitted in the isolation and food-anticipation contexts. Although some variation is visually apparent, no significant differences were obtained from the Wilcoxon signed rank test (Table 1).

Ontogenetic change in the whistle

Considering the absence of differences between the isolation and the food-anticipation whistles revealed by the previous analyses, we decided to look for changes over time considering the dependency between observations in the same individual. Table 2 shows the results of the descriptive statistics of the parameters of the whistles emitted by our subjects along the recording periods.

The parameters for which the normality assumption was satisfied were evaluated in search of tendencies over time. From the results displayed in Figure 2 and Table 2, it can be observed that whistles presented a significant tendency of increase in duration and low frequency and of decrease in rhythm.

Discussion

Our results indicate that there are no statistical differences between the structure of the isolation and the food-anticipation whistles. The differences noted by other authors (Arvola, 1974; Coulon, 1982) were probably related to their methods of analysis based on the description of typical sonograms. On the other hand, our results also show that the basic structure of the whistles changes ontogenetically. As guinea pigs grew, whistle notes became longer and presented higher low frequency, while the rhythm of whistles emission became slower.

These changes are consistent with anatomical changes in the vocal tract that occur as the animal grows: the increase in the larynx length and the thickening of the vocal cords produce narrower band calls (a significantly rise in the low frequency and a tendency of fall in the maximum and peak frequencies). The increase in lung capacity would facilitate the emission of longer, more intense notes that are consequently produced in a slower rhythm. Our results are similar to those displayed in the literature for other species – squirrel monkeys in Winter, Handley, Ploog et al. (1973); Lieblich, Symmes, Newman et al. (1980); vervet monkeys in Seyfarth & Cheney (1986); pigmy marmoset in Elowson, Snowdon & Sweet (1992) – that describe variations in the duration and frequency of the calls along development.

Together, these results lead us to conclude that the isolation-whistle is the same as the food-anticipation whistle; both present the same basic structure. A question that follows from this conclusion is the occurrence of this vocalization in two different contexts. During separation from conspecifics only pups emit whistles, and in response to food cues both pups and adults emit whistles. What is the mechanism modulating the emission of this vocalization?

A possible mechanism can be related to the functioning of the pituitary – adrenocortical (PAC) system, responsible for stress responses. It is well known that, during a 30 min isolation period, as the rate of guinea pig pup´s whistles emission decrease, cortisol levels and immobility increase (Ritchey & Hennessy, 1987; Hennessy, 1988; Hennessy & Sharp, 1990; Sachser et al., 1998; Hennessy et al., 2001). The increase in the cortisol level seems to be a response to the separation of the mother and from the surrogate environment indicating that this is a stressful event to the pup (Wewers, Kaiser & Sachser, 2003). In species that form attachment bonds, the increase in plasma concentrations of PAC hormones has been demonstrated in response to the separation from the attachment object (Henessy, Maken & Graves, 2000). But while cortisol level presents a linear increase in response to time in isolation, the emission rate of whistles present a decrease (Pettijohn, 1979a; McInturf & Henessy, 1996). This relation suggests that low levels of stress trigger the emission of whistles, but, as levels increase, vocalization and locomotion decrease. Our proposal is supported by the results of some studies that obtained simultaneously decreases in whistles emission and increases in cortisol levels (Henessy, Ptstick, O'Leary, Maken & Farré, 2001) or the inverse relation (Henessy, Maken & Graves, 2000; Henessy, O'Leary, Hawke & Wilson, 2002; Wewers, Kaiser & Sachser, 2003).

Our suggestion is that **mild stress** is the physiological trigger for the emission of whistles vocalization and this kind of stress occurs both during brief isolation periods and upon the perception of stimulus related to food delivery (Mistlberger, 1993; Ott, Friedrich, Prilop, Lehnert, Chara, Born & Hallschmid, 2011). We propose that the triggering of whistles by mild stress and its suppression by higher levels of stress was possibly selected in the context of naturalistic mother-pup separations that can occur during foraging. As already demonstrated (Pettijohn, 1979; Berryman, 1981; Coulon, 1973; Tokumaru, Ades & Monticelli, 2006) isolation-whistles function as signals which elicit approach of the mother. Elsewhere (Monticelli, Tokumaru & Ades, 2004), we argued that immediately after separation in a naturalistic foraging context, as the mother is likely to be not very far, it may be advantageous for the pup to keep moving and to emit fast repetitive sequences of whistle notes, maximizing the probability of the vocalization being heard and responded to. As time passes in isolation, probability of recovery by the mother decreases and less conspicuous vocal signals and immobility may represent the best tradeoff between calling and defense against predators. Considering that this hypothesis is correct, we further propose that this mechanism could be activated in the guinea pig under laboratory conditions in the food-anticipation context because it mimicries the mild stress condition produced during brief isolation periods.

This explanation can also be used to understand why both wild and domesticated species of cavies emit the isolation-whistles but only the domesticated guinea pigs emit the food-anticipation whistle. In comparison to at least on wild cavy species (*Cavia aperea*), guinea pigs showed decreased reactivity of PAC system (Künz & Sachser, 1999). This difference is robust as it was seen even in the comparison between guinea pigs and wild cavies reared in captivity for 30 generations (Künzl, Kaiser, Meier, & Sachser, 2003). The authors argue that the decreased reactivity of the guinea pigs' stress axes is one of the results of domestication that helped these animals to adjust to man-made housing conditions. Reinterpreting these results, we could say that guinea pigs are less responsive to environmental changes or that they have a higher threshold for the activation of the stress axes in response to these changes.

This effect of domestication would give the guinea pigs the opportunity to answer to food-anticipation stimuli with whistle calls because, in comparison to the wild cavies, the human keeper does not enhance their levels of stress. It is probable that both guinea pigs and wild cavies learn to expect food when food-associated stimuli are present. Personal (PFM) observations in a wild cavy colony have shown that animals approach the border of the cage, sniff the air and look in the direction of the keeper when he/she starts the feeding routine. But although both species show food-anticipation behavior and, probably, the corresponding changes in physiology, wild cavies probably present an extra level of stress as food stimuli include the presence of a human keeper. This higher level of stress in response to food-anticipation stimuli would prevent the emission of whistles by wild cavies. If this hypothesis is correct, we could expect that wild cavies treated with antidepressants during exposition to food-anticipation stimuli would emit whistles after a period of habituation. We do intend to perform this experiment to test this hypothesis.

In the light of our results and the proposed mechanism controlling whistles emission, we suggest a change in the form of reference to the whistles vocalizations. Researchers have categorized the whistles referring to the context in which they occur. But, as they are structurally the same between contexts, we suggest that the categorization is made in reference to the mechanism triggering the whistles, i.e., anxiety whistles or mild distress whistles. Of course, this suggestion is subjected to the confirmation of the hypothesis described above.

A limitation of this study is that we were not able to collect isolation and food-anticipation whistles from the same individual at all developmental stages. Guinea pig pups do emit isolation whistles and food-anticipation whistles. But while the first ones are easily individually collected, the second occurs when pups are responding to the feeding routine in the colony. We did not try to adopt individuals as pups, because it would imply bottle-feeding them, increasing mortality chances, but it is possible that adopted pups would emit whistles to the feeding routine while in the familiar cage and in response to isolation in an unfamiliar environment. Another possibility is the use of individual microphones permanently attached to the animals to record the whistles emitted in the colony. Both suggestions can be subjected to future testing depending on technical adjustments in our lab.

Although we did not compare isolation and food-anticipation whistles at the same developmental stages, the comparison of adult food-anticipation whistles and pups isolation whistles showed both the absence of structural differences and ontogenetic change in the basic structure. The evo-devo hypothesis offered here to their emission, by guinea pigs in different contexts and by wild cavies only in the isolation context, is supported by these results and others in the literature and offers the possibility of future testing and demonstration.

Ethical note

This work followed the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) and was approved by the Committee of Ethics for Animal Use (CEPA) from Instituto de Psicologia [Psychology Institute] of the University of São Paulo (proc. 005.2009).

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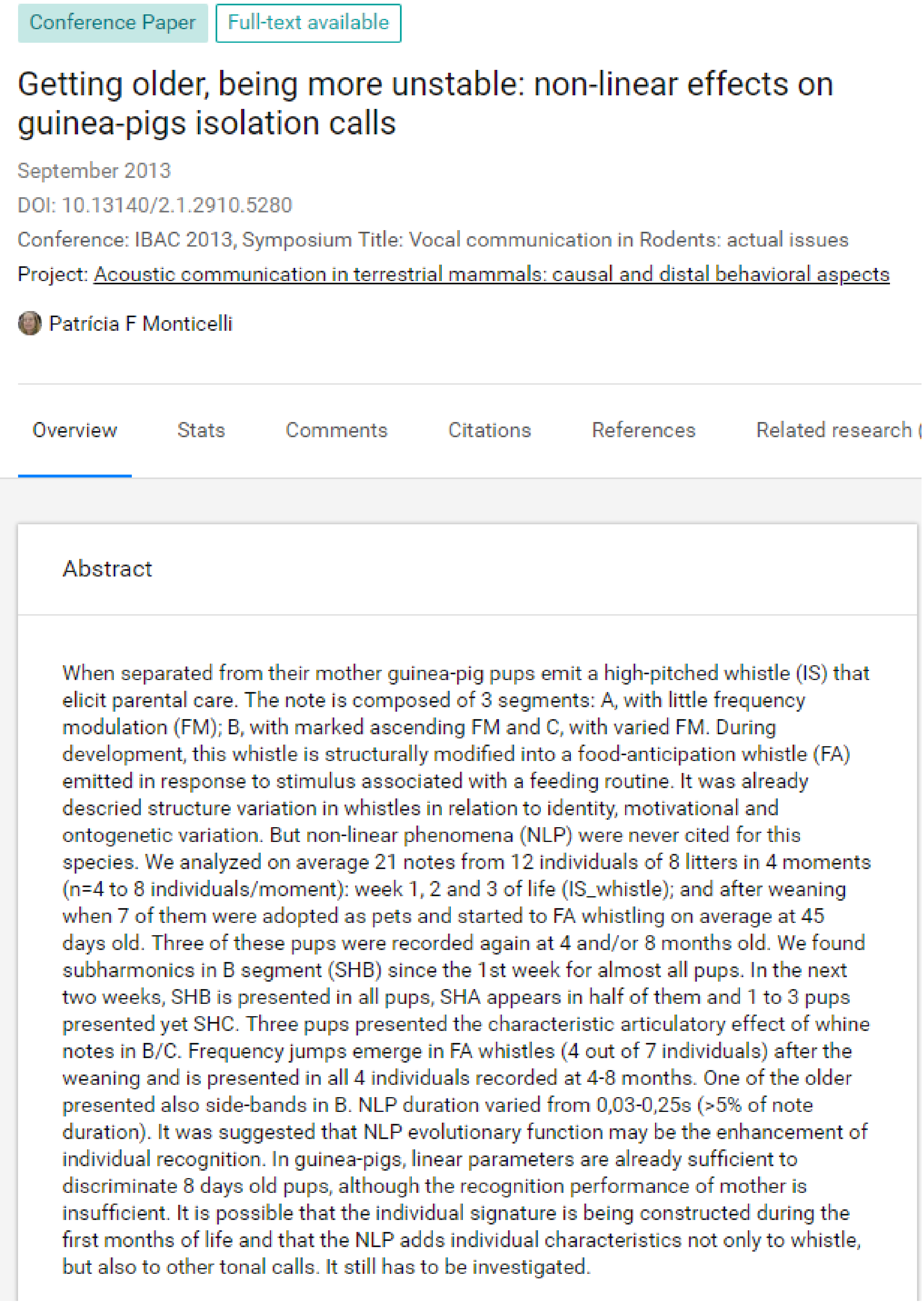
Apêndice C – *Getting older, being more unstable*



Conference Paper | Full-text available

Getting older, being more unstable: non-linear effects on guinea-pigs isolation calls

September 2013
DOI: 10.13140/2.1.2910.5280
Conference: IBAC 2013, Symposium Title: Vocal communication in Rodents: actual issues
Project: Acoustic communication in terrestrial mammals: causal and distal behavioral aspects

Patrícia F Monticelli

Abstract

When separated from their mother guinea-pig pups emit a high-pitched whistle (IS) that elicit parental care. The note is composed of 3 segments: A, with little frequency modulation (FM); B, with marked ascending FM and C, with varied FM. During development, this whistle is structurally modified into a food-anticipation whistle (FA) emitted in response to stimulus associated with a feeding routine. It was already descried structure variation in whistles in relation to identity, motivational and ontogenetic variation. But non-linear phenomena (NLP) were never cited for this species. We analyzed on average 21 notes from 12 individuals of 8 litters in 4 moments (n=4 to 8 individuals/moment): week 1, 2 and 3 of life (IS_whistle); and after weaning when 7 of them were adopted as pets and started to FA whistling on average at 45 days old. Three of these pups were recorded again at 4 and/or 8 months old. We found subharmonics in B segment (SHB) since the 1st week for almost all pups. In the next two weeks, SHB is presented in all pups, SHA appears in half of them and 1 to 3 pups presented yet SHC. Three pups presented the characteristic articulatory effect of whine notes in B/C. Frequency jumps emerge in FA whistles (4 out of 7 individuals) after the weaning and is presented in all 4 individuals recorded at 4-8 months. One of the older presented also side-bands in B. NLP duration varied from 0,03-0,25s (>5% of note duration). It was suggested that NLP evolutionary function may be the enhancement of individual recognition. In guinea-pigs, linear parameters are already sufficient to discriminate 8 days old pups, although the recognition performance of mother is insufficient. It is possible that the individual signature is being constructed during the first months of life and that the NLP adds individual characteristics not only to whistle, but also to other tonal calls. It still has to be investigated.

Apêndice D – *Isolation induced changes in guinea-pig* Cavia porcellus *pup distress whistles*

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Isolation induced changes in Guinea Pig *Cavia porcellus* pup distress whistles

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ABSTRACT

Guinea Pig *Cavia porcellus* pups emit high-pitched distress whistles when separated from their mother. In order to assess the influence of the duration of a brief isolation period on whistle acoustic structure, we recorded the distress whistles of six 8-day old pups separated for 15 min from their group in a novel environment and compared the mean values of the first and last 30 whistle notes. Acoustic analysis revealed, throughout the session, a significant decrease in whistle duration, an increase in mean frequency and a tendency for a decrease in number of harmonics in the first part of the note. Results demonstrate that, throughout a brief isolation period, the vocal response of Guinea Pig pups to isolation undergoes structural changes possibly related to time-dependent changes in motivational state.

Key words: acoustic communication, isolation calls, Guinea Pig.

INTRODUCTION

In socially-bonding species, separation from familiar attachment figures stimulates physiological and behavioral stress responses. Guinea Pig *Cavia porcellus* pups exhibit high rates of whistling and increases in plasma concentrations of cortisol when separated from their mothers and from other members of the group (Hennessy 2003, Ritchey and Hennessy 1987, Tokumaru 1995). Isolated pup's distress whistles are high-pitched and are composed of repeated harmonic notes with marked frequency modulation (Berryman 1976, Pettijohn 1979, Tokumaru 2000).

Variation in the intensity and/or structure of vocalizations throughout a separation period has been interpreted as produced by motivational changes (Marler et al. 1992). Newman and Goedeking (1992) found that the mean and peak frequency of the vocalizations of Common Marmoset *Callithrix jacchus* pups increased and duration of the call decreased, from the beginning to the end of a 15 min isolation period. There were also significant stable inter-individual differences in initial frequency, final frequency and pitch variation of the marmoset calls.

Changes in the rate of the emission of whistles of Guinea Pig pups throughout an isolation period have already been described. The rate of whistle notes emitted decreases (Pettijohn 1979), while plasma cortisol levels and time spent in immobilization increase during a 30 min isolation period (Ritchey and Hennessy 1987, Hennessy 1988, Hennessy and Sharp 1990, Sachser et al. 1998, Hennessy et al. 2001).

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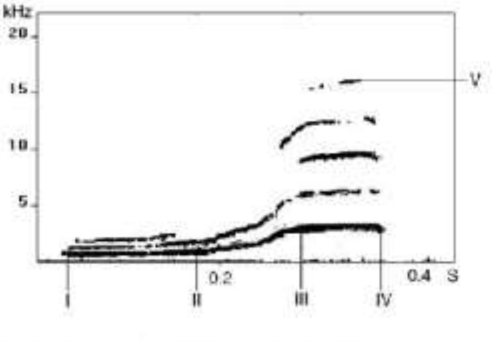

Fig. 1 – Sonogram of a whistle note, showing its harmonic structure (V = fifth harmonic). The note is divided into three segments: A, from I to II; B, from II to III and C, from III to IV.

We here address the question of whether time in isolation induces structural changes in distress whistles. Such changes may be relevant to an understanding of the functional aspects of distress calling in Guinea Pig, and to the time course of behavioral distress reactions.

MATERIALS AND METHODS

Six Guinea Pig pups, from three litters born in the colony of the Department of Experimental Psychology (São Paulo University), descendants of a heterogeneous stock, served in the experiment. Each litter was housed with the mother and the father in 90 × 60 × 30 cm white polypropylene boxes. Water, rabbit chow and fresh vegetables were offered once a day.

On the eighth day of life, each pup was transported from the colony to the test room, put into a 49 × 47 × 27 cm wooden box and left in isolation for 15 minutes. Recordings of vocalizations were obtained with a unidirectional microphone Sennheiser ME88 connected to a Sony DATTCD-D8 (sampling frequency: 48 kHz, frequency response: 20 Hz to 22.000 Hz ± 1.0 dB; dynamic range > 87 dB). The microphone was located 50 cm above the center of the box.

The first 30 and the last 30 whistle notes emitted during the isolation period were selected for analysis. Recordings were digitized with an 8-bit acquisition card using Avisoft-SASLab Pro 3.2 (Raimund Specht, Berlin, Germany). This software generates sonograms with frequencies between 0 and 24 kHz (sampling frequency, 48 kHz). Settings used for the generation of the sonograms were FFT size: 512; bandwidth: 111 Hz; frequency resolution: 86 Hz; weighting function: Hamming; time resolution: 0.73 ms.

Whistle notes were divided into 3 segments (Fig. 1): (A) an initial segment with little or no frequency modulation; (B) a segment with marked ascending frequency modulation, and (C) a segment with variable (generally descending) frequency modulation (Tokumaru 2000).

The following acoustic parameters were measured: (1) duration of the note; (2) mean interval between consecutive notes; (3) number of harmonics in segment A; (4) number of harmonics in segment C; (5) frequency modulation of the note (difference between starting and ending point of fundamental frequency): (6) dominant frequency, i.e. frequency with the highest intensity; (7) mean frequency. Mean values of the first and last 30 whistle notes of each pup were compared with a nonparametric Wilcoxon test (SPSS 11.0 software).

RESULTS

Mean values and standard deviations of acoustic parameters of the whistle notes of Guinea Pig pups are shown in Table I. Throughout the isolation period, there was a significant decrease in whistle duration ($Z = -2.232$, $p < 0.05$), an increase in mean frequency ($Z = -1.992$, $p < 0.05$) and a tendency for a decrease in number of harmonics in A ($Z = -1.782$, $p = 0.075$). The internote interval increased, throughout the isolation period, in 4 pups and the number of harmonics in C decreased in 5 out of 6 pups, at the end of the period (Table I).

DISCUSSION

Our data are the first demonstration of time-dependent changes in the acoustic structure of Guinea Pigs distress whistles. Whistle notes were shortened, mean frequency increased and there was a small but significant increase in number of harmonics in A, at the end of a 15 min isolation period. Such temporal changes are analogous to those observed by Newman and Goedeking (1992) in the calls of Common Marmoset pups: duration of isolation correlated negatively with call duration and positively with mean frequency and frequency peaks of calls. Frequency and structure of vocal emissions of Squirrel Monkey *Saimiri sciureus* pups vary as a function of different kinds of isolation contexts. Total separation from the mother is associated with a smaller number of vocalizations, higher frequency peaks and higher plasma cortisol levels than a partial (with visual contact) separation (Wiener et al. 1990). Wiener et al. (1990) interpret the calls Squirrel Monkey pups emit in isolation as stimuli that elicit recovery behaviors in the mother.

Vocalizations of isolated Guinea Pigs, as other separation calls, function as signals which elicit approach of the mother. Immediately after separation, as the mother is likely not to be very far, it may be advantageous for the pup to keep moving and to emit fast repetitive sequences of notes, maximizing the probability of the vocalization to be heard and responded to. As time passes in isolation, probability of recovery by the mother decreases and less conspicuous vocal signals and immobility may represent the best trade off between calling and defense against predators. There is indeed evidence that, in Guinea Pig pups, immobility and plasma cortisol levels increase and vocalization rate decreases with time in isolation (Hennessy et al. 1991, 1997). The long-note, high rate whistling recorded at the start of an isolation period may be interpreted as the pup's investment in short-term recovery; the short-note, low rate whistles recorded at the end of the isolation period, as a compromise between attention getting and defensive tendencies.

The whistle, characterized by short, high pitched pulses, with a broadband frequency spectrum uttered in fast repetitive sequences, is similar in structure to other distress or isolation calls (Maestrepieri and Call 1996) and can be interpreted, according to Morton's (1977) structural-motivational rules, as indicating that the sender is fearful and not hostile.

In adult Barbary Macaques *Macaca sylvanus*, duration and internote interval of disturbance calls decrease with time from the onset of a disturbing situation, but increase in infant-crying episodes (Fischer et al. 1995). Thus, infants seem to be more distressed than adults as time from disturbance onset increases. In Guinea Pigs, isolation vocalization, which is similarly restricted to pups, can reflect time-dependent changes in motivation related to attachment and fear.

The variability here demonstrated in a strongly stereotyped vocal call, such as the distress whistle of Guinea Pig pups, opens a set of interesting questions about motivational and social determinants of communication in this species.

RESUMO

Quando isolados de sua mãe, filhotes de Cobaia-doméstica *Cavia porcellus* emitem assobios agudos. Para avaliar os efeitos do tempo de isolamento sobre a estrutura acústica do assobio, registramos as vocalizações de 6 filhotes de 8 dias de idade, isolados durante 15 minutos, e com-

TABLE I

Mean values and standard deviations of acoustic parameters of six Guinea Pig pups whistle notes. Number of cases for internote interval values is indicated below each value; in all other cases, n = 30.

Parameters	Pup	11a	11b	8b	8d	8e	9c
Note duration (s)	30 first notes	0.20 (±0.03)	0.22 (±0.02)	0.21 (±0.04)	0.19 (±0.02)	0.19 (±0.02)	0.19 (±0.02)
	30 last notes	0.18 (±0.03)	0.16 (±0.04)	0.15 (±0.03)	0.18 (±0.02)	0.17 (±0.03)	0.17 (±0.04)
Internote interval (s)	30 first notes	0.13 (±0.01) (n=4)	0.14 (±0.00) (n=5)	0.124 (±0.02) (n=7)	0.10 (±0.02) (n=30)	0.10 (±0.01) (n=9)	0.10 (±0.02) (n=21)
	30 last notes	0.11 (±0.01) (n=4)	0.24 (±0.22) (n=9)	0.119 (±0.01) (n=6)	0.12 (±0.08) (n=20)	0.14 (±0.04) (n=5)	0.11 (±0.02) (n=23)
Number of harmonics in A	30 first notes	2.27 (±0.45)	2.33 (±0.55)	2.23 (±0.68)	3.00 (±1.11)	2.27 (±0.64)	2.77 (±1.17)
	30 last notes	1.93 (±0.25)	1.27 (±0.45)	1.43 (±0.68)	2.53 (±1.41)	1.13 (±0.35)	3.23 (±1.43)
Number of harmonics in C	30 first notes	4.10 (±0.40)	3.47 (±0.63)	3.73 (±0.69)	4.13 (±0.73)	3.97 (±0.56)	4.10 (±0.80)
	30 last notes	3.97 (±0.18)	3.40 (±0.50)	3.67 (±0.66)	3.80 (±0.81)	4.27 (±0.58)	3.70 (±0.65)
Frequency modulation (kHz)	30 first notes	2.73 (±0.78)	2.89 (±0.19)	2.49 (±0.20)	2.58 (±0.17)	2.61 (±0.16)	2.49 (±0.18)
	30 last notes	2.55 (±0.38)	2.40 (±0.94)	2.56 (±0.34)	2.63 (±0.29)	2.91 (±0.30)	2.59 (±0.44)
Dominant frequency (kHz)	30 first notes	6.31 (±3.02)	3.37 (±2.51)	4.55 (±2.95)	3.30 (±3.31)	4.96 (±3.25)	3.62 (±3.78)
	30 last notes	7.06 (±3.18)	3.73 (±0.12)	6.08 (±3.54)	2.20 (±1.36)	5.24 (±3.03)	2.64 (±1.25)
Mean frequency (kHz)	30 first notes	3.25 (±2.97)	2.40 (±2.07)	2.40 (±3.06)	1.62 (±2.51)	3.25 (±2.66)	2.72 (±3.63)
	30 last notes	5.99 (±3.41)	3.71 (±0.12)	3.70 (±2.88)	1.85 (±1.43)	4.09 (±2.38)	2.57 (±1.35)

paramos os valores médios das 30 primeiras e das 30 últimas notas emitidas. A análise acústica mostrou que, no final do período de separação, as notas de assobio se tornavam mais curtas, com freqüências médias maiores e uma tendência à diminuição do número de harmônicos na parte inicial. Os resultados demonstram que, ao longo de um breve período de isolamento, a resposta vocal de filhotes de cobaias sofre mudanças estruturais possivelmente relacionadas a mudanças no estado motivacional.

Palavras-chave: comunicação acústica, assobio de separação, Cobaia-doméstica.

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Apêndice E _ *The rich acoustic repertoire of a precocious rodent, the wild cavy* Cavia aperea

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The rich acoustic repertoire of a precocious rodent, the wild cavy *Cavia aperea*

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The Caviomorph rodents have precocious young and vary in social complexity, resorting to acoustic communication to efficiently regulate social interactions intra-group and to avoid intruders and predators. The wild cavy *Cavia aperea*, one of the species with the largest geographic distribution, is considered a wild representative of the domestic *Cavia porcellus*, a species with a remarkable acoustic repertoire. We conducted a descriptive study on the acoustic behaviour of a captive population of *C. aperea*. We observed animals in groups, in pairs and isolated pups, and conducted sonographic and descriptive analyses. Ten distinct call types were identified: teeth-chattering, structurally variable contact calls, whines and squeals, a scream, alarm whistle, almost exclusive pup-isolation whistle and tweet calls, and the previously described alarm *drrr* and courtship *purr* calls. As in other caviomorphs, some of these signals may carry information about specific contexts and moods and others may communicate arousal. The similarity of this repertoire size to that of *C. porcellus* shows that the richness of the latter is not a product of domestication. Instead, it may be associated with a relatively complex social life and other ecological factors. Our data set the groundwork for comparative studies of the evolution of acoustic communication.

Keywords: acoustic repertoire; alarm whistle; guinea pig; hystricognath rodents; social behaviour

Introduction

Hystricognaths are rodents with a complex and interesting social behaviour. They characteristically produce precocious young, occupy a variety of environments and make remarkable use of the acoustic channel for communication (Eisenberg 1974). The Hystricognaths include the chinchilla, the mole rat, the spiny rat and the South American caviomorphs (Woods 1993) as the Capybara (CP), the rock cavy (RC) (locally known as *mocó*) and the wild cavy (WC) (locally known as *preá*).

Comparative studies of the acoustic behaviour of these species are essential for understanding their phylogenetic relationship, given the conflicting phylogenies derived using morphological versus molecular data (Graur et al. 1991; Luckett and Hartenberger 1993; Woods and Kilpatrick 2005; Honeycutt et al. 2007). Such studies may also be important to test hypotheses concerning ecological constraints on sociality and behaviour such as that proposed by Lacher (1981). Based on the vocal repertoire size of the Caviinae

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[†] Patrícia Monticelli would like to dedicate this paper to the memory of César Ades.
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genera *Kerodon*, *Cavia* and *Galea*, Lacher suggested that polygynous species (*Kerodon*) have a richer vocal repertoire than promiscuous species (*Cavia* and *Galea*). The problem with this interpretation is that we now know that *Cavia* is more similar in terms of their behavioural repertoire, social and reproductive biology to *Kerodon* than Lacher believed (Alencar 2012). The acoustic behaviours of *Cavia* and *Kerodon*, however, are as yet undescribed.

The wild cavy *Cavia aperea*, a species with broad distribution in Brazil and neighbouring countries (Massoia 1973; Ximénez 1980; Cherem et al. 1999), is particularly interesting because of its relationship with *Cavia porcellus* (Bonatto et al. 1995; Spotorno et al. 2004). *C. aperea* is a social and territorial animal that establishes a stable home range on the banks of rivers and lakes, in the Cerrado and in secondary forest areas (Ximénez 1980; Asher et al. 2004). It lives in small groups of 1–3 females and their unweaned offspring, under a mating system described as female-defence polygyny (Asher and Sachser 2001; Asher et al. 2004). In high-density populations (30 adults/ha, Asher et al. 2008), two other male strategies are found. There are satellite males, described as small resident males that associate themselves with a group and participate in regular social feeding events; and there are intermediate-sized roaming males that have no stable home range and circle the territory of other males, being able, at times, to copulate with a female, producing a low degree of multiple paternity (Asher et al. 2008).

The mother and pup bond is strong and involves the use of specific sounds for attraction and maintenance of contact (Rood 1972; Monticelli 2005). In captivity, alloparental care of pups was described, with some degree of aggression between the participating females, which were members of the same group (Monticelli and Ades 2003). The male of this captive group was tolerant to approaches from and contact with pups, and at times licked them, but came to be extremely aggressive with the males once they reached 30 days of age.

According to Lacher's hypothesis, this rich social network should correspond to a rich acoustic repertoire with varied and graduated signals capable of modulating the various types of behavioural contacts, providing information about internal state and the readiness to perform certain behaviours.

This is the first sonographic description of the acoustic repertoire of a wild species of *Cavia*. Recordings were made from as wide a variety of situations and social contexts as possible in order to characterize the vocal repertoire of *C. aperea* comprehensively.

Materials and methods

Subjects

The study of vocal behaviour of *C. aperea* was conducted in 1999 with a captive population raised for about 25 generations. The animals were kept in indoors and outdoors enclosures in the husbandry facility of the *Institut für Neuro- und Verhaltensbiologie* (Münster, Germany). Because members of this population descended from a stock captured in the Buenos Aires region of Argentina in 1995 (Künzl and Sachser 1999; Künzl et al. 2003), we labelled them as wild cavy from Argentina (WCA). The colonies were composed of a male and 2–4 females and their litters and groups of same sex animals. The study included a total of 44 females, 22 males and 13 litters of 1–2 pups.

The animals were individually identified by hydrogen peroxide stains or cuts made to their fur on different regions of the body (Künzl and Sachser 1999). In the animal husbandry facilities, the cavies experienced dark–light cycles of 12:12 h and an average temperature of 20°C.

Recordings and sound analysis

After a period of a week of habituation to occasionally new grouping conditions and to the presence of the experimenter, we started *ad libitum* observation sessions (one to six 30–60-min sessions in each of the groups). After this initial exploratory phase, we conducted systematic recordings of behavioural and acoustic data using focal animal sampling in four controlled contexts: PI – pup isolation ($n = 28$ pups from 8 litters, from 1 to 30 days old); MM – pairings of two males ($n = 7$ pairs); FF – pairing of two females ($n = 9$); MF – pairing of a male and a female ($n = 10$). All pair types were composed of animals that came from different social groups in the animal husbandry facility.

To establish the experimental conditions, the animals were transported individually from the animal husbandry facility to a nearby experimental room. There, they were placed in a wooden box (50 × 50 × 30 cm) covered with a wooden frame covered with a 2-inch galvanized wire mesh, with no food or water available. Each of these sessions lasted 10 min. All procedures followed ASM (American Society of Mammalogists) guidelines for the ethical use of research animals (Gannon and Sikes 2007).

Sound recordings were made using a Sony TCD-D8 professional portable digital audio tape (DAT) recorder (sampling rate: 48 kHz, frequency response: 20–24,000 Hz ± 1.0 dB; dynamic range >87 dB) and a hypercardioid directional microphone (Sennheiser ME 67 with K6 powering module and bass roll-off, with the filter switch on) supported by a tripod and situated 30 cm above the floor forming with it an angle of 90°.

All of the acoustic signals produced in the pairings and during pup isolation were digitized with 8-bit accuracy from DAT to a personal computer using Avisoft SASLab 3.4 (Raimund Specht, Berlin, Germany) via a Sound Blaster Audigy A/D converter at a sampling rate of 48 kHz. After digitization, we used the "main window" of Avisoft SASLab to scan the records and classify emissions into acoustic categories according to the descriptions by Arvola (1974), Berryman (1976) and ours (Monticelli 2005) of guinea pig vocalizations. Once the categories were defined, we generated spectrograms (*spectrogram window*) with different frequency ranges according to call range (of at least two times the higher frequency of the call) and either a narrow bandwidth (20–60 Hz) for frequency measurements, or wide bandwidth (81–176 Hz) for temporal measurements. We treated the acoustic properties of basic patterns distinguishing units, phrases and bouts as described elsewhere (Struhsaker 1967; Monticelli and Ades 2011).

The acoustic parameters measured in all the calls were note duration, internote interval, rhythm (number of units/time), lower frequency (the first frequency component of the note in the vertical axis, usually, but not always, the fundamental frequency), higher frequency (the last frequency component in vertical axis), dominant frequency (the frequency with the most energy of the note) and number of visible harmonics.

Results

We identified here eight acoustic categories that, together with the previously described alarm *drrr* and the courtship *purr* calls (Monticelli and Ades 2011), compose an acoustic repertoire of at least 10 signals in *C. aperea*. There are low-pitched nasal vocalizations such as contact subsounds, *drrr* and *purr* (Figure 1); high-pitched varied vocalizations such as whines and squeals and less variable screams (Figures 2 and 3); trilled calls such as the alarm whistle and voiceless tooth-chattering (Figure 3) and vocalizations that are almost exclusively emitted by pups, such as the isolation whistle and the tweet (Figure 4).[1] Table 1 presents descriptions in relation to median, minimum and higher values of acoustic parameters of each category, except for the scream, for which we were only able

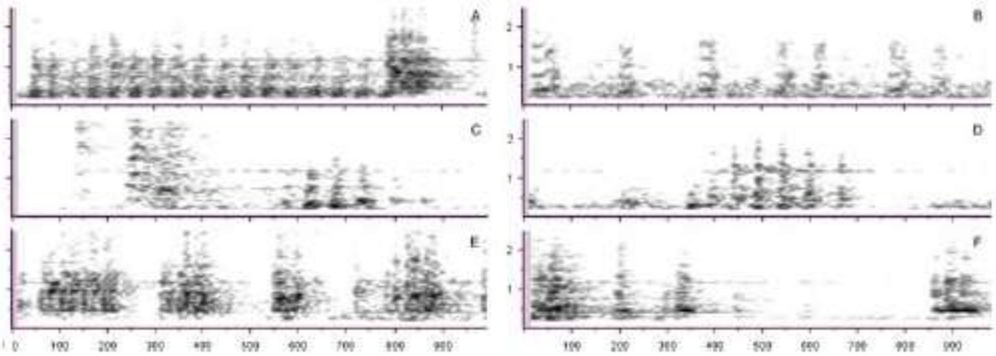

Figure 1. The low-pitched subsounds in comparison with the *drrr* alarm and the *purr* courtship calls. (A) *Drrr* emitted after noise outside the animal husbandry room. (B) *Drrr* emitted by a male paired with a female, after a noise outside the room, but interspersed with social investigation behaviour. (C) *Purr* emitted by this same courting male and ending in a bark type of subsound. (D) Bout of non-regular rhythm of harmonic structure contact calls, initially emitted as single notes, than as double-note syllables, with ascending frequency modulation. (E) Pulsed subsounds syllables emitted by male during exploratory activities in MF pairing. (F) Mix of pulsed sylables and single noise notes emitted by the female of that previous MF session, also during environment exploration.

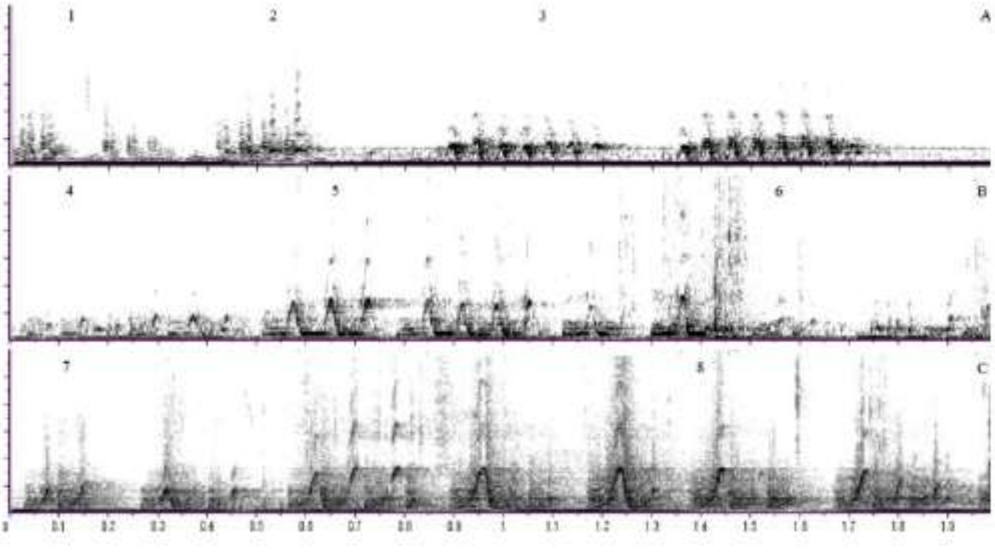

Figure 2. The whine calls. (A) Pulsed subsounds (1) and transitory type (2) precedes male LSFM (3) during initial environment exploration in the presence of a non-familiar female in MF. (B) Pre-whine transitory elements (4) precede HSFM (5) of the female in that MF session, ending in pre-whine again (6). (C) Mixed bout of pre-whine and HSFM (7) and squeal (8) at the end of that session, produced by the female.

to obtain one low-quality recording and, therefore, is only described in the text and is visually represented in Figure 3.

Contact nasal subsounds

This category is composed of slightly varying types that have in common an extremely low intensity, suggestive of proximity between participants, and that are short, monotonous and low-pitched (Table 1). All of them sound like primarily nasal emissions, like the

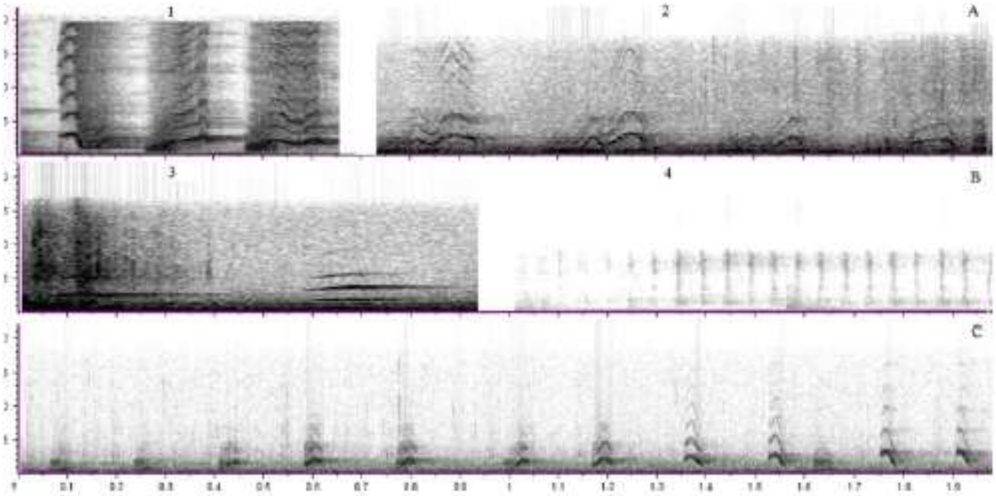

Figure 3. The higher-pitched and the voiceless calls. (A) (1) A phrase with three different notes of squeals emitted by a female when being approached by a male in MF; (2) sample of squeals from a longer bout emitted by a young female avoiding contact with an adult female introduced into her housing box. (B) A phrase of two notes of scream (3) recorded from a distance emitted by an unknown animal, and the initial part of a longer bout of teeth-chattering (4). (C) A small part of a longer bout of the alarm chirp whistle.

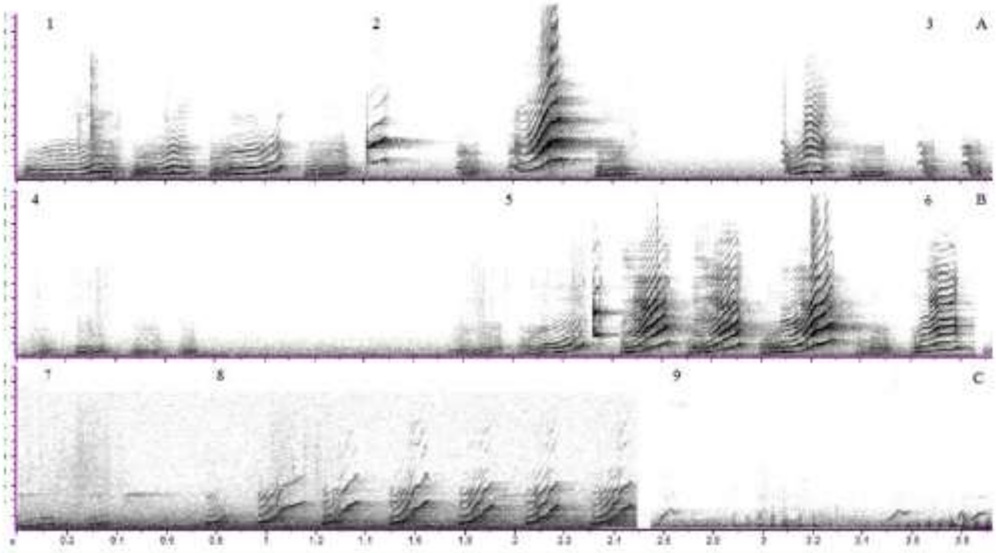

Figure 4. The pup calls. (A) Long unmodulated whine sequence (1) followed by a B squeal (see text), a chirp single-note chirp, BCB squeal (see text), a transitory element of pre-whine repeated (starting at 2), and ending with two single-note chirps (3). (B) Contact calls (4) preceding an isolation whistle bout of a 21-day-old female (5), ending with a squeal (6). (C) Another isolation whistle (8) preceded by contact calls (7) (6-day-old pup); a fragment of a tweet bout (9), with three notes. All emissions, except that from C, were obtained from isolation and pairing of pups. The tweet was obtained from an infant being licked by the mother in a group kept in one of the animal husbandry facilities.

Table 1. Median, minimum and higher values of the acoustic parameters of the seven calls described here (*n* represents the number of notes used in this description).

	Tooth-chattering ($n = 72$)	Contact calls ($n = 136$)	Whine calls ($n = 46$)	Squeal/Scream ($n = 34$)	Alarm whistle ($n = 46$)	Isolation Whistle ($n = 12$)	Tweet ($n = 10$)
	16	5[a]	4	5	4	3	3
Rhythm (notes/s)	*14–21*	*2–6*	*3–5*	*3–8*	*3–12*	*3–5*	*3–4*
	4.29	1.99[a]	1.69	0.62	2.64	2.05	2.60
Pharse duration	*1.49–64.61*	*0.96–4.70*	*1.06–3.65*	*0.36–1.86*	*0.25–4.50*	*0.88–2.44*	*0.67–6.21*
	0.03	0.06	0.14	0.13	0.03	0.19	0.05
Unit duration	*0.01–0.06*	*0.02–0.56*	*0.22–0.38*	*0.06–0.24*	*0.01–0.06*	*0.14–0.30*	*0.03–0.12*
	0.02	0.26	0.11	0.13	0.08	0.12	3.85
Inter-unit interval	*0.01–0.05*	*0.01–1.92*	*0.01–0.23*	*0.07–0.84*	*0.07–0.08*	*0.06–0.13*	*1.17–5.87*
	0.45	0.28	0.39	0.43	0.92	0.47	0.68
Lower frequency	*0.25–0.94*	*0.10–0.83*	*0.16–0.75*	*0.21–2.48*	*0.36–1.52*	*0.30–0.72*	*0.50–0.82*
	7.48	1.81	3.62	16.57	13.43	16.00	1.93
Higher frequency	*0.54–15.20*	*0.51–5.00*	*1.22–14.00*	*5.32–24.00*	*3.24–19.11*	*0.77–24.00*	*1.36–2.87*
	0.84	0.46	0.84	2.51	1.97	0.94	1.13
Dominant frequency	*0.41–2.31*	*0.10–1.25*	*0.19–3.19*	*0.47–5.06*	*1.22–2.44*	*0.75–4.41*	*0.89–1.28*
	–	0[b]	2	7	6	5	1
Number of visible harmonics		*0–5*	*1–11*	*1–15*	*2–8*	*5–16*	*1–2*

Notes: Temporal parameters are presented in seconds and spectral parameters in kHz.

[a] Subsounds syllables and harmonic clucks of single or multiple units.

[b] Zero represents the absence of harmonic structure.

subsounds described by Arvola (1974). Within this category, calls vary in three ways (Figure 1): (1) in temporal structure, there may be (a) single notes lasting from 0.02 to 0.10 s (Figure 1(D), all notes; F, from 200 to 400 ms), which may be emitted at non-regular intervals, or in (b) double or triple syllables (multiple syllables, Figure 1(D), the last two pairs of notes) lasting from 0.09 to 0.256 s. In addition, there may be (c) pulsed syllables like *drrr* and *purr* calls, consisting of 2–6 pulses (Figure 1, compare *drrr* represented in A/B and *purr* in C with pulsed subsounds represented in E and F). These pulsed syllables lasted 0.03–0.21 s and were usually repeated at non-regular intervals. Eventually, 2–4 syllables would be grouped in phrases of regular inter-unit intervals of 0.3–0.14 s (as in Figure 1(E)) in a 4–6 unit/s emission rhythm. There may also be bouts of 6–7 phrases of 1–5 s. (2) In terms of spectral dimension, units are generally atonal (noise) and seen as a blob on the sonogram like the notes and pulsed syllables in Figure 1(E),(F), and the bark-type vocalization emitted at the end of the *purr* call represented in Figure 1(A). At times, the notes have a harmonic structure of 2–4 frequency components with almost no modulation (Figure 1(D), the first four notes) or they may also have an ascending frequency component at the end of the note (Figure 1(D), the last four notes). This ascending modulation is the transition form between the contact call and pre-whine calls (Figure 2(B),(C), the first two notes), as described below.

All variants were emitted during passive and affiliative behaviours linked to proximity between individuals or recognition behaviour (sensu Holmes 1984) and grooming (sniffing, naso–naso or naso–anal contacts). In the pairings and in the groups, these sounds were emitted by the females when they were in proximity, marked or courted by a male and in interactions of animals of the same sex during approximation or exploration. During the isolation of pups these sounds were registered in only two sessions, before isolation whistle bouts (Figure 4(B),(C)) and tweet bouts.

An even lower frequency pulsed syllable type was emitted by adult animals in a dominant posture during approach of another unfamiliar animal in the pairings. This may correspond to an aggressive grunt, instead of a contact call, in a more detailed analysis, but is very similar to the pulsed syllable of contact. In MM and FM pairings, it was registered during exploration with rapid locomotion, exploration and rearing posture (standing erect during exploratory activities) performed by larger males. Also, when these males were urinated upon by females in pairings and stopped to sniff the urine mark. It is also emitted when members of a group jointly explore the territory in the colony situation, and when nursing females come in proximity with the young and place themselves in a posture of nursing.

Contact calls elicit the same vocal response in familiar or non-aggressive receivers and behaviours of contact or coordinated movement between animals of the group. Otherwise, the absence of vocal response to contact calls appears to favour avoidance or agonistic behaviour, as observed in a pairing of subordinate females (the smallest of their groups). One of the females moved during the contact calls, while the other remained immobile and silent. The absence of a response from the other female appears to have served as a stimulus for the first to throw herself at times against the mesh wire of the test-box top and then freeze.

Whine or cry

The whine category is also composed of various types of notes, according to the frequency modulation contour (Figure 2). They were classified together in the same category because they are typically interspersed in long bouts. They also have in common a naso–oral form of production that is noted by ear. There is a low-range successive frequency modulation (LSFM) type (Figure 2(A-3)) used in agonistic interactions, very often simultaneously with tooth-chattering, usually by males. A higher-pitched variant, high-range successive frequency modulation (HSFM, Figure 2(B-5)), usually emitted by females or by submissive males, was related to situations of mild discomfort (sensu Berryman 1976) produced by proximity and contact initiated by another individual: an animal being approached when eating, or a female in response to an approach by contact or courtship displayed by a male; the closer and more persistent the male is towards the female, the greater the peak and the number of successive frequency modulations.

In the pairings, the LSFM variant was emitted by males that apparently tried to dominate other males, pressing them with the chin and trying to mount them, during nasal investigation of the urine marks left by females and in agonistic displays directed towards other males or females. In these situations, the emitter was able to tooth-chatter at the same time. Females usually emitted the HSFM whines, when paired with males or during interaction with them in the groups, and only one female emitted this type of sound when paired with another female.

All kinds of whine notes have a harmonic structure (up to 11 harmonics in the HSFM type) and are composed of different arrangements of three subparts, as described for the isolation whistle of the guinea pig (Tokumaru et al. 2004). The A part has a low-frequency range and no frequency modulation; the B part is a fast ascending frequency modulation and the C part is the fast descending frequency modulation that links the other two. In the LSFM variant (Figure 2(A)), there is no A part; it is composed of successions of B and C parts. In HSFM (Figure 2(B)), A part is usually the initial part of the note followed by B and C parts and variable terminations (ABCABCAB or ABCA as in the first and fourth notes of Figure 2(B) starting at 5). The C part may also be absent as was the case for emissions produced by females that were being pressed in the rump or mounted by males. The absence of C part is related to faster bouts. Less commonly, a type of AB note with longer A part and smaller B part (Figure 4(A), initial notes) is emitted by submissive or smaller animals after the aversive situation has ended, usually after squeals.

In cases of prolonged mid aversive interactions, the emitter appears to respond in an increasingly aggressive manner to the presumptive offensive action of the receiver and whines may be interspersed with squeal notes (Figure 2(C)) and elicit tooth-chattering and purring in the receiver in agonistic and courtship contexts, respectively. Pulsed subsound syllables and almost similar pre-whine transitory variants (Figure 2(B-4),(C-7)) frequently precede whine and isolation whistle bouts and appear at the end of whine and squeal bouts when the aggressor retreats. Whines produced by isolated pups are interspersed with squeals and with isolation whistles at moments of greater exploration and attempts to escape from the test-box.

Squeals/scream

The squeal note can also be subdivided into A, B and C parts. It is usually composed of B and very short C compositions, like an incomplete isolation whistle note (Figure 3(A)). But, uncommon notes may be formed, such as the BCAB represented by the first note of Figure 3(A-1). Squeals are emitted when the interactions between the animals reach a more severe point in which an attack is about to occur or has just occurred. They are produced with the mouth open (as in the whistles and unlike the whine) during a dispute for food, or by pups that try to nurse and receive a blow from the female or by animals that received light injuries from others. Where more severe injuries result, the scream may be used (Figure 3(B-3)).

In the pairings, squeals and screams were emitted (1) close to the time of an attack (squeal for imminent attacks and screams after being attacked), (2) by small males or subordinate animals that have been attacked in the past by that individual (3) or in response to sudden movements by the adversary or the researcher, or, most commonly, by females avoiding mounting by a male.

Although squeals were frequently emitted in a burst of whines, they are different from them because of the fast and sharp rise of frequency (like a whistle). In general, they are somewhat shorter, emitted in longer intervals, have a much higher number of harmonics and reach higher frequencies (Table 1). The dominant frequency is also higher than that of the whine, and bouts of squeals are shorter than bouts of whines, restricted to brief escape behaviours. The higher squeals are more difficult to distinguish from whistles because they share the ABC subdivision parts of the note, but usually have an ascending instead of a descending conclusion (part C) (Figure 4(A-4),(B-6)).

The scream is a sharp type of squeal, with a long and slow ascending B note (Figure 3(B-3)), which reaches higher frequencies, even ultrasonic (exceeding 24 kHz), and has many harmonics (up to 11 harmonics were counted in other wild-captured lineages of *C. aperea* from São Paulo, Brazil).

Alarm whistle

The alarm whistle has been described with onomatopoeic words such as chirp (Eisenberg 1974) and chirrup (Berryman 1976) and as the "most pure oral vocalization of the domestic cavy" (Arvola 1974, p. 90), and has also been called song (Figures 3(C) and 4(A-3)). It lasts from 2.6 s up to a few minutes. In long bouts, there are short intervals between phrases or a variation in rhythm (deceleration and acceleration, according to the distance or reoccurrence of the stimulus). We registered bouts of up to three phrases, which lasted from 0.2 to 6.6 s. The notes last from 0.02 to 0.07 s and were separated by intervals of 0.09–0.16 s. The minimum frequency of the notes was greater than that in the other calls: from 0.4 to 0.8 kHz. The higher frequency varied from 3.3 to 13.6 kHz, and 2–8 harmonics were visible (Figures 3 and 4).

The alarm whistle is a vocal response to variation in the physical or social environment such as (1) after the introduction of animals in already established groups, when a resident or the introduced animal would withdraw to a corner of the box, emit the chirp and all would stop; (2) in outside enclosures and in the breeding husbandry facility, during the day or at night, when the handler came close or after sudden noises or voices outside the room (as soon as the door opened the chirps stopped) and (3) in pup isolation sessions, after the sixth day of life. In this case, a short chirp sequence was emitted soon after a sequence of separation whistles (Figure 4(A)).

When a WC emits the chirp, other animals from neighbouring colonies in the same room at times also respond with chirps. All of the animals in the room would remain frozen for the duration of the emission. Later, there would be general agitation, with movement in the colonies and bouts of pulsed syllables at regular intervals. Individual notes or short sequences of 2–3 chirp notes were also emitted, as if the animal was beginning a sequence and stopped, or in the middle of other signals, such as the squeal.

Isolated chirp notes, which to the ear can sound like a snap, were emitted by females paired with males. The female was moving through the box sniffing, when the male came into contact. She reacted aggressively, giving it a head attack, or charging in his direction with her body. This occurred a couple of times in this 10-min pairing session, with no vocal response. It appears to be an emission that communicates intolerance to physical proximity, but is less aggressive than a fight.

Isolation whistle

The isolation whistle note is the archetypal ordered ABC composition with a very clear tonal structure of 5–16 harmonics. It lasts 0.14–0.30 s and is repeated at 0.06–0.13 s intervals, forming bouts of 0.88–2.44 s in 3–5 notes/s rhythm.

It was emitted exclusively by pups, up to the fourth week of life (but commonly in the first 2 weeks) when they found themselves apart from the group in the bigger outside enclosures. In 28 isolation sessions, this call was emitted only once by a 6-day-old pup (Figure 4(C-8)), and a second time in pairing of 3-week-old female pups (Figure 4(B-5)).

Tweet

This is another onomatopoeic term presented by Berryman to represent an extremely low-intensity tone that is nearly imperceptible to the human ear. It sounds like a pure whistle formed by a rapid and sharp rise in frequency, of around 1 kHz represented by a nearly vertical mark on the sonogram. It can reach up to 3 kHz and have a second harmonic. The notes have the form of an inverted V or a kind of rotated J, as shown in the sequence in Figure 4. The notes are never emitted alone. Instead, they consist of bouts that vary from 0.67 to 6.21 s, consisting of 3–8 units spaced to form long and irregular intervals of 1.17–5.87 s.

The tweet was promptly emitted in the context of maternal ano–genital grooming of the pup in the first days of life (the tweet could be heard in the two maternal ano–genital grooming of pups observed). It was also emitted, however, by adult animals as a more general response to ano–genital stimulation in interactions between a male and a female with signs of oestrus (vaginal aperture) or by males smelling receptive females' urine marks.

Teeth-chattering

This non-vocal sound results from the rubbing of the upper incisors and molars against the lower incisors and molars. It was emitted by adult and sub-adult males and females in threatening conditions accompanied by visual agonistic displays described in the literature (Messias 1995; Monticelli 2005). It occurred both in groups (between the dominant male and the sexually maturing juvenile males, or between females) and in pairing conditions between females (FF) and between males (MM) during chasing and agonistic interactions. In MF, it was emitted by females in response to a courting male or to a male that occasionally approached during exploratory activities. It also occurred during handling of most aggressive animals by the experimenter and when attempts were made to capture animals from the groups. In these cases, the resident males of two groups faced the experimenter by displaying a frontal threatening posture (Messias 1995; Monticelli 2005). It also occurred when placing animals in unfamiliar or aversive environments (the pairing box).

The sound produced by teeth-chattering corresponds to a rapid succession of very short units (pulses of 0.01–0.06 s) repeated at extremely short intervals (0.01–0.05 s; Table 1). Each unit consisted of two parts: the first appeared with a higher frequency range (0.25–

15.20 kHz) and another appeared in the sonogram as a small blur (Figure 1). There is normally a frequency band of greater energy around 0.41 and 2.31 kHz and another nearly as intense from 0.47 to 3.53 kHz. The duration of the phrases (or of the bouts, when more than one phrase is present) varied considerably, according to the interaction, and lasted as long as the confrontation between emitter and opponent continued. The longer phrases registered in the pairings were a little more than a minute (64.61 s). The rhythm of emission that varied from 14 to 21 notes/s was the fastest in the entire repertoire. There seems to be a relationship between rhythms and the quality of the confrontation: faster emissions and longer phrases accompanied the more severe confrontations, like those between two males of the same size. In contrast, when only one animal engaged in tooth-chattering, the rhythm was slower. In agonistic interactions, tooth-chattering initiated by one animal normally elicited the same response in the other.

Discussion

The wild cavy *C. aperea* produces 10 basic patterns of acoustic signals in different social contexts and in isolation. Some of these categories artificially pool across a higher number of acoustic signals if we consider the structural variation in notes and temporal patterns of emissions as distinct calls instead of placing them together. That can only be done after a deeper analysis of the communicative function of the subcategories – do they vary according to the status or identity of participants, or according to other variables?

The contact call category is one that is composed of at least four types of structurally different notes: the tonal note, the click note (a single non-tonal note), the broadband noise bark and the pulsed subsounds. All these forms are emitted in isolation, in rhythmic phrases or in bouts of varied duration, enhancing the variability within this category. All of these contact calls were also described as domestic cavy (DC) calls by the names of chut, chutter (Berryman 1976), low-pitched, high-pitched and high-pitched double subsounds (Arvola 1974). Although they are structurally different, these forms are emitted in the same situation: by amicable or exploratory encounters (in the case of animals from different groups) and in the maintenance of contact between group members.

Similarly, the whine and squeal categories are also composed of different forms that cannot yet be classified as different signals. They are presented here as graded forms related to the quality and latency of interactions, and to the identity of participants. For instance, does the difference between the whines LSFM and HSFM represent signals with different meanings or do they reflect levels of aggression (those that pursue and those that are pursued)?

Whines seem to communicate attentiveness during intimate interaction and slight or modest discomfort from the emitter in relation to the behaviour of the receiver. That could be an initial approach or a second meeting after a slightly aversive experience (such as courtship and forced mounting in young or non-receptive females), an attempt at physical contact (naso–anal, nose-to-nose, grooming and pursuit) or a dispute over food among the participants. A whine can become a squeal if the interaction becomes truly agonistic and dangerous and into a scream if the emitter is attacked.

Our description of the *C. aperea* repertoire coincides with descriptions of other hystricognaths in having intermediary or transitory forms. In fact, this gradation was described by Eisenberg (1974) as characteristic of the group. In the repertoire of the 17 species he compared, including the Domestic Cavy, *C. porcellus*, there are "basic syllables" (one of a harsh structure, another harmonic and others overlapping between harsh and harmonic), which are combined in two ways. Firstly, they produce calls associated with situations involving readily predictable stimuli, such as specific contexts and moods – in the case of WC, they are the *drrr*, *purr*, tweet, alarm whistle, tooth-chattering and isolation whistle (the latter three are shared by all 17 species, even if they are structurally different). The second way promotes the enhancement of a series of calls with a more general communicative function, which act as excitation indicators and/or for the localization of the emitter, such as calls used for maintenance of contact during movement – the contact calls also shared by all of the species – and whine and squeals in WC.

The richness of the DC repertoire, represented by the number of different calls and signalling contexts, surprised Eisenberg, who was unaware of the repertoire of WC. In fact, this study is the first to describe the repertoire of a wild *Cavia*. We can now say that the rich repertoire of DC is not an effect of the domestication process. All of the 10 patterns of WC are present in DC (Arvola 1974; Berryman 1976; Monticelli 2005). These two very closely related species (Spotorno et al. 2004) have, however, one difference in repertoire size: the emission by the domesticated species of a food-anticipation whistle (Monticelli et al. 2009) or the caretaker whistle (Ades et al. 1994). Other differences previously cited are related to the perception or reaction to signals, and to the physical structure of alarm *drrr* and courtship *purr* calls, more precisely, to temporal dimension parameters (Monticelli 2005; Monticelli and Ades 2011).

Another difference between DC and WC concerning threshold response appeared in our attempts to obtain the isolation whistle of pups. The whistle is a vocalization that occurs promptly in DC young when separated from the group in an experimental situation (Niciporciukas et al. 1999; Monticelli et al. 2003, 2004; Tokumaru et al. 2004, 2006). In WC, however, under the effects of predation, the lost pup may have to make a decision: to choose between calling for the mother and improving the chance to re-establish contact with her *or to* be quiet and freeze to avoid being detected by a potential predator whose arrival promoted the escape responses that caused the fissioning of the group. The first response of the wild pups to any manipulation from us was to freeze. They were unlikely to emit any vocalization after that. We once kept a WCA pup isolated for more than an hour before it began to move again, but it never vocalized. There were 28 attempts to create experimental situations of isolation from 1 to 30 days with different pups. We registered the whistle on only two occasions: from an isolated 6-day-old female that also emitted *drrr*s and contact calls, and from a pairing of female pups, one 21 days old and the other 25 days old. They also emitted whines, alarm whistles, many contact calls and *drrr*s. DC pups never emit *drrr*s or alarm whistles when isolated, or even during spontaneous interactions in colony groups (Arvola 1974; Berryman 1976; Coulon 1982; Monticelli 2005; Table 2). Thus, unlike the DC, which may whistle during an entire 10-min isolation session, a WCA pup emitted only short bouts of high-frequency whistles, which in and of themselves may limit their detectability by predators (Wilson and Hare 2004, 2006).

It is interesting to note the presence of two kinds of alarm calls in WC. The *drrr* is an alert call used for communication with a nearby individual, which is a member of the group (a small harem of up to four individuals, Asher 2004; Asher et al. 2008). The alarm whistle may be a warning signal used by members of stable groups dispersed over large distances among their sub-territories (the average size territory of a male, which overlaps that of the females in its harem, is 880 ± 217 m^2 and that of the females is 549 ± 218 m^2; Asher et al. 2004). At this distance, the *drrr* would not be functional. Both alarm calls are common to the DC repertoire but are rarely emitted; the chirp whistle is even absent in most guinea pigs (Berryman 1976).

Table 2. Situations of emission of calls by WC and DC following Berryman (1976) and Monticelli (2005) to DC.

	Tooth-chattering	Contact call	Whine	Squeal	Scream	*Drrr* alarm call	*Purr* courtship call	Chirp alarm whistle	Tweet	Isolation whistle
Contact seeking/maintiaining		WwDd		wd		Ww				wd
Exploratory activities		WwDd				WwD				
Sexual enconter										
Male		D	WD		WD		WD			
Female	WD	WD	WD	WD	WD					
Mother–young interaction										
Mother		WD								
Pup		wd					d		wd	
Isolation										
Adult from group		WD								D
Pup from mother		wd		wd						wd
Disturbance										
Human present	WD					WwD		W		
Change in social environment	W	WwDd	WD	WD	WD	W	WD	WwD		
Change in physical environment		wd				WwD		WwD?		
Aversive situation/pain				WwDd	WwDd					wd
Aggressive enconter	WD		WD	WD			WD	W		

Notes: Lowercase represents pups and uppercase represents adults.

In terms of the structure and situation of emission, the alarm whistle can be compared with the alarm whistle of the RC or mocó (*Kerodon rupestris*, Lacher 1981), with the bark of the CP (Barros et al. 2011), with the alert whistle of marmots (Blumstein and Armitage 1997; Blumstein and Armitage 1998), with the rhythmic alarm call of gerbils (Randall and Rogovin 2002) or with the repeated alarm whistle of Richardson's Ground Squirrels (Warkentin et al. 2001). All of these calls are high pitched, repeated in bouts and difficult to locate. All of them are associated with the distance of potential predators and the urgency of response and promote attention reactions (body reorientation and a frozen posture). For all of these reasons, we suggest that the WC chirp would be aimed at a predator, as was suggested for Yellow-bellied Marmots (Blumstein and Armitage 1997) and gerbils (Randall and Rogovin 2002). In situations of greater risk, the call becomes more conspicuous (with an accelerated rhythm and longer bandwidth), and the emitter keeps the predator in sight while vocalizing as part of a group or alone. We often saw the WCA doing exactly this with a fixed stare at the researcher during emission. When approached, the emitters would run to shelter or throw themselves against the pairing box or the group enclosure. In nature, we did not see WCs leaving the shelter offered by tall grass to chirp in our presence. When we heard chirps produced by *C. aperea* populations living in the wild in the municipality of Itu (São Paulo State, Brazil; Monticelli and Ades 2003; Monticelli 2005), they came from an animal hidden in reeds more than a metre tall.

According to Lacher's (1981) ecological constraints hypothesis, habitats vary according to the distribution and productivity of their resources (shelter, food, females and males available for reproduction) and the risk presented by predators. Where resources are clumped, it is possible for an animal to monopolize them. On the other hand, fields with resources distributed throughout make monopolizing resources costly in time and energy, favouring the dispersal and the domination of a small area (territoriality). In this case, there would be advantages for a dominant male to attract and monopolize a small number of sexual partners through aggressive behaviour and elaborate courtship displays.

Similar to Lacher (1981), Eisenberg (1974) attributed the level of social complexity and the richness of the acoustic repertoire to the degree of resource distribution. In 1974, Eisenberg had no information about the acoustic repertoire of the CP or WC, though we now have that information. The largest of the hystricognaths, the CP, lives in harems, much larger than those of WC (10–25 individuals, Herrera and Macdonald 1993) and has a comparable repertoire size. Descriptions have been published of seven signals functionally characterized as contact, alarm, distress and agonistic calls used to regulate social encounters, to maintain the cohesion of the group during long distance movement and to alert members of the group about environmental cues, such as predation risk and juvenile isolation (Barros et al. 2011). New signals will probably be discovered when the courtship behaviour of the species is documented and natural populations are studied, probably revealing an acoustic repertoire comparable with that described here for the *C. aperea*.

A similar comparison can be made with the RC that has been recently grouped with CP within Caviinae (Woods and Kilpatrick 2005). Lacher has studied RC in captivity and described it as living in harems, performing resource defence polygyny and as a specialist in inhabiting semi-isolated rock piles in the Brazilian Caatinga, a relatively productive habitat, but where resources are highly clumped. The dominant male would need a rich repertoire of contact and aggressive behaviours and vocalizations to maintain high levels of communication in the group, ritualizing aggressive interactions and thus potentially reducing aggressive encounters among members, thereby allowing the defence of the rocks from invasion by outsiders. In fact, the repertoire size of RC is similar to that of the CP and WC. The WC enjoys high food abundance and perennial food availability that reduces competition for food. The home range is small and stable, compared with the much larger home ranges of *Microcavia australis* and *Galea musteloides* (Rood and Weir 1970; Rood 1972; Asher et al. 2008).

Qualitative data about acoustic repertoire size of other Caviinae support Lacher's ecological constraints hypothesis. The Desert Cavy (*Microcavia* sp.), considered closest to the ancestral prototype of the Caviinae, also lives in a region of clumped resources in thorn-bush associations, but with a limited number of shelter sites and greater risk from predators. Their behavioural and acoustic repertoires are considered to be simpler than those of RC and WC, and the level of social complexity to be lower than that of RC and WC. The Yellow-toothed Cavy *G. musteloides* was described as having an intermediate repertoire. It lives in regions with sparse vegetation in a semi-desert habitat, under intense food competition, the females have large home ranges with almost no overlap, are promiscuous and have multi-paternal litters that result from sperm competition (Hohoff et al. 2000, 2003).

This relative richness in the diversity of forms (repertoire size) and level of variation of acoustic signals of WC, RC and CP must have been selected in an environment favourable to the use of the acoustic channel and in a social system that would select for the variation that only the acoustic channel offers (Grier and Burk 1992; Vielliard 2004). There must be a relationship between the level of social complexity as described, for example, and the level of detail of information that the animal needs to communicate. It would be useful to communicate identity (Hare 1998) – that one is a member of the group, is from a neighbouring group or is a roaming male that threatens the reproductive success of the owner male – or to communicate social status in the linear hierarchy that exists between male and female WCs. These advantages (or balancing factors) may compensate for the costs of forming aggregations (Blumstein and Armitage 1998). If on one hand life in a group imposes a need for a division of resources, thus increasing inter-individual competition, the conflicts of interests can be resolved by behaviours and vocalizations that indicate appeasement or through ritualization of aggressive behaviours, reinforced by stereotyped acoustic signals, which reduce the probability of direct and escalated confrontations (Parker and Rubenstein 1980; Beaugrand 1997).

Until now, very little was known about the use of the acoustic channel by WCs. This study offers the first and most comprehensive description of their acoustic repertoire to date. As the acoustic repertoires of other species of phylogenetic interest become known, we will improve our understanding of the evolution of acoustic communication in this important group of rodents with different levels of social complexity and precocious production of offspring.

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Note

1. Sound files and other sonograms representative of the emissions of wild and domestic cavies are available at http://portal.ffclrp.usp.br/sites/patriciamonticelli.

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Apêndice F _ *The acoustic repertoire of* Cavia intermedia *as a contribution to the understanding of the Caviidae communication system*

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The acoustic repertoire of *Cavia intermedia* as a contribution to the understanding of the Caviidae communication system

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ABSTRACT

Wild cavies from the taxon *Cavia aperea*–*Cavia porcellus* are known for their extensive use of acoustic signals for communication including a courtship call that is a rarity in Mammalia. The genus *Cavia* includes an insular species (*Cavia intermedia*) derived from a continental "asocial" *Cavia magna*. We hypothesized that the courtship call and all the other 12 acoustic signals previously described for *C. aperea*–*C. porcellus* were synapomorphies of the genus and took the opportunity of recording the critically endangered *C. intermedia*. Four field trips lasting 10 days were conducted in 2008 and 2009. After collecting and analysing nearly 350 sound files of 38 individuals, we found all the 12 calls of wild and domestic cavies previously described, including the courtship call, and a new call, a peep. Despite the presence of the peep, our description of *C. intermedia* acoustic repertoire showed the evolutionary stability of the acoustic repertoire of this rodent genus of 6 species, even after domestication of *C. porcellus* and 8000 years under insular selective forces in *C. intermedia*. We present a discussion about the communicative function of *Cavia* calls in a comparative context and its repertoire size in accordance with the complexity level of sociality of this caviomorph rodent.

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Introduction

Throughout evolutionary history, the interaction between animals, whether of the same species or not, presumably became more efficient as a result of natural selection. The acoustic channel of communication is considered very efficient because of its flexibility (Hauser 2000). The sound wave is sufficiently flexible to permit an almost infinite variability in its time, frequency and intensity dimensions and this variability can be associated with different reactions (Bradbury and Vehrencamp 2011), which, on an evolutionary scale, can, under the constraints of the vocal apparatus, create a repertoire of different signals that can be associated with identity, species, sex, motivational state, hierarchical status and so on (Vielliard and Silva 2006). As biological phenomena, acoustic signals present ontogenetic

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and phylogenetic features that represent their evolution (Vielliard 1997). Thus, it is expected that species derived from a common ancestor share similarities in their acoustic repertoires.

The caviomorph rodents are a good model to discuss the evolutionary history of the acoustic repertoires in mammals. Among them are species that make an extensive use of the acoustic channel, as noted by Eisenberg (1974), and there are some shared signals, as the tooth-chattering and a contact sub-sound, that can be used to backtrack their origins and selective forces. In the Caviinae subfamily, *Cavia porcellus* has had its acoustic repertoire extensively studied (Arvola 1974; Eisenberg 1974; Berryman 1976; Coulon 1982; Monticelli and Ades 2011), and it was suggested that its extensive repertoire was selected during the domestication process (Eisenberg 1974). Only after the acoustic repertoire description of *Cavia aperea* was it made clear that the extensive use of acoustic signals had an earlier origin than domestication: *C. aperea* has the same repertoire as its domesticated relative (Monticelli and Ades 2013).

The genus *Cavia* comprises four other species that have not yet been studied for their acoustic abilities (*Cavia magna*'s repertoire was described in an unpublished work; Olivio 2014). One of them is *Cavia intermedia*, an endemic species of Moleques do Sul Archipelago, Santa Catarina, Brazil (Cherem et al. 1999), supposedly derived from a continental population of *C. magna* about 8000 years ago (Olimpio 1991; Gava et al. 1998). It is one of the rarest mammals in the world (Salvador and Fernandez 2008a) and it is listed among the 100 most endangered species on the planet (Chapman 2008; Baillie and Butcher 2012). *C. intermedia* shares similarities in genetic (Gava et al. 2012) and morphological traits (e.g. interdigital membrane, three subdigital pads, masculinized genitalia) with the most common cavy along the Santa Catarina coastline, *C. magna* (Cherem et al. 1999; Furnari 2013).

C. intermedia's reproductive system and social organization were recently described by Furnari (2011; unpublished thesis) as governed by female defence polygyny with no territorial defence, i.e. females distribute themselves according to food availability and males move according to female concentration. Animals have no fixed or preferred territory to forage in. Most of the interactions between the animals are non-aggressive; the adult males mainly interact with adult females and females interact with both sexes in the same proportion. The infants interact primarily with their mothers and young males, and show courtship displays and agonistic behaviours.

In order to understand the evolutionary history of the extensive use of sound signals for communication in caviomorph rodents, the acoustic repertoires of many more species still needs to be described. In this study, we introduce the description of *C. intermedia*'s repertoire and consider the other Caviidae species we have been studying in our lab, offering a comparative scenario for discussion.

Materials and methods

Subjects and study area

Wild cavies living in Archipelago Moleques do Sul were studied from February 2008 to December 2009 during four field trips lasting 10 days each. Vocal emissions of 38 animals were recorded (10 males, 9 females and other 19 not sexed individuals) from all age classes (infants, juveniles and adults, determined by body mass following Salvador and Fernandez 2008b), some of which were previously captured and individually marked by Furnari (2011).

Moleques do Sul is located in the Southern Brazil and consists in 3 islands 14 km from the mainland portion of Santa Catarina State (27°51′S 48°26′W) (Cherem et al. 1999). The cavies are found only on the largest island (area: 10 ha). It is covered with granitic rocks and herbaceous and shrubby vegetation, including their alimentary items *Paspalum vaginatum* and *Stenotraphrum secundatum* (Cherem et al. 1999), and the *Verbesina glabrata* and *Cortaderia selloana* that they use as shelter (Salvador and Fernandez 2008a). The local weather is mesothermal humid, rainfall occurs all over the year and the average wind speed reaches values between 1.37 and 3.60 m/s (i.e. 5 and 13 km/h), being higher in the most exposed areas (Furnari 2011). A more detailed description of the study site is presented in Salvador and Fernandez (2008a) and details of the capture procedure and marking in Furnari (2011).

Recordings

Most of the sound recordings were made during *ad libitum* observations of free-living animals in three different areas of the island, using a 5-m long microphone cable that put the mic at no more than 3-m from the farthest animal, not disturbing them with our presence. Additional recordings were obtained from animals inside traps, during handling of individuals (males and females, adults and infants) for measuring and individual marking procedures, as well as during pairing of animals in a cage box, following Monticelli and Ades (2013) paradigm. Animals were captured with Fermarame® live-traps, and after handling or pairing, they were immediately released. Concerning the pairing sessions, animals were put together in a portable wooden cage (50 × 50 × 30 cm) covered with a 2-inch galvanized wire mesh structure. Animals remained together for about 30 min and neither water nor food was offered. Three different kinds of pairs (male–male, female–female and male–female) were formed using 12 animals (7 males and 5 females). All methods followed the Guidelines of the American Society of Mammalogists for the use of wild mammals in research (Sikes and Gannon 2011) and Guidelines for the Capture, Handling and Care of Mammals (Animal Care and Use Comittee 1998).

Both *ad libitum* observations and pairing session were recorded with a Marantz PMD660 recorder (sampling rate: 44.1 kHz; frequency response: 20–22,000 Hz ± 1 dB; dynamic range > 87 dB; Japan) and a directional shotgun microphone Sennheiser ME67 + K6 powering module (bass roll-off and filter switch on; frequency response: 40–20,000 Hz ± 2.5 dB; minimal nominal impedance: 200 Ω; equivalent noise level: 10 dB; sensitivity in free field, no load [1 kHz] 50 mV/Pa ± 2.5 dB; Germany). In order to reduce wind noise effects on the recordings, we used a combined foam and hair covered windshield (Rycote, Sennheiser; Germany).

Acoustic analysis

We used Raven Pro 1.4 (Cornell Lab of Ornithology; United States) to scan and select a sample of calls with enough sound-to-noise ratio within all files recorded ($n = 356$). We used ear and visual inspection to categorize the recorded sounds of the species following previous *Cavia* calls categorization (specifically Berryman 1976; Monticelli and Ades 2011, 2013). This is not the first description of a cavy species' repertoire and previous classifications of cavy calls into distinctive categories were already made by four independent researchers (Arvola 1974; Eisenberg 1974; Berryman 1976; Coulon 1982; Monticelli and Ades 2013). As the previous descriptions obtained similar results, and, as our sample of calls of different individuals (independent variable) was not enough to allow a classification test (e.g. DFA), we present here a description of the physical structure and a characterization of the situation of emissions of what we could observe and record from this rare species. In Table 1 (description of call structure), the individuals were the sample unit (when more than one note was measured from the same individual, the individual was represented by his average value).

Measurements of acoustic parameters were obtained in spectrograms with the following settings: Hann window; FFT 512 (pulsed and lower-pitched calls, as purr, drr, tooth-chattering and sub-sounds, Figures 3–5) or 1024 (to structurally complex harmonic and higher pitched calls, as whine, scream, neigh and peep, Figures 1 and 2); 90% overlap, 0.113 ms resolution time. We used the minor measurement unit (note) to describe the properties of each call. The spectral and time parameters determined were: minimum frequency (set at F0), maximum frequency, dominant (peak) frequency, note duration, rhythm (notes/s) and frequency range (maximum frequency minus minimum frequency). The communicative function of the calls was inferred by the general context of emission, i.e. immediately before/after behaviours of the involved individuals, following Berryman (1976) and Monticelli and Ades (2013) observations and descriptions to other *Cavia* species.

Results

Simultaneous visual and aural inspection allowed us to identify all the previously described acoustic categories and a new one, a peep, as follows: harmonically structured notes (whines, neigh, scream, peep, isolation whistle and tweet) (Figures 1 and 2), guttural sub-sounds (*sensu* Arvola 1974) emitted as single units (grunt) or as multiple pulse syllables (multiple-sub-sound: sounds like exploding soap bubbles that may correspond to the chut/chutter of Berryman [1976]; and pulsed-sub-sound: a rhythmical and lower pitch syllable sounding as "pru-pru") (Figure 3), rhythmical sounds as the courtship call (purr) and short-distance alarm call (drr) (Figure 4) and the voiceless tooth-chattering (Figure 5). Samples of each one of these calls can be heard at Fonoteca Cesar Ades (FOCA), our sound library of neotropical mammals. Table 2 summarizes the categories with gradations and lists the situations when the calls occurred.

In the higher frequency calls, such as the neigh and the scream, non-linear phases were common and interspersed with harmonic ones (frequency jump, deterministic chaos and sub-harmonics). Eleven out of the 13 quantitatively described categories were uttered by both sexes except for the male courtship call, the purr (that may be used also by females, as it was observed in other cavies). Exclusive to infants were possibly the isolation whistle and tweet, as described for the other cavies.

The average, standard deviation and range (minimum and maximum values) of the acoustic parameters used to describe these call types are presented in Table 1, except for chirrup, which was accidentally recorded in mp3 format, and tweet, isolation whistle and drr, which could be heard by the experimenter (PFM) in the island, but could not be recorded with enough quality to allow structural description (see Table 2 for details). Both infant calls are distinctive sounds, the tweet (onomatopoeia label from Berryman 1976) is a low intensity short whistle that has a *J*-contour in the spectrogram, and the whistle is indistinguishable

Table 1. Measurements (average, standard deviation and minimum and maximum values) of acoustic parameters of the ten quantitatively described calls.

	Time (s)		Frequency (kHz)			
	Note duration	Rhythm (notes/s)	Minimum frequency	Maximum frequency	Frequency range	Dominant frequency
Multiple subsounds (*n* = 6 individuals; 54 notes)	0.036 (0.007) 0.028–0.044	7.288 (1.089) 6.319–9.375	0.934 (0.127) 6.319–9.375	1.508 (0.175) 1.274–1.793	0.573 (0.116) 0.458–0.799	1.150 (0.111) 0.995–1.307
Pulsed subsounds (*n* = 10 inds; 47 notes)	–	50.823 (10.425) 33.506–68.282	0.173 (0.052) 0.125–0.271	2.550 (0.902) 1.708–4.395	2.376 (0.883) 1.546–4.240	0.399 (0.169) 0.204–0.740
Purr (*n* = 5 inds; 63 notes)	0.025 (0.006) 0.017–0.030	12.156 (3.119) 8.891–16.667	0.257 (0.023) 0.242–0.296	1.486 (0.055) 1.436–1.573	1.228 (0.069) 1.139–1.328	0.352 (0.048) 0.279–0.395
Tooth-chattering (*n* = 2 inds; 15 notes)	0.016 (0.003) 0.014–0.018	10.983 (3.365) 8.604–13.362	1.042 (0.059) 1.000–1.084	19.659 (2.599) 17.821–21.497	18.617 (2.658) 16.737–20.497	3.553 (2.934) 1.478–5.628
Whine (*n* = 11 inds; 63 notes)	0.043 (0.023) 0.015–0.102	–	0.625 (0.196) 0.264–0.897	2.684 (1.294) 1.614–5.633	2.059 (1.242) 1.018–5.051	1.047 (0.343) 0.460–1.593
Wideband whine (*n* = 4 inds; 28 notes)	0.257 (0.122) 0.158–0.409	–	0.990 (0.466) 0.654–1.670	13.132 (6.938) 4.008–20.806	12.142 (6.550) 3.354–19.136	1.678 (0.556) 1.078–2.354
Neighs (*n* = 3 inds; 20 notes)	0.499 (0.176) 0.362–0.697	–	0.288 (0.208) 0.166–0.529	10.982 (7.563) 5.032–19.494	10.693 (7.360) 4.863–18.965	1.362 (0.154) 1.184–1.464
Scream (*n* = 4 inds; 8 notes)	0.280 (0.68) 0.219–0.353	–	0.604 (0.158) 0.424–0.721	16.357 (4.980) 12.573–22	15.753 (4.858) 12.149–21.278	2.501 (0.978) 1.453–3.390
Peep (*n* = 10 inds; 28 notes)	0.046 (0.011) 0.024–0.063	–	3.151 (0.481) 2.492–4.228	16.652 (4.554) 8.083–21.230	13.500 (4.399) 5.224–18.041	4.553 (0.990) 3.477–6.718
Grunt (*n* = 4 inds; 6 notes)	0.041 (0.019) 0.019–0.056	–	0.234 (0.020) 0.211–0.248	2.602 (0.667) 1.867–3.171	2.368 (0.684) 1.619–2.960	0.453 (0.121) 0.323–0.562

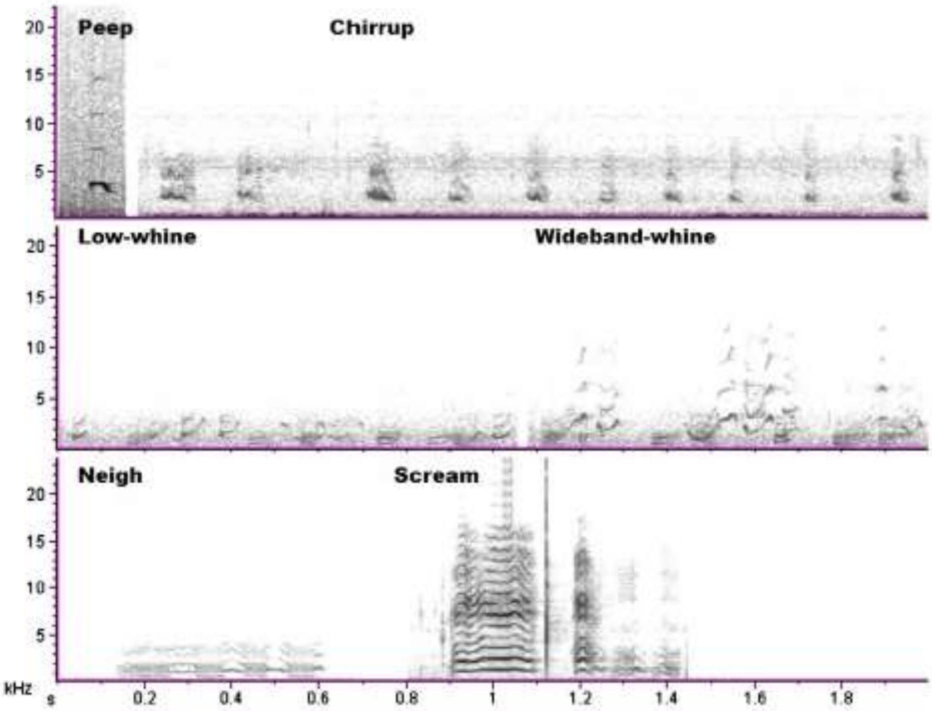

Figure 1. High-pitched and harmonic calls. In the first line a note of peep and a sequence of chirrup. In the middle, the two variants of whine, and on the bottom, a note of neigh and a phrase of scream (2 quite different notes emitted in sequence). Note the presence of non-linear effects in neigh and scream.

from the guinea-pig's whistle. The alarm call (drr) is a low intensity sound, composed of repeated units exactly as described by Monticelli and Ades (2011).

Based on the situations of emission, the calls could be grouped into general functional categories: contact maintaining (sub-sounds), warning/predator intimidation (drr, peep and chirrup, that we proposed was directed to the predator in *C. aperea*), social regulation (whines and neigh; this latter may be a distress call, but we cannot say from our small sample), aversive situation/pain (scream), aggressive encounter/opponent intimidation (grunt, tooth-chattering), sexual encounter (courtship purr), mother–young interactions modulation (tweet and whistle), following Berryman (1976) and Monticelli and Ades (2013). A more detailed description of the structure of calls can be seen in Table 2.

Discussion

The acoustic repertoire of *C. intermedia* described here is composed of the same calls of the *C. aperea – porcellus* clade (e.g. Berryman 1976; Monticelli and Ades 2013), with one added call, the peep. This call, that we name and physically describe here for the first time, had previously been recorded by us from its sister species, *C. magna*, and has a very particular structure that makes it clearly distinguished from any other cavy call (compare the peep note in Figure 1 to the other calls). It was emitted by animals from inside the trap, before being caught by the experimenter in hand, and whenever a cavy escaped from a trap or human hand, after the animal reached the protection of the vegetation (see Table 2). From

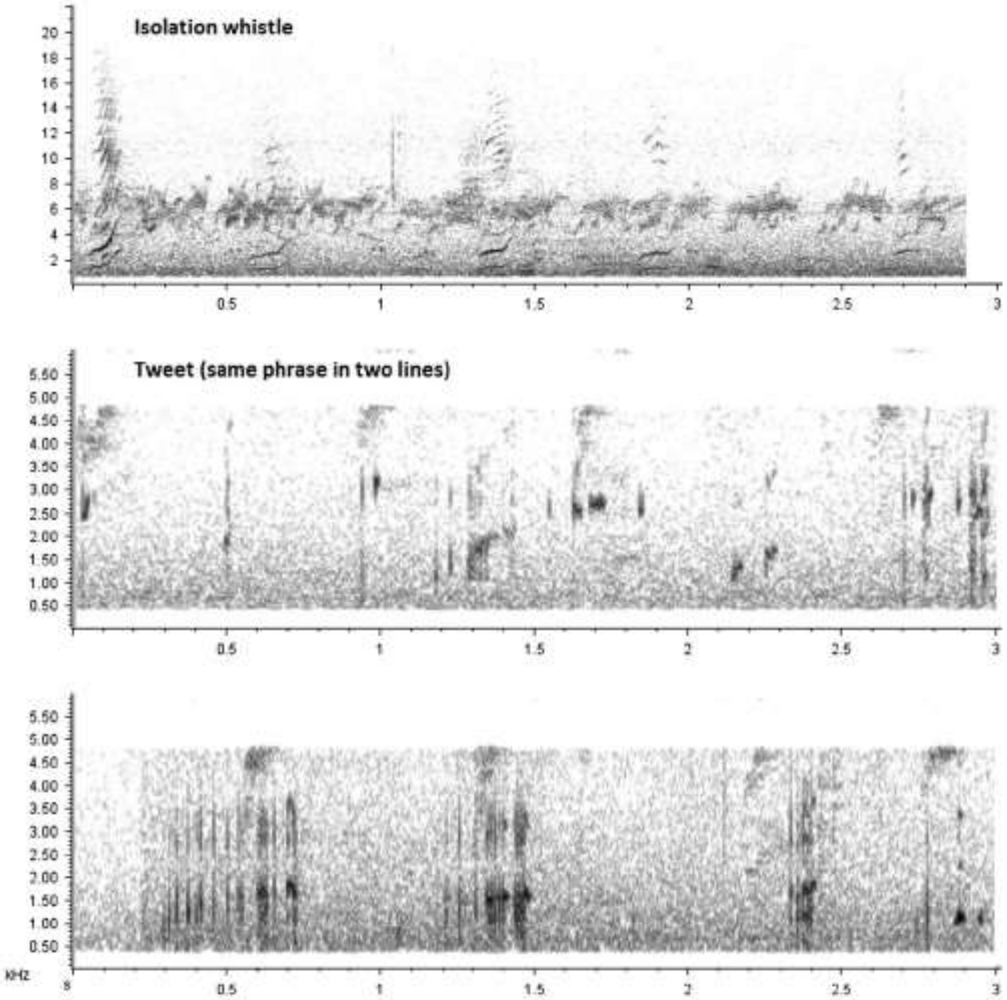

Figure 2. Infant calls. The Isolation whistle (above) and tweet (sequence divided into two lines) are high-pitched harmonic calls usually uttered in phrases.

our experience, we would expect *C. aperea* to chirrup in this situation (Monticelli and Ades 2013). If *C. intermedia* emits both chirrup and peep (Table 2), what is the functional distinction between them? This question remains to be understood and could be investigated in *C. magna* populations in the continental region of Florianopolis. *C. intermedia* is now considered under high risk of extinction and the access to the island is highly restricted by Brazilian federal agencies.

In this sense, the knowledge of the size and composition of the repertoire of *C. intermedia* has its importance. It is possible now to say that a basic communication system of 12 categories is shared by almost all Brazilian cavies (*C. porcellus*, *C. aperea*, *C. intermedia* and *C. magna*, from unpublished data of our own). It is reasonable to expect that even the Andean species *Cavia tschudii* uses this same system, given its genetic proximity to *C. porcellus* (Spotorno et al. 2004) and the stability of behavioural repertoires during domestication (Hale 1969). If this is true, throughout the evolutionary history of the genus, the acoustic repertoire was kept almost unchanged, except for minor structural changes in some of the

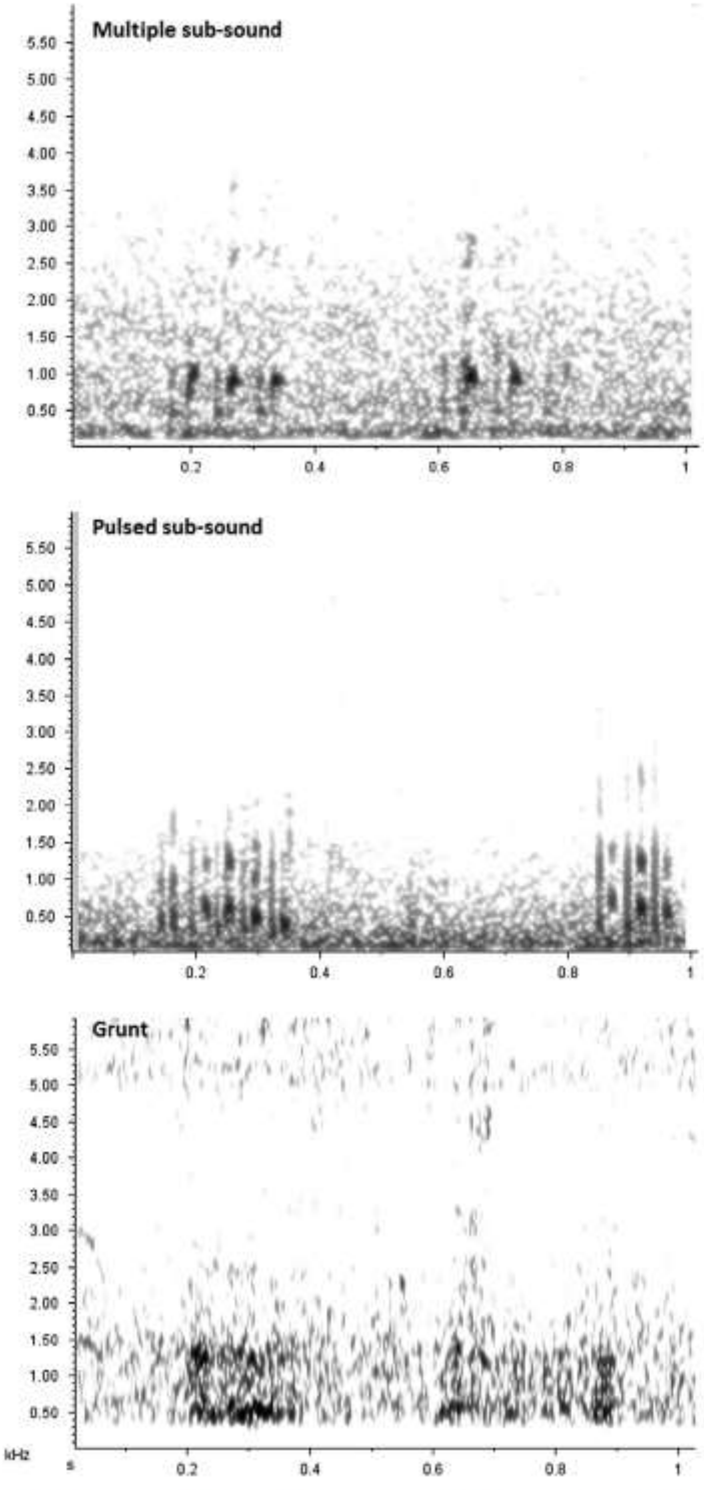

Figure 3. The guttural sub-sounds. They are low-pitched non-harmonic calls. Multiple sub-sounds (top) and pulsed sub-sounds (pru-pru, middle) are issued in two or three notes grouped in a second level unit, a syllable (in both, two syllables represented), and grunt is issued as single notes, eventually repeated, as in the last figure. All espectrograms were generated in 12kHz sampling frequency.

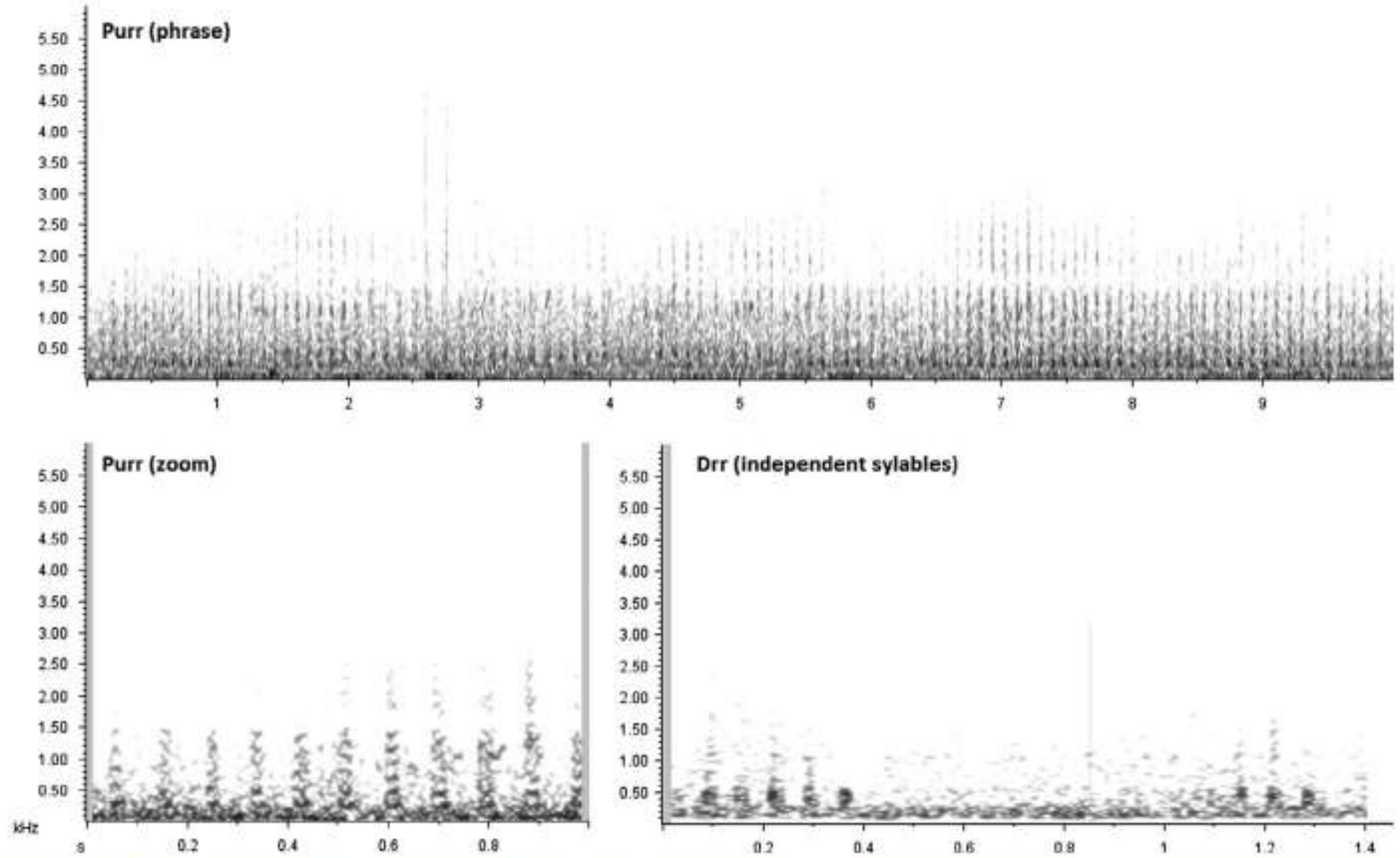

Figure 4. The rhythmical calls, Purr (part of a 20s phrase, above, and in detail bellow) and Drr, in sampling frequency of 12kHz. They are low-pitched and rhythmical emissions of very short pulses that are never uttered singly. The purr is the courtship call of *Cavia* and drr is the short-distance alarm call.

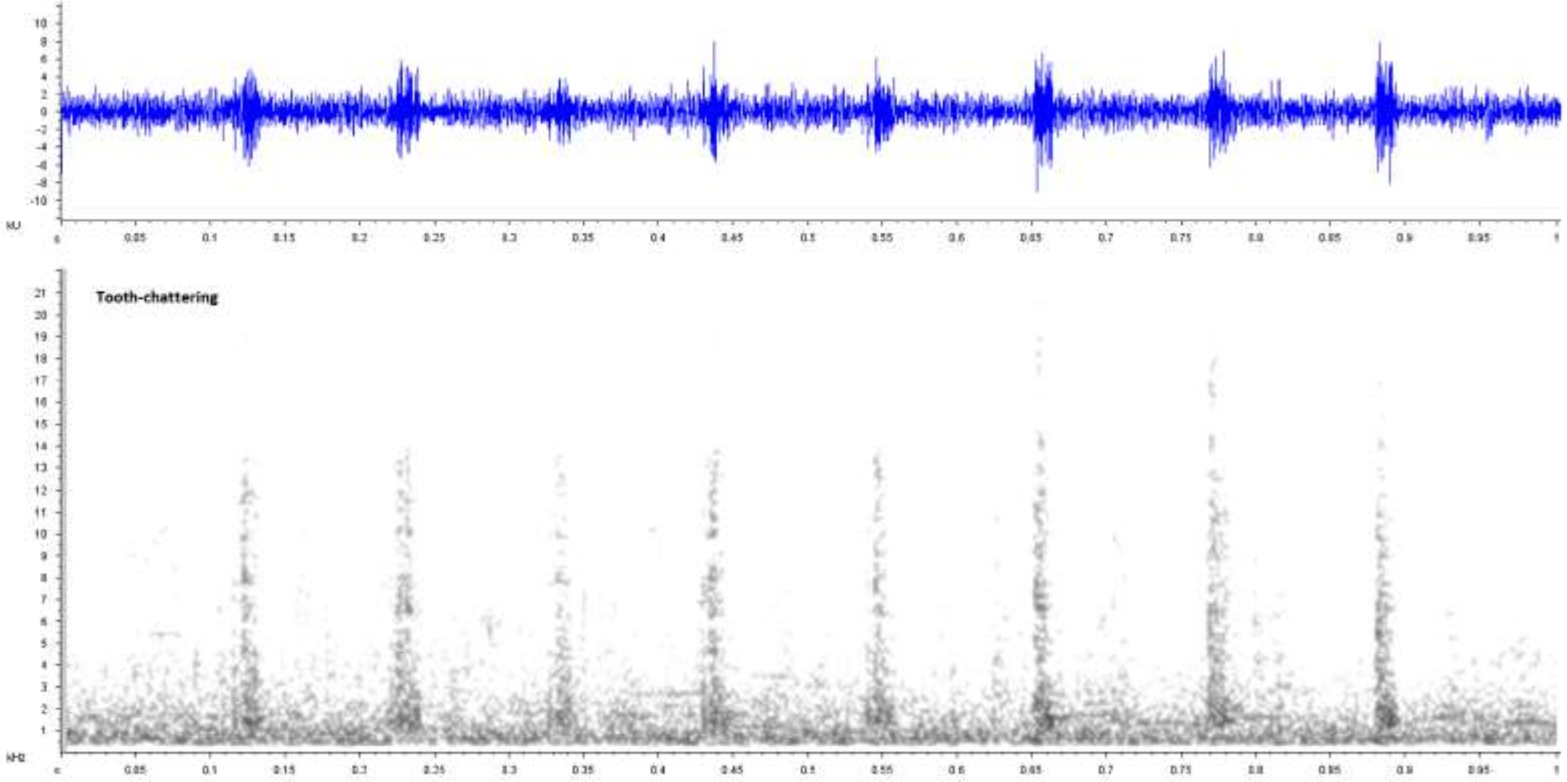

Figure 5. Tooth-chattering, the voiceless call. This sound form of production was precisely described by Arvola (1974) and occurs in a variety of caviomorph rodents.

calls (Monticelli and Ades 2011, 2013). The peep is an extra category that was not in that 12-units basic repertoire. As the last review of cavies' phylogenetic relationships suggests that *C. magna* was the first species that diverged from the common ancestor of the group (Dunnum and Salazar-Bravo 2010), it is more parsimonious to suppose that it may have evolved after the divergence of *C. magna* from the common ancestor.

Contact and close-proximity pacific calls

The contact calls of *Cavia*, i.e. the one that promotes the contact within or between group members keeping cohesion and assuring coordination of the group movements (Caine and Stevens 1990; Marler 2004; Kondo and Watanabe 2009), are the sub-sounds. They occurred in amicable or exploratory encounters of familiar individuals that approached others, moved together, during foraging in the company of others, in sexual encounter and in mother/offspring interactions. Arvola (1974) named it sub-sounds as they are short, nasal monotonous pulse calls. He already noticed that they were uttered singly or in pairs and had a maximum intensity of about 70 dB at 10 cm. All three species, *C. aperea, C. intermedia* and *C. porcellus* emit sub-sounds in the same context, but the domestic ones (whose sub-sounds were previously called chuts, Berryman 1976) may be composed of harmonic notes singly emitted, that only when the movement gets more intense turns to the multiple notes syllables (2 or 3 notes) described here and also before (Monticelli and Ades 2013). The other type, the pulsed sub-sound (that sounds as "pru-pru") is related to fast movement and was emitted while the animals (only wild ones) were moving around their home range. Sub-sounds seems to correspond to Eisenberg's clucks (Eisenberg 1974), that would be one of the basic syllables of hystricomorph acoustic repertoire, that when combined would constitute the complex sounds of all the 17 species of hystricomorph rodents.

Other caviomorph rodents present analogous calls to multiple and pulsed sub-sounds. The Capybara (*Hydrochoerus hydrochaeris*) click call, besides having a different physical structure (it is also a pulse but with a wider frequency band), is also emitted while the group is in movement, supposedly to keep individuals together (Nogueira et al. 2012). Rocky cavies (*Kerodon rupestris*) produce also a sub-sound (named chutter by Lacher 1981) that is similar in context and in physical structure to *Cavia*'s.

Close-proximity non-affiliative calls

The grunt was not described for *C. aperea* because it was grouped together with sub-sounds (Monticelli and Ades 2013). The confusion was due to its rare occurrence and the superimposed with tooth-chattering. It was noted for guinea-pigs, that vocalize in higher amplitude, but only recently heard also in wild species as *C. magna* and *C. intermedia*. The grunt is not uttered in amicable situations; it is an aggressive call, supposedly used to intimidate a rival, simultaneously uttered with tooth-chattering and during agonistic display. The voiceless tooth-chattering is produced by the inter-shock of the incisive tooth (a precise description of this mechanism can be found in Arvola 1974). It is present in most caviomorph acoustic repertoires, always related to aggressive contexts (Eisenberg 1974).

Mother–infant interaction calls

Communication signals between mother and infants in *Cavia* are the isolation whistle and the tweet. As in *C. aperea* and *C. porcellus* (Monticelli et al. 2004; Tokumaru et al. 2004), a sequence of whistles was heard here when an infant was released from the trap and supposedly ran to find his mother inside the brushes. Isolation whistles are also part of capybara's repertoire, not only uttered by infants but also by adult animals separated from the group (Lord 2009; Barros et al. 2011). *C. porcellus* adult individuals do also whistle, but it may be an effect of domestication (Corat et al. 2012). Guinea-pig and *C. aperea* infants also uses a very particular vocalization, that Berryman (1976) labelled using the onomatopoeia "tweet", during ano-genital grooming performed by the mother on her infant. We recorded that here, from a pair of mother-infant caught in a trap, but it was not possible to determine exactly that the mother was licking the infant in that moment.

The distress call

C. intermedia screamed while been caught and in aversive or painful situations, as in other cavies and Caviidae (e.g. capybara and rocky cavy; Lacher 1981; Barros et al. 2011), in some cases interspersed with wideband whines and neighs. Scream and neighs follow the pattern described by Morton (1977) in his Motivational-Structural rules, being tonal and high-frequency and used when animals would be frightened. Morton's theory was tested by August and Anderson (1987), who found a wide variation in some fear sounds related to different motivational states. This gradation pattern of transitory forms was present in *C. intermedia*' s calls, supporting Eisenberg's (1974) theory that this is a common feature of caviomorph rodents (it was also evident in *C. aperea*, Monticelli and Ades 2013; and *C. porcellus*, Coulon 1982).

Scream and neigh had non-linear phenomena, another feature normally related to urgent and intense emotional state (Caldwell and Caldwell 1979; Killebrew et al. 2001), described before in quite different mammals, such as the domestic dog (Volodina et al. 2006) and chimpanzee (Riede et al. 2004), among many other species. Non-linear effects found in *C. intermedia* calls were jump frequency, subharmonics and deterministic chaos supposedly produced by irregularities caused by desynchronization in vocal fold patterns of vibration. It was suggested that it could indicate individual status and motivation (Wilden et al. 1998; Fitch et al. 2002; Schneider and Anderson 2011).

Warning and intimidation calls

Drr is the short-distance alarm call shared by all the other *Cavia* species that was used in the same way by *C. intermedia*, as in the others: it promptly elicits a warning (Rood 1972) or a freezing (Monticelli and Ades 2011) posture. In some cases, animals may even run away after hearing a drr. Generally it is emitted in response to abrupt environmental changes. During this study, we were able to record only one good sample of this call, but heard it other times, even though it seemed to us that it was used in a lower rate than in *C. aperea* and *C. magna* which we studied before. On the island there is no predator of cavies (there is no snake, other mammal or bird of prey living in the island, Salvador and Fernandez 2008a; Furnari 2011). It was already noticed before, when we comparatively

Table 2. Calls structure and situations of emission.

Call categories and gradations	Structure	Behavioural context
1. (Multiple) Sub-sound (sounds like soap bubbles exploding)	Narrowband, short duration and non-harmonic notes, emitted in phrases composed of two or three notes repeated at short intervals	Uttered during peaceful contact between individuals, and whenever two or more individuals approached others or moved together. The sub-adult male Joca uttered this while he was in the experimenters hand to fur discoloration. Once uttered by an infant after a new individual approached the foraging area where it was with another adult. An infant (Manuelito) that was captured with his mother uttered sub-sounds from inside the trap and after that, while he was in the hand of the experimenter
2. Pulsed sub-sound (sound as *pru-pru*)	Narrowband, short duration and non-harmonic notes, emitted in phrases or bursts composed of little groups of notes with short intervals between them	It was more frequently emitted by males than females and we did not observe it in immobile animals. It was uttered by two adult males during handling and fur brushing. Repeated sequences of these notes were associated with faster locomotion in the patrol behaviour (Monticelli and Ades 2013)
3. Peep call	Wideband, short duration and harmonic call, emitted only as single notes. Its harmonics showed different modulations, ranging from straight line to "tilde" shapes	Most commonly uttered by animals released from the trap (once by one that escaped from the experimenters hands), after hiding on the bushes. It also was uttered by animals from inside the trap, before being caught by the experimenter, and by Téo, an adult male that frequently visited the camping area and used to steal corn pieces. When the food was taken away from him, he peeped just after retreating
4. Chirrup	A fast sequence of harmonic notes, longer than the peep and lower in frequency (F0 and maximum frequencies)	Heard and recorded 6 times during *ad libitum* observations of animals in the foraging area, twice (in different days) it happened when one or more animals were slowly approaching the recently inserted microphone with the furry windshield. When it was swayed by the wind, the animals quickly retreated and that may be the cause of the chirrup in at least these two occurrences. All the other chirrups occurred inside the vegetation
5. Whine (social regulation calls)	Harmonic, narrowband and short duration call. It occurred as single notes, phrases or bursts. Usually is preceded by multiple sub-sounds	Whines and neigh were registered during animal handling and during social interactions involving male-female persecution, naso-anal contact, mounting, or as food defence response following an intruder approach. The short note whines were uttered by a male after ear piercing (the wide-band by 2 sub adult males in the same situation) and by a female evading a male. It was commonly heard in foraging, when animals approach each other
Wideband-Whine	It sounds like a whistle, but it is longer and wider in band than the whine. It occurred as single notes or as a sequence of notes interspersed with whine notes in a phrase. There may be intermediate shapes between whine and wideband-whine	
6. Neigh	Two-harmonic phase note with a chaos phase in the middle. Registered only as phrases	The neigh was the most extreme discomfort call uttered by at least 2 females (sub adult and adult) after male-female persecution got more intense, and by a male and another adult animal while evading an attack. In pairing sessions, was emitted by females during interactions with males
7. Scream	Harmonic call, wider band than whine, wideband-whine and neigh. Its maximum frequency almost reaches ultra sounds. It occurred as single notes or phrases. Some notes showed non-linear phases (jump frequency and sub-harmonics)	The scream was only heard by animals at the experimenter hand, during hydrogen peroxide application, or during ear piercing. Possibly it is the distress call of the species

(Continued)

Table 2. (*Continued*).

Call categories and gradations	Structure	Behavioural context
8. Tooth-chattering	Voiceless call produced by shocking of incisive teeth (Arvola 1974). Notes are of very short duration repeated in regular intervals	Shared with all Caviidae rodents. Used in agonistic interactions or even when a male entered the foraging area where another was already present. Also uttered by two males (the sub adult Joca and the adult Alaor) inside the trap, after experimenter approach and being lightly poked with a stick
9. Grunt	Narrowband and non-harmonic call. Its minimum frequency reaches very low frequencies.	It was emitted by aggressive animals simultaneously to tooth-chattering in pairing cage (4 times by one male), once by a male when another one entered the foraging area where he was, and twice by adult males from inside the trap, after the experimenter approach and being lightly poked with a stick.
10. Purr	Pulsed, narrowband, short duration and non-harmonic notes, emitted in rhythmic phrases or burst composed by identical notes in regular intervals	The male stereotyped courtship call of *Cavia*, uttered by males during approaching or following a female; the male exhibits the rumba movement (the courtship display described by King 1956), that is lighter in the wild species. It was produced by two sub adult males (Junior and Joca) during fur brushing and discoloration
11. Drr	Narrowband and non-harmonic call, composed by rapidly repeated and very brief identical notes	Short distance alarm call uttered in response to sudden sounds. It was produced by a sub adult male (Junior) and the adult male Alaor during fur brushing and discoloration.
12. Isolation whistle	Wideband and harmonic notes emitted in a short sequence	We recorded only in video what sounds exactly as the isolation whistle of other cavies. Adults were being observed in foraging area and someone from inside the *Verbesina* bush vocalized
13. Tweet	Low intensity short whistle that has a ./fundamental contour in wide-band spectrograms	An infant close (maybe suckling) to his mother in the trap. In other cavies it is produced by infants days after birth, when being licked by mother in the genital region

studied *C. aperea* and *C. porcellus* (Monticelli and Ades 2011), that it was very hard to obtain a spontaneous drr from a guinea-pig, but we frequently recorded in all the three wild populations. The domestication process seems to have elevated the response threshold to abrupt noise that would promptly frighten wild animals, as was seen in other domesticated species (Price 1984). It was suggested that the insular speciation would produce similar effects to the domestication process (Stamps and Buechner 1985; Blumstein 2002). In fact at Moleques do Sul the predation rate is so low that alarm responses may have become less and less useful, progressively decreasing its rate of emission until hitting the low level of response described in *C. porcellus*. The island effect on alarm emission was described by Blumstein and Daniel (2005) in island macropodid marsupials (kangaroos, wallabies and their relatives). They demonstrated a loss of group size effects in these animals that means a reduction in anti-predation behaviours and an increase in foraging time, i.e. the insular macropodid marsupial species are less vigilant than mainland species.

In contrast to drr, and even to chirrup (heard only in one out of four field trips), that we would expect to happen more frequently, we found a high rate of peep call emission. We cannot currently explain that, as discussed before. But it is the chirrup, with its high-pitch and quickly repeated pattern, that seems analogous to the long distance alarm whistles or trills of other species (*K. rupestris:* Lacher 1981; marmots: Blumstein and Armitage 1997a, 1998; and *S. cyanus*: Veitl et al. 2000). All these calls, peep, chirrup, whistles and the trill, would fit Marler and Hobbett's description of an alarm call (Marler and Hobbett 1975): high in pitch and extremely short, which makes it difficult to locate the sender. Both peep and chirrup were most frequently emitted after the animal hid in the vegetation (not in the case of Teo, the cavy that was used to stealing corn in the camping area), challenging the mobbing or intimidation hypothesis that it would be used to communicate with the predator. The different functions of peep and chirrup in *Cavia* remain to be explained.

The courtship call

As expected, the purr is also present in *C. intermedia* and accompanies the *rumba* display (King 1956). More than a courtship call, the purr may express arousal and/or sexual motivation (Monticelli and Ades 2011). The simple approach of a female by a male may be accompanied by a purr. The female responds emitting a sequence of multiple sub-sounds, which gets faster in rate of emission and then is replaced by whines, neighs and even short screams. *C. intermedia* polygynous mating system is based in direct female defence with no resource item offer. Males may be selected for their qualities (e.g. Runaway selection – Fisher 1930; Handicap principle – Zahavi 1975) that they exhibit to the female during the courtship display (Monticelli and Ades 2011). In fact, cavies are one of the few mammals that show a courtship call and a visual display that is more common in birds and anurans (Monticelli and Magrini 2012). Purr could have been selected, as suggested in Monticelli and Magrini (2012), by its effects of calming down the female caused by its efficient pattern, pulsed, low pitch and continuous (Lacey 2000).

C. intermedia's *repertoire in a comparative perspective*

The conservative characteristic of the acoustic repertoire of close related species living in distinct ecological conditions was already noticed by the meta-analysis of Eisenberg (1974) of caviomorph rodents acoustic repertoires. Besides *Cavia* species, Capybara (*H. hydrochaeris*) (Barros et al. 2011), rocky cavy (*K. rupestris*) (Alencar 2011 – unpublished data; Lacher 1981), and a more distant caviomorph species, the Echimidae spiny rats of the Genus *Trinomys* (Manaf and Spinelli Oliveira 2000; personal observation) keep a large acoustic repertoire, with similar number of calls and usage (behavioural context of emission). Similar evolutionary histories have been supposedly subject to similar selective forces, making acoustic traits representative of phylogenetic lineages (Meyer et al. 2012). In Southern African Gerbils (*Gerbillinae*) (Dempster and Perrin 1991), the six species use the same ultrasonic call during male–female encounters, with minor structural differences (in note duration and in maximum and minimum frequencies). This conservative characteristic of the acoustic repertoire of closely related species was also seen in other species, even outside rodents, as in *Lynx* species (Peters 1987) and dingos (*Canis lupus dingo* compared to *C. lupus*, Déaux and Clarke 2013).

The acoustic repertoire seems not to evolve as a single unit; it may better be seen as a system of communication signals that respond independently to selective forces and to genetic drifts (Phillips and Johnston 2009). Even being evolutionarily flexible, the acoustic system (i.e. the species repertoire) still retains phylogenetic constraints, probably related to the fact that the sound production mechanism is the same. Thus, as any biological trait, the evolution of acoustic signals may not be explained by a single selective pressure (Ryan and Brenowitz 1985).

A great selective force on the acoustic behaviour of the species may be sociality. Animals that live in large and stable social groups may need a more variable signal system, with gradation forms, to communicate and regulate others behaviour (that may even be cooperative partners) than animals living in small or no-social groups (Blumstein and Armitage 1997b; Freeberg et al. 2012). Following this idea, Freeberg and colleagues (2012) proposed the "social complexity hypothesis" to explain the idea that complex social groups are related to complex acoustic repertoires. Vocal complexity can be expressed in features as size and/or density of social groups and vocal complexity is usually characterized in relation to repertoire size and the diversity of contexts in which vocal signals are emitted (Krams et al. 2012). The caviomorph rodents are a good model to test this theory (Francescoli et al. 2016). Among them are social species such as the cururos (*S. cyanus* – Veitl et al. 2000), the degu (*Octodon degus* – Nakano et al. 2013) and the plain vizcachas (*Lagostomus maximus* – Branch 1993) that have a repertoire size similar to cavy species and more extensive than solitary species. Also the mole-rats, quite different species among rodents, (Bednářová et al. 2013); and the sciurognathi marmots (Blumstein and Armitage 1997b) were shown to support the theory of social complexity. Outside rodents, it is also possible to observe these features in birds (e.g. Paridae family – Krams et al. 2012) and primates (McComb and Semple 2005).

In this sense, the present description raises the knowledge about wild cavies' natural lives and offers insights in the discussion of acoustic communication signals evolution. In contrast with the domesticated cavy (*C. porcellus*), the wild cavies repertoire had not been exhaustively studied. Acoustic studies, such as the present one, improve knowledge about the phylogenetic relationships among species of the genus *Cavia*.

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Apêndice G _ *Monografia: Descrição comparativa dos repertórios acústicos dos preás* Cavia magna e Cavia intermedia

Os resultados abaixo resumidos, são parte do trabalho de conclusão de curso de Paula Verzola Olivio, que deu continuidade à linha de estudos de cobaias e preás. Hoje, a Paula está desenvolvendo seu doutorado, também com apoio da FAPESP. Neste trabalho inicial, ela analisou as gravações que eu havia coletado durante o pós-doc, na Ilha Moleques do Sul, em Santa Catarina (preás *C. intermedia*). Essa população deve ter se originado a partir da separação da população de *C. magna* que ocorria ali, quando o nível do mar subiu, há estimados 8 mil anos (). Pelo número reduzido e a alta taxa de endogamia, a população está em risco de extinção.

No projeto de IC, fomos capturar a espécie que deu origem a essa, em Florianópolis, *C. magna*. A Paula fez uma descrição comparativa do repertório dessas duas espécies, tendo como base a descrição que eu havia feito do repertório das outras cavias. A monografia foi apresentada ao depto de Biologia da FFCLRP, em 2014. Os dados de *C. magna* ainda não puderam ser publicados, por dificuldades que tivemos com parte da amostra obtida. Publicamos só a descrição do repertório da espécie da ilha. O processo FAPESP foi o 13/06606-8, da linha de Bolsas no Brasil - Iniciação Científica.

Olivio, Paula Verzola
"Descrição do repertório acústico dos preás do Sul, *Cavia magna* e *Cavia intermedia* e comparação da diversidade e da forma dos sinais entre espécies brasileiras do gênero *Cavia*".
Ribeirão Preto, 2014.
84 p.
Monografia apresentada à FFCLRP/USP. Área de concentração: Biologia Geral.
Orientadora: Profa. Dra. Patrícia Ferreira Monticelli
1. Bioacústica. 2. Filogenia. 3. Repertório acústico. 4. Comportamento animal.

RESUMO

OLIVIO, P. V. "**Descrição do repertório acústico dos preás-do-sul, *Cavia magna* e *Cavia intermedia*, e comparação da diversidade e da forma dos sinais entre espécies brasileiras do gênero *Cavia***". 2014, 84 p. Monografia – Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brasil.

Através da comunicação, os animais são capazes de modular suas relações intra- e interespecíficas. Entre os grupos que fazem uso deste canal acústico, cada um deles pode mantem uma estrutura definida do som, que pode auxiliar no reconhecimento específico. Espera-se que duas espécies atuais derivadas de um mesmo ancestral mantenham características acústicas em comum. No presente trabalho, pretendeu-se testar a hipótese de que as espécies do gênero *Cavia* mantiveram a diversidade e a estrutura de emissão dos chamados de seus repertórios vocais ao longo da evolução do gênero, tendo apenas variado as medidas dos parâmetros acústicos entre as espécies. Para isso, foi descrito o repertório acústico ainda desconhecido das espécies *C. magna e C. intermedia*. Em seguida, os sinais encontrados para estas espécies foram comparados de forma quali-quantitativa com aqueles já descritos para as espécies *C. porcellus* e *C. aperea*. Assim, foi possível observar a manutenção da diversidade e da estrutura geral (macro) dos chamados nestas quatro espécies estudadas, com 10 categorias comuns e o pio como exclusividade das espécies do sul. As amostras fora comparadas através de analise de variância e comparações dos grupos dois a dois. A classificação multivariada (análise discriminante) do chamado de corte colocou as espécies do sul mais próximas entre si do que em relação às outras duas, que, por sua vez, estavam mais próximas entre si. Além disso, comparando os parâmetros acústicos das quatro espécies, *C. porcellus* apresentou os chamados mais longos e *C. magna/C. intermedia* apresentaram os valores mais altos de frequência. A maior proximidade entre as espécies do sul e a presença exclusiva do pio sustentam a hipótese da ancestralidade comum dessas espécies enquanto a manutenção geral do tamanho e diversidade do repertório reforçam a constância do repertório acústico das espécies ao longo da evolução.

Palavras-chave: Biacústica, *Cavia*, filogenia, repertório acústico.

Apêndice H _ *Bioacoustics of domestication: alarm and the courtship calls of* Cavia

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BIOACOUSTICS OF DOMESTICATION: ALARM AND COURTSHIP CALLS OF WILD AND DOMESTIC CAVIES

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ABSTRACT

Comparisons of wild (*Cavia aperea*) and domestic (*C. porcellus*) cavies promote an understanding of the physiological and behavioral effects of domestication. The richness and peculiarities of *Cavia* acoustic repertoires encourage the use of this model for testing how domestication alters repertoires and the physical structure of calls. We present a comparison between alarm and courtship calls of domestic and two populations of wild cavies from different geographic regions, one of them with a short-term captivity history of 25 generations. We found significant differences between domestic and wild cavies in both calls, particularly in temporal parameters, and only spectral differences between two wild populations in alarm calls. There were also differences in the frequency of emission of calls: alarm calls were more frequent in the wild and courtship calls were more frequent in the domestic species. Our results suggest that domestication has influenced the temporal parameters of both alarm and courtship calls of *C. porcellus*, but not the spectral parameters that, instead, may be influenced by environment or population factors.

Keywords: alarm call, *Cavia*, courtship call, domestication, guinea-pig.

INTRODUCTION

Caviomorph rodents (New World Hystricognaths) constitute an interesting group for the study of the evolution and ecology of acoustic communication. Their intense social life and foraging activities require coordination of activities and movements, providing conditions in which acoustic signals may offer an advantage in terms of the transmission of information about position, identity and motivational conditions of senders (Lillehei & Snowdon 1978; Cheney & Seyfarth 1980; Smith *et al.* 1982; Newman & Goedeking 1992; Boinski *et al.* 1994; Mateo 2003; Tokumaru *et al.* 2004; Ebensperger & Blumstein 2006; Lingle *et al.*

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*Patricia Ferreira Monticelli is now at Depto Psicologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto – Universidade de São Paulo (Av Bandeirantes, 3900, Monte Alegre, Ribeirão Preto, SP-14.094-613), and can be contacted on pmonticelli@ffclrp.usp.br ecotypic differences present in the same wild species. Our research also represents the first study of *C. aperea* vocalizations.

MATERIALS AND METHODS

Subjects

Domestic Cavies (DC) from a heterogeneous stock of animals were maintained in the animal husbandry facility of the Psychology Institute, University of São Paulo, in 58 × 60 × 100 cm polypropylene cages. Water, rabbit chow and fresh vegetables were offered once a day, except on the weekends.

Wild cavies from Argentina (WCa) descended from a stock captured in the Buenos Aires region of Argentina in 1995 and raised for about 25 generations in the animal husbandry facility of the *Institut für Neuro- und Verhaltensbiologie*, University of Münster, bred without goal-directed selection (Künzl & Sachser 1999; Künzl *et al.* 2003). The animals were housed in groups of one male and up to four females in 1-square-meter enclosures, in indoor and outdoor pens. Commercial guinea pig diet, rolled oats, and water were provided ad lib. The diet was supplemented regularly with fruits and hay (Künzl & Sachser 1999). The WCa population, although in captivity for some 5 to 10 years (Künzl & Sachser 1999), showed no statistical difference in behaviour or endocrine parameters when compared to wild captured animals from their original locality (Künzl *et al.* 2003).

Wild cavies from Brazil (WCb) were captured in the wild, on a rural property near the town of Itu, State of São Paulo (Monticelli 2000). The animals were kept in 1-square-meter enclosures in an outdoor pen close to the capture site, with one male and one or two females in each enclosure. WCb individuals were identified through skulls and fur as *C. aperea* at the Museu de Zoologia de São Paulo (wild specimens identification numbers: MZUSP32294, MZUSP32295, MZUSP32296, MZUSP32297, MZUSP32299, MZUSP32302, MZUSP32303, MZUSP32308, MZUSP32309, MZUSP32300, MZUSP32310, MZUSP32311, MZUSP32312, MZUSP32313, MZUSP32304, MZUSP32301; domestic specimens identification numbers: MZUSP32307, MZUSP32315).

At all animal husbandry facilities cavies were exposed to a 12:12 L:D cycle: photoperiod 0700-1900 h; temperature 20±2°C; relative humidity about 60%. The floors were covered with wood shavings. *Cavia aperea* groups (WCa and WCb) were provided with nest boxes in their cages so that they could be hidden from human presence (without these, they would throw themselves at the wall and get injured; this never happens with the domestic species). Domestic cavies were individually identified through natural colouring of the fur: wild cavies were identifyed by selective shaving or dyeing the fur with hydrogen peroxide (Künzl & Sachser 1999).

Recordings and sound analysis

We obtained quality recordings of alarm and courtship calls from the highest possible number of individuals from each group. Following Berryman (1976) and Eisenberg (1974), we recorded vocalizations: (1) from animals in groups without handling, and (2) pairing individuals from different housing groups, for 10 minutes in a test-cage (50 × 50 × 30 cm) in the lab, as follows: FF – pairing of females (DC= 10 pairs, WCa= 9 pairs, WCb= 5 pairs), FM – pairing of male with female (DC = 10 pairs, WCa= 10 pairs, WCb= 6 pairs) and MM – pairing of males (DC= 8 pairs, WCa= 7 pairs, WCb= 4 pairs), totaling 260 minutes of recording for wild cavies and 280 for domestic cavies in these pairing sessions

All procedures followed ASM guidelines for the ethical use of animals in research (Gannon & Sikes *et al.* 2007).

Sound recordings were made using a Sony TCD-D8 professional DAT recorder (sampling frequency: 48 kHz; frequency response 20-24.000 Hz ± 1.0 dB; dynamic range >87 dB) and a hypercardioid directional microphone (Sennheiser ME 67 with K6 powering module and bass roll-off filter switch on). Spectrographic analyses were carried out on a personal computer using Avisoft SASLab 3.4 (Raimund Specht, Berlin). Calls were transferred from DAT to the Avisoft from the phono/line out port of the DAT into the computer via an Sound Blaster Audigy A/D converter (sampling rate 48 kHz, 8 bits).

Alarm and courtship calls constitute a sequence of *pulses* (a continuous tracing along the temporal axis of a sonogram) grouped in *phrases* (a group of pulses separated from other similar groups by a time interval greater than any time interval separating the units within a phrase) and, eventually, grouped in *bouts* (1 or more phrases separated from other similar groupings by a time interval greater than that separating the phrases) (Struhsaker 1967).

Acoustic parameters were measured in a spectrogram window (FFT 256 Hz, frame size 100%, Hamming windowing function) after adjusting the sampling frequency to 5 kHz. We measured: (1) *phrase duration*, (2) *number of pulses* per phrase, (3) average *pulse duration*, (4) average *interpulse interval duration*, (5) *rhythm* (number of pulses per second), (6) *minimum frequency* (lower limit of the 1st frequency band), (7) 1st *band maximum frequency* (upper limit of the 1st frequency band), (8) *maximum frequency* of the phrase, (9) *dominant frequency* (frequency with highest energy concentration in a phrase); (10) *number of reinforced bands* above the dominant frequency; and (11) *number of phrases per bout* in courtship call. For courtship calls that occurred in bouts, *bout duration* and *interphrase interval duration* were also measured and presented in the descriptive analysis.

Analysis of call structure

An initial descriptive analysis of call structure was conducted for each group based on the measurements of the acoustic parameters of the emissions produced by adult individuals, with sexes pooled for drrr calls (purr were emitted just by males). Five measures of typical examples of each acoustic parameter were taken from five different calls (phrases of drrr or purr) from each individual. The average of these five measures per parameter per individual was obtained as a representative case. The number of cases obtained were: alarm call: DC = 14, WCa = 13, WCb = 7; courtship call: DC = 8, WCa = 8, WCb = 2. The smaller sample of calls obtained from WCb is a product of the smaller number of individuals in this group. Given that the number of courtship call cases for WCb was insufficient, these data were not considered in the quantitative analyses.

Comparison between groups

The difference among groups (DC × WCa × WCb) in terms of the parameters of the alarm call were evaluated using univariate variance analysis (SPSS 7.5 for Windows 2003, SPSS Inc., Chicago, Illinois), with $p = 0.01$, and pairwise contrasts through the post-hoc Tukey test. For the courtship call, the comparison was made between DC and WCa only by univariate variance analysis. The use of parametric analysis was made after verification of the normal distribution of the acoustic parameters ($p<0.05$ in Kolmogorov-Smirnov Normality Tests) and the homogeneity of variance.

Comparison between sexes

To test whether there was a difference between alarm calls emitted by males and females (DC × WCa), we used a univariate variance analysis (SPSS 7.5) with two factors, sex x group. The small number of recordings obtained from WCb precluded their inclusion in this analysis.

Discriminant analysis

For a multivariate analysis that considered the set of parameters of the signals for classification of cases into groups, we used a discriminant function analysis for each vocalization type – *drrr* and *purr* (Smith *et al.* 1982; Jorgensen & French 1998; Rendall *et al.* 1998; Jouventin *et al.* 1999; McCulloch *et al.* 1999; Ilmann *et al.* 2002; Tokumaru *et al.* 2004; Aubin *et al.* 2007). We used the program SPSS 7.5 and adopted the stepwise method with probability of F to enter less than or equal to 0.01.

RESULTS

Alarm call (*drrr*)

Structural characteristics

The alarm call of the domestic and wild cavies is a relatively low intensity signal composed of rapidly repeated, very brief and broadband units (Figure 1). The average values and standard deviation of the acoustic parameters of typical alarm calls of the domestic and wild cavies, as well as the significant differences between groups (post hoc Tukey test, p<0.01) are presented in Table 1.

The ANOVA revealed significant differences between the groups in 9 of the 10 parameters analyzed: number of pulses ($F_{2,32}$ = 5.6; $p<$ 0.01), pulse duration ($F_{2,32}$ = 36.9; $p<$ 0.001), interpulse interval duration ($F_{2,32}$ = 19.5; $p<$ 0.001), rhythm ($F_{2,32}$ = 48.2; $p<$ 0.001); minimum frequency ($F_{2,32}$ = 22.1; $p<$ 0.001), 1st band maximum frequency ($F_{2,32}$ = 36.3; $p<$ 0.001), number of reinforced bands ($F_{2,32}$ = 6.9; $p<$ 0.01), maximum ($F_{2,32}$ = 9.0; $p<$ 0.001) and dominant ($F_{2,32}$ = 6.8; $p<$ 0.01) frequencies. Only phrase duration did not differ significantly between the 3 groups ($F_{2,32}$ = 0.815; $p>$ 0.05).

In the *posthoc* comparisons using the Tukey Test (Table 1) a large number of differences in the acoustic structure were found in the comparison between DC and WCb. They differed in all the parameters except maximum frequency and number of reinforced bands. DC and WCa differed in the duration of pulses and interpulse intervals, rhythm, number of reinforced bands and maximum frequency.

DC differ from both the populations of wild cavies (WCa and WCb) in temporal and spectral parameters of the *drrr*. *Drrrs* of DC had average longer phrases, pulses and intervals between pulses, slower rhythms and lower number of pulses per phrase. Minimum frequency, 1st band maximum frequency and dominant frequency were on average higher and there were a lower number of reinforced bands.

WCa and WCb differed between each other in only spectral parameters: minimum and maximum frequency and 1st band maximum frequency (Table 1).

In the discriminant analysis, all of the acoustic parameters were considered, but the important variables for the reclassification of the emissions in their original groups (DC, WCa, WCb) were rhythm (F = 28.049; lambda = 0.283) followed by the 1st band maximum frequency (F = 22.884; lambda = 0.249). The discriminant function analysis yielded an average rate of correctly classified selected calls of 95.07%. Figure 2 illustrates the conspicuous separation of analyzed alarm calls according to the three groups. The discriminant function analysis revealed clear assignment of calls to the respective species.

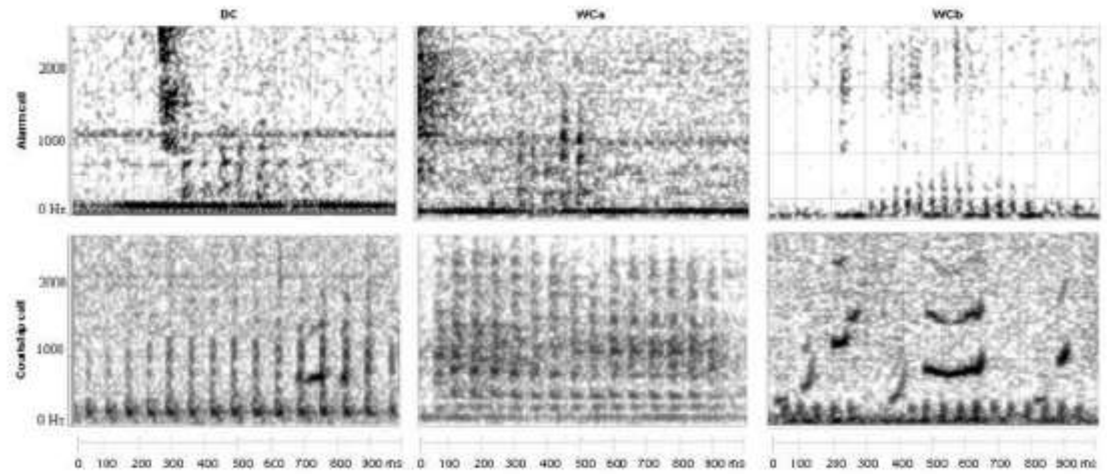

Figure 1. Typical alarm (*drrr*) and courtship (*purr*) calls of domestic (DC) and wild cavies from Argentina (WCa) and from Brazil (WCb). In each sonogram, frequency is plotted as a function of time.

TABLE 1

Mean values (SD between brackets) of the parameters of alarm calls (*drrr*) of domestic cavy (DC), wild cavies from Argentina (WCa) and Brazil (WCb). Temporal parameters in milliseconds and spectral parameters in Hz. Significant post-hoc Tukey tests indicated in the last column.

	DC	WCa	WCb	Post-hoc tests (p<0.01)
Phase duration	337(104)	300(52)	307(61)	
Number of pulses	5(1)	6(1)	7(2)	DC<WCb
Pulse duration	44(6)	32(3)	29(3)	DC>WCa; DC>WCb
Interpulse interval	25(6)	17(2)	17(2)	DC>WCa; DC>WCb
Rhythm	16(2)	22(2)	24(2)	DC<WCa; DC<WCb
Minimum frequency	187(44)	160(43)	73(19)	DC>WCb; WCa:WCb
1st band maximum frequency	320(65)	317(44)	142(62)	DC>WCb; WCa>WCb
Maximum frequency	852(401)	1342(459)	650(216)	DC<WCa; WCa>WCb
Dominant frequency	289(59)	268(83)	175(70)	DC>WCb
Number of reinforced bands	3(2)	5(2)	5(1)	DC<WCa

showing that the measured acoustic parameters provided reliable information for identifying and discriminating the three samples.

The analysis of variance with two factors, sex (male n=11; females, n=10) × group (DC, n=10; WCa, n=11), did not reveal sex differences (all $p > 0.05$), but indicated significant differences between the domestic and the WCa wild cavies concerning the length of the phrase ($F_{1,21} = 25.06$; $p < 0.01$) and the rhythm ($F_{1,21} = 31.97$; $p < 0.01$).

Context of emission

The behavioral context of emission of the *drrr* confirmed the defensive nature of the call. In both species, the call occurred in response to sudden environmental changes, most commonly sounds, and in response to social stimuli such as the introduction of a non-familiar animal in the colony or sudden movements of opponents during agonistic encounters. In the wild cavies, the *drrr* also occurred as a response to short bouts of *song* (a high-pitched signal described as "the purest voiced oral vocalization of the guinea-pig" (Arvola 1974, p. 90) which is apparently used to provoke silence and immobility in other animals; this vocalization was also found and sonographically described in *C. aperea* by Monticelli (2005) and during environment exploration. *Drrr* was one of the most frequent calls of the wild cavies – as it was registered at least once, in almost all recordings sessions – and one of the rarest of the domestic cavy – as it was registered in only three DC pairing sessions. Sometimes we had to evoke alarm calling in DC using a brief and intense sound produced by clicking fingers.

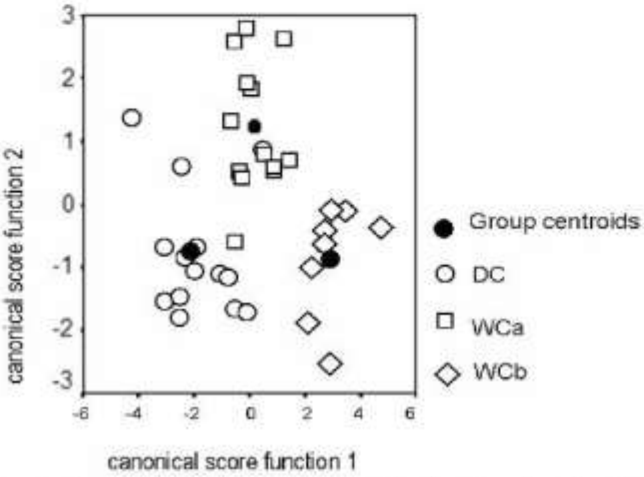

Figure 2. Scatterplot of the canonical variates based on the discriminant analysis of the acoustic parameters of the alarm calls of domestic cavy (DC), wild cavies from Argentina (WCa) and Brazil (WCb). Geometric symbols represent the average value of an analyzed alarm call and different symbols represent different groups. Filled circles indicate the group centroids.

Drrr was at times emitted in bouts only by the wild cavies, even if the triggering stimulus was not repeated. In DC *drrr* occurred only as singular emissions and animals did not take more than 2 or 3 seconds to go back to their previous activity after emission.

We registered 29 singular *drrrs* (5 emissions by females and 24 from males) in 28 sessions of pairing of DC. In this species the call of one animal never provoked a vocal response in the other. We registered 42 *drrrs* (24 emissions of females and 18 of males) in 26 sessions of pairing of WCa, 10 of which were bouts and 32 were singular emissions. During pairings, one of the wild cavies would vocalize after the other, which suggests a possible social contagion of the calls. In WCb cavies, singular *drrrs* were registered three times during the 15 pairing sessions.

Courtship call (*purr*)

Structural characteristics

The courtship call, like the alarm call, is a low intensity broadband and pulsed signal, but has a lower frequency, and its phrases and bouts are usually longer (1-5 phrases may be present and a bout can reach 10 seconds; Figure 1). The pulses are brief and identical, as in the *drrr*, and their rhythm (number of pulses per second) are positively related to the velocity of the courtship display performance, called *rumba* (a slow approach with a rhythmic oscillation of the hindquarters laterally; King 1956; Rood 1972).

The ANOVA that compared acoustic parameters of DC and WCa *purrs* revealed a significant difference in 4 of the 11 parameters analyzed (Table 2): pulse duration ($F_{1,14}$ = 15.2; p=0.002), interpulse interval duration ($F_{1,14}$=8.5; p=0.011); rhythm ($F_{1,14}$=25.6; p=0.000); and 1st band maximum frequency ($F_{1,14}$=10.7; p=0.006).

The *purr* of the WCa is similar to that of DC, but it is generally more fragmented: the bouts are on average shorter in duration and have more phrases that are shorter than those of DC. Those breaks in *purr* emissions occur when the emitter pauses courtship and performs anti-predator defensive behaviors (rearing and scanning around aparently attending to environmental stimuli). As in the alarm call, the courtship call of domestic cavies is also slower and has pulses and interpulse intervals on average longer than the wild species (Figure 1; Table 2). In relation to the spectral parameters, WCa have lower average values than DC, except in the minimal frequency, in which they practically do not differ.

In the discriminant analysis, just as for the alarm call, the rhythm is the strongest variable and sufficient for discriminating between groups (F= 26.47 and Wilks lambda = 0.33). The discriminant function analysis yielded an average rate of correctly classified selected calls of 87.5%.

Context of emission

Both DC and WCa males emitted *purr* during courtship of females, but also during a social investigation period (dominant animals – those most aggressive in their familiar groups and that had preferential access to food and nest – investigate the anal-genital region, conduct a *rumba* display and vocalize for non-familiar individuals introduced in their cages, upon pairing, or upon returning to their own colonies).

In DC, WCa and WCb, in the colonies or in the pairings, the *rumba* was always accompanied by the *purr* call. Since domestic cavies engage in courtship more frequently than wild cavies, even in the case of the non-receptive females, they also emitted *purrs* more frequently.

DISCUSSION

The common architecture found in DC, WCa and WCb calls (low frequency and low intensity calls, formed by broadband pulses

TABLE 2

Mean values (SD between brackets) of the parameters of courtship calls (purr) of domestic cavy (DC), wild cavies from Argentina (WCa) and Brazil (WCb). Temporal parameters in milliseconds and spectral parameters in Hz. Significant differences are indicated in the last column by the direction of inequivalence for the contrasts.

	DC	WCa	P<0.01
Number of phrases per bout	2 (1)	3 (2)	
Bout duration	4500 (2410)	3600 (2650)	
Interphrases interval	400 (120)	310 (140)	
Phrase duration	2819 (2259)	1842 (1006)	
Number of pulses	46 (3)	39 (34)	
Pulse duration	41 (3)	34 (5)	DC>WCa
Interpulse interval	20 (4)	14 (5)	DC>WCa
Rhythm	16 (1)	21 (3)	DC<WCa
Minimum frequency	28 (8)	30 (30)	
1st band maximum frequency	385 (50)	330 (40)	DC>WCa
Maximum frequency	1816 (490)	1587 (428)	
Dominant frequency	189 (61)	135 (61)	
Number of reinforced bands	3 (2)	5 (2)	

repeated at equal intervals, 50% shorter than the pulses, grouped in phrases of variable duration) confirm the phylogenetic proximity between domestic and wild cavies and indicate the preservation of ancestral characteristics in the domestic species, as found in signals of other domesticated animals (Güttinger 1985; Price 2002).

The *drrr* and the *purr* in the domestic cavy has also been preserved with respect to the emission context. The first is a short distance alarm call – not modulated and of low intensity – that evokes, in both species, a posture of attention or freezing. The other is the call that accompanies the courtship display in the two species, but is also emitted in a number of other social situations, indicating dominance or excitation. These results corroborate others that indicate the preservation of the initial function of signals in domesticated species (Hale 1962; Desforges & Wood-Gush 1976; Price 1984; Miller & Blaich 1986; Price 2002; Jensen 2009).

If we adopt the genetic species concept (Bradley & Baker 2001), *C. aperea* is conspecific to *C. tschudii* and a wild ancestor very closely related to *C. porcellus*. This assumption permits us to attribute the differences between these species to selective effects resulting principally from domestication. The DC and WCa individuals studied were all born and raised in the laboratory and have an analogous breeding context: the differences in their calls can thus not be attributed to factors of past experience. Our results also indicate the existence of differences between the two populations of *C. aperea* (WCa and WCb), which may stem from the selective effects that distinguish populations of different regions, as habitat structure and the nature of interactions with predators (Slobodchikoff *et al.* 1998), and also from captivity experience effects. The WCa population were composed of wild-caught cavies bred in captivity for 25 generations (short-term captive), and thus tested indoors like the domesticated cavies (long-term captive). The WCb animals had very little time in captivity: all, except for two males, were animals born in nature and brought into captivity. This potential effect of indoor housing and testing could have produced similar effects in particular aspects of *drrr* calls in the spectral parameters in DC and WCa (as suggested by the intermediate values in minimum, 1st band maximum and dominant frequencies shown by WCa, between DC and WCb, as shown in Table 1). But it must not be confused with domestication effect (Price 1984; Grandin & Deesing 1998; Ruiz-Miranda & Kleiman 2002). Although rapid changes may have occurred with lab breeding of cavies it must be completely different from being domesticated through the domestication process that produced the guinea-pigs 6,000 years ago. In addition, Künzl *et al.* (2003) found no statistical differences in behaviour or endocrine parameters when the WCa population was compared to a new population of wild animals captured from WCa original locality.

Interspecific differences in the conditions of emission

The differences between domestic and wild cavies in the situations of *drrr* and *purr* emissions obey the general principle that follows from the observations of Künzl and Sachser (1999): domestic cavy react less than wild ones to environmental changes that generate defensive behavior and the males are more ready for courtship than females, even outside the reproductive period. These differences can be attributed to the context of domestication: in the absence of predatory pressure, genetic variants of guinea pigs that are less reactive to new and potentially threatening stimuli would have a chance to spread through the population. From a perspective of immediate causation, the difference between domestic and wild cavies can be the result of (1) an increasing threshold for triggering defensive behavior: more intense stimuli would be necessary to evoke defensive responses, and among them, the *drrr* vocalization; (2) a lowering of the threshold for triggering courtship behavior, a generalized effect of domestication with a direct consequence on the emission of the signal that accompanies this behavior; (3) an increase of selectivity in relation to the stimuli capable of eliciting defensive responses; and, (4) a decrease in the propensity for the production of sequences of *drrrs*, in the absence of immediate environmental stimulation (Hale 1962; Crockford 2002).

The captive environment can alter the thresholds for the emission and use of signals even in the absence of genetic differences, depending on the stimulation offered, as found in lion tamarins (Ruiz-Miranda & Kleiman 2008). The difference in the frequency of signals by animals born in captivity and wild populations was attributed to the difference in the threshold of reaction to external stimuli or the absence of specific context for emission. The long calls that lion tamarins use for long distance communication between and within the group are emitted less frequently by animals in captivity than those in nature.

Interspecific differences in the structure of the calls

Both in the *drrr* and in the *purr*, DC differ from WCa and WCb in all the temporal parameters evaluated, except in phrase duration, which appears to be strongly influenced by the emitter's social conditions and motivation, as proposed by Arvola (1974) and Coulon (1982). DC had *drrrs* and *purrs* with pulses that were longer and had greater intervals between them and were repeated in a slower manner than those of the WCa and WCb.

Interspecific differences in the spectral dimension are not as homogenous as intraspecific ones and vary among contrasts. For the *drrr*, DC differed from WCa because it had a lower number of reinforced bands and a lower maximum frequency, and from WCb because it had higher values of minimum, 1st band maximum and dominant frequencies. In the *purr*, DC had higher 1st band maximum frequency than WCa.

Differences in acoustic structure of calls resulting from domestication have been found in other species both in temporal as well as spectral parameters (Güttinger 1985; Feddersen-Petersen 2000; Yin 2002; Yin & McCowan 2004; Monticelli *et al*. 2009). For instance, meows of domestic cats *Felix catus* are significantly higher in mean fundamental frequencies (comparable to our 1st band maximum frequency) and in mean formant frequencies (which correspond to our dominant frequency) and are shorter in mean duration than cries of their wild relative *Felis silvestris lybica* (Nicastro 2004). The notes of the maternal alarm call of the Peking lineages of the domestic mallard duck are longer than those of wild *Anas platyrynchos* (Miller & Gottlieb 1981).

Nevertheless, our results point to similar effects in such funcionally different rhythmic vocalizations – alarm and courtship calls – that are concentrated in temporal parameters. Temporal parameters were more rigid and preserved species-specific characteristics, whereas frequency parameters were more flexible and susceptible to environmental characteristics. Gautier (1989) also found preservation of temporal parameters when comparing four species of *Cercopithecus* monkeys that were separated by a speciation process different from domestication. The author found differences principally in temporal parameters of the alert, contact, social cohesion and loud calls (the only spectral difference was related to the frequency modulation of the contact and cohesion calls).

This difference in flexibility to change between temporal and spectral parameters could be derived from those two dimensions being controlled by distinctive subareas of the auditory cortex and asymmetric neural activities between hemispheres, as suggested for humans (Zatorre & Belin 2001); spectral aspects are predominantly processed in the right hemisphere, whereas temporal elements are predominantly processed in the left one, as supported by Okamoto *et al.* (2009).

Difference between populations of Cavia aperea

Differences in sonographic structure of the *drrr* between WCa and WCb cavies, based on spectral parameters, with lower minimum and maximum frequencies of the 1st band and the maximum frequency in WCb cavies point to a differentiation of these populations, which is to a degree surprising if we accept the idea that *drrr* is a species-specific signal.

Unlike temporal parameters, spectral dimensions may require lower nervous system control and have greater flexibility to adjust the emitter to the environment to which it is adapted (Okamoto *et al.* 2009). For instance, Slabbekoorn and Smith (2002), studying parapatric bird populations along an ecological gradient from rainforest to ecotone forest bordering the savanna in central Africa, found significant differences between individuals in different habitats in minimum and maximum frequency, and delivery rate of song notes (which do not correspond to any of our parameters). Spectral variations were observed even during the course of a day in a single habitat, which corroborates the notion of their having greater flexibility. Similarly, Perla and Slobodchikoff (2002), examining the relationship between habitat structure change and alarm call characteristics in six colonies of Gunnison's Prairie Dogs *Cynomys gunnisoni*, found significant differences in calls (alarm call dialects) between colonies in frequency components, but not in temporal components. Playback experiments conducted afterwards revealed that differences in alarm call structure affected acoustic transmission of calls through the local habitat. Blumstein and Armitage (1997) also reported a small (but not significant) difference in the warning whistles of Yellow-bellied Marmots in two locations (Colorado and Utah, USA), in frequency parameters including: maximum frequency, bandwidth and frequency at peak amplitude. Populations of Barbary Monkeys *Macaca sylvanus* from different locations also had differences in frequency parameters of the shrill barks, but not in temporal parameters: dominant frequency (first and third frequency of greater energy of the note), frequency range occupied by the call (difference between minimum and maximum frequency) and other parameters related to distribution of energy between the frequencies (Fischer *et al.* 1998). The authors discuss this variation as an effect of "vocal accommodation" and as a result of the founder effect, in the sense that the vocal characteristics of some individuals had served as a prototype for the group.

We consider WCa and WCb cavies to be populations of *C. aperea* instead of using the separation of Ximenez (1980) in *C. aperea pamparum* and *C. aperea* ssp. This is the species with the broadest distribution within the genus, occupying areas with very different ecological and climatic characteristics and with variation in size and coloration from north to south of their known distribution range. It is probable that there are ecotopic differences among populations of this species and reduced gene flow among populations across their range, resulting from different selective forces of each environment (differences in climate or altitude, or other differences that may exist between São Paulo and Buenos Aires). Acoustic structure of *drrr* calls may also be associated with genetic differenciation between those populations as suggested by Slobodchikoff *et al.* (1998) for Gunnison's Prairie Dogs.

The recognition of differences in the calls

The discovery of differences in the acoustic quality of calls is not sufficient to guarantee that these differences have a meaning as message, as an element of social communication. Variation must be recognized and this recognition in general takes place through parameters such as frequency range or dominant frequency (Newman & Goedeking 1992; Hailman & Ficken 1996). The dominant frequency of the *drrr* of cavies from the two locations did not vary, which leads us to think that the perception of the signal had not been altered, that is, that there is recognition and response (attention and emission of *drrr*) between the populations. Evans (1993) presented mallard ducklings with playbacks of the maternal warning call with synthetic alteration of the dominant frequency. Although the latency of the response was lower in relation to the altered playbacks and decreased proportionately with the degree of alteration of this parameter, the fact that the ducklings still responded to the mother's call suggests that other acoustic parameters (such as the frequency modulations and the temporal characteristics) are also involved in conspecific call recognition (Evans 1993).

Whether temporal and frequency differences between the *purrs* of domestic and wild cavies act as reproductive barriers is unknown. A wild male cavy that produces a *purr* like a domestic one would have less chance of coupling with a wild female cavy and easier access to a female guinea pig. The same can be expected of a domestic male that emits a *purr* like a wild one, but symmetry in interspecific effects would not necessarily prevail.

What is the origin of the changes in the vocalizations of alarm and courtship in domesticated animals?

It appears unlikely to us that they occurred from direct selection by humans, in a manner parallel to that which produced dog breeds. First, these calls are not clearly distinguished by the human ear; secondly, there is no clear reason for a breeder to selectively reproduce individuals with higher pitch or those with a slower rhythm.

Artificial selection may instead have been indirect, that is, the consequence of the role that another characteristic associated to the vocalizations has in the selective vicissitudes of reproduction in captivity, an effect of pleiotropy (Belyaev *et al.* 1981; Crockford 2002). For example, if the pitch of the food-anticipation whistle (Monticelli *et al.* 2009) emitted in the presence of the handler was a factor that led some animals (those that had higher pitch whistle) to receive more food and thus experience a higher probability of reproducing, perhaps, as being part of the same sensory-motor system, other vocalizations like the *drrr* and the *purr* would likely become higher in pitch.

The selection of morphological or physiological characters also could be at the base of an indirect process of genotypical change (the selection of these traits thus generating effects in behavioral traits (Belyaev 1979). Guinea pigs were selected principally for their meat and one of the effects of domestication on this species is increased body size (Weir 1974; Spotorno *et al.* 2006; Jensen 2009). Larger animals could have been selected by domesticators, based on obtaining more meat per individual raised. Changes in length and diameter of the vocal tract of the guinea pigs could have altered their filtering and resonating capacities, causing variation in the distribution of energy in the harmonics and in other characteristics of the calls (Schrader & Hammerschmidt 1997). Anatomical modifications in the respiratory system could also alter temporal parameters. Animals with greater lung capacity can perhaps emit longer calls during longer exhales and take more time inhaling (interval between notes or phrases). Nevertheless, there does not appear to be any simple relationship between the size of the body of a cavy or of a guinea pig and the spacing of the pulses in a warning call.

Other explanations include factors such as the relaxation of natural selection, because of the protection offered by captivity (against predators, illness and the supply of food and water that all but eliminate the need for natural foraging abilities), which would allow less favorable characteristics to be maintained and gain in proportion in the population; this could explain, for example, the increase of dominant frequency, making the vocalization of the guinea pigs more conspicuous. Effects of genetic derivation or restriction of variability due to inbreeding (Price 1984, 1998) are other possible factors to explain the changes among guinea pigs and wild cavies and all of them could act together, making it difficult to separate the partial contribution of each factor, even in laboratory conditions. The discovery of differences in vocal behavior between *C. porcellus* and *C. aperea* and between populations of *C. aperea*, whatever the precise factor that generated them, opens the door to very interesting hypotheses about the role of these differences, especially those that are related to *purr*, in the maintenance and strengthening of reproductive barriers among species.

In 1974, Eisenberg raised a question about the origin of the rich acoustic repertoire of guinea pigs: could this be a product of domestication? Do wild populations have the same variety of signals in their repertoire? We now have concrete bases to affirm that yes, wild cavies in fact have the same range of vocalizations as domesticated species (Monticelli 2005; Monticelli & Ades 2007), without additions or losses, as could be expected from discussion regarding the effects of domestication by Hale (1962), Ratner & Boice (1975) and Price (1998). The similarity between the vocal repertoire of the species, together with the results of the work of Künzl & Sachser (1999), reveal the phylogenetic proximity of guinea pigs and *C. aperea* cavies.

Our study, however, also provides information to affirm that domestication brought novelties in the way that the guinea pig produces two of its most important vocalizations, the warning *drrr*, and the reproductive *purr*. Although guinea pigs were domesticated relativey recently – about 6,000 years (Wing 1986; Lavallée 1990) – this was enough time for the vocal behavior of the guinea pig to change significantly. These data regarding vocalizations could be taken, together with other behavioral data, to indicate that *C. porcellus* and *C. aperea* have entered, potentially irreversibly, into a process of speciation.

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Apêndice I _ *Social support does not require attachment: Any conspecific tranquilizes isolated guinea-pig pups*

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Social support does not require attachment: Any conspecific tranquilizes isolated guinea-pig pups

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ABSTRACT

Guinea pig pups produce typical distress whistles when isolated. Whistles' frequency is decreased or abolished when they contact with the mother and, to a lesser degree, a sibling or even an unfamiliar female, is regained. Those non-aggressive companions were considered social support providers for reducing pup physiological stress responses and whistling rate in an unfamiliar environment. However, what would happen if the isolated pup would be in contact with an adult male, normally indifferent to pups, in such distress situation? The role of attachment and familiarity to males in promoting changes in distress responses of isolated pups was verified. Tests consisted of separating three week old pups from their family, in a familiar or an unfamiliar environment, and introducing a conspecific in the cage after one minute (mother, sibling, father or a strange male). Whistling and other behaviors were compared between the alone period and the accompanied period. Main factors were *prior presence/absence of father* (pups were raised with father until testing or only for the first week after birth), *sex of pup*, *novelty of test environment* and *companion*. It was verified that (1) all conspecifics reduced whistling rate ($F_{4,88} = 77.89$, $p < 0.001$), but pups behaved differently with different conspecifics; (2) suppression of isolation induced behavior did not necessarily occur because of previous attachment (e.g., pups in the PAF condition spent more time pausing, $F_{1,22} = 7.68$, $p < 0.05$, less time in passive contact with companions, $F_{1,22} = 10.63$, $p < 0.01$, and ate/drank less, $F_{1,22} = 6.18$, $p < 0.05$). It was concluded that the suppression of pup's isolation induced behavior by companions must not be used alone as a measure of attachment. It must be seen in an evolutionary perspective where the presence of any conspecific represents security offering self-protective behavior cues as finding a place to hide, and providing dilution effect against predation.

1. Introduction

Mother–infant bonding is well described in guinea pigs (*Cavia porcellus*). Pups actively search and follow their mother shortly after birth and most of the time, will remain close to her until weaning, usually at the fourth week of life (Pettijohn, 1979a; Porter et al., 1973b). Such behavior has been interpreted by some authors as being dependent on a process analogous to imprinting (Shipley, 1963; Sluckin, 1968). Nevertheless, infants do not exclusively approach their mother. Pups 3 or 4 days old frequently follow adult guinea pigs other than the mother (King, 1956) and nurse from other lactating females (Fullerton et al., 1974; Takamatsu et al., 2003).

Infants frequently show signs of distress when separated from their mothers (Ainsworth et al., 1978; Harlow and Harlow, 1965) such as vocalizations, changes in locomotion and in self-directed behavior. Such reactions which diminish or stop when the contact with the mother is restored are commonly taken as indicating the existence and intensity of mother–child attachment in both humans (Ainsworth et al., 1978; Bowlby, 1984) and other mammals (Hennessy and Weinberg, 1990; Wiener et al., 1990).

Isolating guinea pig pups increase the production of high-pitched whistles and cortisol levels. These reactions are prevented

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Abbreviations: Prior presence of father (PPF), the father was kept with pups and mother throughout the experiment; Prior absence of father (PAF), the father was taken to a separate cage at day 8 after the birth of the pups.

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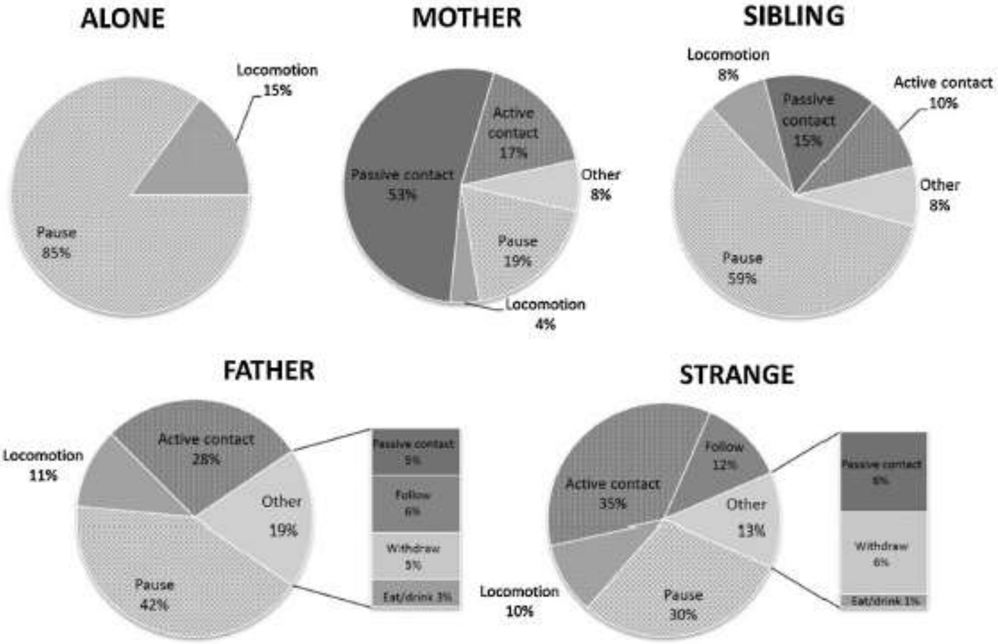

Fig. 1. Percentage of time invested by pup in non-vocal behavior categories during the 3-min period of (1) still alone (Alone) or (2) in the presence of a companion (Mother, Sibling, Father or a Strange male) after the 1 min isolation test. Data of pup behavior was taken together in *familiar + unfamiliar cage* and in *father present + father absent* conditions.

from day 15 to day 21 of age. For each test, the following procedure was used: (1) a family was transported from the husbandry facility to an experimental room and left there for at least 1 h in its own (familiar) cage before being transferred to a new one; (2) the pup was carried to the test room and put either in its *familiar* cage or in an *unfamiliar* one; (3) the pup was left alone for a 1 min period; (4) a companion was introduced in the same cage for a 3 min period; (5) all animals were then, put back into the familiar cage and taken back to the colony. Test conditions (familiarity of cage and companion) were randomized. Behavior of both, the pup and the companion, was videotaped during (3) and (4) with a Sharp VHS Slim Camcorder Camera positioned one meter above the center of the cage.

2.3. Measurements

Behavior measured categories were: *whistles* (number of whistles produced); *locomotion* (pup wanders around the cage); *passive contact* (pup stays in contact with companion); *active contact* (pup nuzzles companion; pushes companion with the head; briefly touches companion with front paws or insert head below companion's body); *following* (pup moves behind companion); *withdrawal* (pup moves away from companion); *pause* (pup displays little or no movement) and *eating* or *drinking*. Rate of whistles and percentage of time spent in each behavior (other than vocal) were obtained for each session. Whistles were counted from the recorded videotapes by hearing and by observing the typical accompanying thoracic movements. Data was collected from March to December, 1994. This research followed the Ethical Guidelines of the International Society for Applied Ethology.

2.4. Statistical analysis

A $2 \times 2 \times 2 \times 4$ mixed-design factorial ANOVA ("split-plot" ANOVA, SPSS17, Norusis, 2008) was used, with *prior presence/absence of father*, *sex of pup*, *novelty of test environment* and *companion* as main factors. Tests assumptions were verified by Shapiro–Wilks and Levene tests to evaluate normality and homogeneity of variances, respectively, as in Norusis (2008).

Vocalization (i.e., number of whistle notes/time whistling) and behaviors (relative duration in 3 min) were compared between the alone and the accompanied periods of testing using separate analysis of variance (ANOVA) followed by a stepwise multiple comparisons procedure (Newman–Keuls tests). This procedure was used as a post hoc test whenever a significant difference between sample means was revealed. PPF and PAF conditions were distinguished whenever analyses showed that a significant (i.e. $p < 0.05$) main effect of *prior presence/absence of father*.

3. Results

3.1. Effects of companion

Isolated pup behavior was significantly affected by the presence or absence of a companion and by the companion type (Fig. 1). All behavior categories, except withdraw and eating/drinking, varied in accordance with companion type: whistle rate ($F_{4,88} = 77.89$, $p < 0.001$), locomotion ($F_{4,88} = 9.42$, $p < 0.01$), pause ($F_{4,88} = 74.85$, $p < 0.01$), passive contact ($F_{3,66} = 63.57$, $p < 0.01$), active contact ($F_{3,66} = 14.64$, $p < 0.01$), following ($F_{3,66} = 6.44$, $p < 0.01$). Post hoc comparison results are shown in Table 1.

When left alone, after the 1-min period of isolation, 85% of the three following minutes, pups spent in Pause (see chart 'ALONE' in Fig. 1). When the companion was a sibling the 3-min test did not elicited much interaction; only 25% of the time was spent in physical contact (sum of passive and active percentages in the chart 'SIBLING' in Fig. 1). The mother increased the time in contact, especially the passive one and the males (father or stranger) rose the

Table 1
Newman- Keuls post hoc comparisons of isolated pup behaviors that were affected by the presence and type of companion in ANOVA mixed design test. As Locomotion, Active contact and Following behaviors had no effect of *Prior presence/absence of father*, PPF and PAF data were summed to be evaluated in the post hoc comparisons. Whistle rate, Passive contact and Pause differed between PPF and PAF conditions and they are shown separately in each condition. Shaded values indicate significant differences in post hoc comparisons.

Comparison	No effect						Prior presence of father						Prior absence of father					
	Locomotion		Active contact		Following		Whistle rate		Passive contact		Pause		Whistle rate		Passive contact		Pause	
	p/df	q	p/df	q	p/df	q	p/df	q	p/df	q	p/df	q	p/df	q	p/df	q	p/df	q
Alone × mother	2/51	8.30**					4/27	24.6**			4/27	17.19**	3/23	13.52**			4/23	11.71**
Alone × sibling	4/51	5.01**					3/27	21.20**			3/27	5.67**	4/23	12.52**			3/23	5.16**
Alone × father	3/51	3.27					2/27	24.31**			2/27	10.53**	2/23	13.42**			2/23	8.21**
Alone × stranger	2/51	4.12*					2/27	24.57**			2/27	14.17**	2/23	12.78**			2/23	9.79**
Mother × sibling	3/51	3.29	2/51	2.74	2/51	0.03	2/27	3.4*	2/27	9.98*	2/27	11.52**	2/23	1.01	2/23	7.10**	2/23	6.56**
Mother × father	2/51	5.02**	2/51	4.34**	2/51	1.64	3/27	0.29	2/27	12.39*	3/27	6.66**	3/23	0.11	2/23	9.48**	3/23	3.5
Mother × stranger	3/51	4.18	3/51	7.74**	3/51	6.33**	5/27	0.03	3/27	12.34*	5/27	3.02	4/23	0.75	3/23	9.16**	5/23	1.92
Sibling × father	2/51	1.73	3/51	7.08**	3/51	1.67	2/27	3.11**	3/27	2.40	2/27	4.86**	3/23	0.90	3/23	2.38	2/23	3.05*
Sibling × stranger	5/51	0.88	4/51	10.48**	4/51	6.36**	4/27	3.37*	4/27	2.36	4/27	8.50**	5/23	0.26	4/23	2.05	4/23	4.63*
Father × stranger	4/51	0.85	2/51	3.40*	2/51	4.69**	3/27	0.26	2/27	0.04	3/27	3.64*	3/23	0.64	2/23	0.32	3/23	1.58

* $p < 0.05$.
** $p < 0.01$.

time in active contact (compare the size of the gray pieces of all the charts in Fig. 1).

3.1.1. Factor effects over whistling

As shown in Table 1, pups vocalized more when alone than when accompanied by any other individual. All adults were equally effective in decreasing the rate of whistles. There was a *companion × prior presence of father* interaction ($F_{4,88} = 5.18$, $p < 0.01$). A sibling companion differed from adult in decreasing whistling rate in PPF condition, but not in PAF condition. Also, there was a *companion × gender* interaction ($F_{4,88} = 3.69$, $p < 0.01$): female pups vocalized more than male pups when alone, but not when accompanied, and a *companion × gender × novelty of test environment* interaction ($F_{4,88} = 2.52$, $p < 0.05$): male pup alone and female pup with the strange male vocalized more in the familiar versus novel environment.

3.1.2. Factor effects over non-vocal behavior

Pups spent more time in locomotion when alone than when accompanied by mother, sibling, or strange male (Table 1) and were more active when accompanied by father than by mother. There was a *companion × novelty of test environment* interaction ($F_{4,88} = 7.04$, $p < 0.01$). Pups spent more time moving in the familiar cage than in the unfamiliar one when tested alone, but not when tested with a companion.

Pups spent more time pausing when alone than when accompanied. When accompanied by siblings, pups spent more time pausing than when accompanied by adults. PPF pups accompanied by the father spent more time pausing than when accompanied by the mother.

In relation to physical contact, pups spent more time in passive contact with their mother than with any other companion. In contrast, they spent more time in active contact with a strange male then when with any other companion, and when accompanied by the father than with the mother.

Pups followed the strange male more than any other companion. There was an interaction among the 4 factors (*prior presence/absence of father, sex of pup, novelty of test environment* and *companion*) considered ($F_{3,66} = 5.18$, $p < 0.01$). Female PPF pups tested in the familiar cage spent more time following strange males than other pups or than themselves in any other circumstances.

Two interactions were significant for withdrawing behavior. *Companion × prior presence of father* ($F_{1,22} = 7.35$, $p < 0.05$) indicates that pups in the PAF condition withdrew more from father than from strange male while pups in the PPF condition withdrew more from strange male than from father.

There was also a significant interaction between companion × novelty of test environment × gender ($F_{1,22} = 4.89$, $p < 0.05$). Male pups spent more time withdrawing from strange males in the familiar cage than female pups. In other circumstances females withdrew more than or as much as males.

3.2. Effects of prior presence of father

Prior presence of father had main effects over some pup's behavior (Table 1). Pups in the PAF condition spent more time pausing ($F_{1,22} = 7.68$, $p < 0.05$), less time in passive contact with companions ($F_{1,22} = 10.63$, $p < 0.01$) and ate or drank less ($F_{1,22} = 6.18$, $p < 0.05$) than pups in the PPF condition. The main effect of this factor on whistling ($F_{1,22} = 5.21$, $p < 0.05$) was previously discussed.

3.3. Effects of gender

Female pups spent more time in passive contact than male pups ($F_{1,22} = 4.61$, $p < 0.05$).

3.4. Effects of test environment

Pups spent more time pausing ($F_{1,22} = 12.47$, $p < 0.01$) and in passive contact ($F_{1,22} = 4.62$, $p < 0.05$) when tested in the unfamiliar cage. The main effects of the test environment over locomotion ($F_{1,22} = 23.45$, $p < 0.01$) and following ($F_{1,22} = 21.17$, $p < 0.01$) were already discussed because of the interaction with *companion*.

3.5. Repetition of isolation tests

A main effect was observed over the vocalization of pups tested alone ($F_{7,175} = 2.65$, $p < 0.05$). Newman–Keuls comparisons were significant for: test 1 × test 3, $q_{2,25} = 3.85$, $p < 0.05$; test 1 × test 5, $q_{4,25} = 4.38$, $p < 0.05$ and test 1 × test 6, $q_{3,25} = 4.85$, $p < 0.05$. The results show that the number of whistles increased from the first to the third test, and then remained constant up to the end of testing.

4. Discussion

We found that all adult companions equally decreased guinea pig pups distress vocalizations, including the father and a strange male. This result contrasts with those of previous studies that found that other companions were not as effective as the mother in decreasing distress vocalizations of isolated pups (Hennessy et al., 2002; Pettijohn, 1979b). It also expands these authors' results showing that even strange males can be effective in decreasing pups' vocalizations. The apparent contradiction can be due to the differences in methodology. Hennessy et al. (2002) tested pups for longer periods (20 min and 60 min) and Pettijohn tested only 9 pups and did not present any statistical analysis. The fact that all companions equally diminished pups distress vocalizations could be interpreted as a ceiling effect, but the differences in the pups' behavior toward companions suggest that they distinguished the companions.

It seems that contact with adult conspecific individual functions as a *source of social support* to pups that were separated from their group. In a species with high levels of predation (Asher et al., 2004) being alone is highly dangerous, the presence of any member of the group or even of a strange conspecific may supply the young guinea pig with some reassurance. In fact, it was recently proposed that wild cavies (*Cavia magna*) utilizes the dilution effect as an anti-predator strategy during foraging activities: the frequency of vigilance related behaviors correlated negatively with the number of foraging animals and foraging animals were more probable to forage in more distant places from the shelter when accompanied by a greater number of conspecifics (Elisa Santos, unpublished dissertation).

The socially tolerant nature of guinea pigs makes plausible that a lost pup may search proximity to a male or female adult without severe rejection: pups follow and remain in physical contact, from birth on, with several members of the group, although, of course, the mother remains the main individual reference (King, 1956). Adult males, at least in the domesticated species, do not openly fight pups and may court them: young may prefer contact with adult males to contact with virgin females (Beisiegel, 1993; Berryman and Fullerton, 1976).

If tranquilizing effects are not exclusively due to contact with individual to which the pup is bonded, we should distinguish, as motivational bases of the pup's behavior, the tendency to reestablish contact with the mother from a tendency to reestablish contact with members of the group or with conspecifics in general. Distinguishing *attachment figure support* from *social support* per se may help interpreting results. In an isolation context, attaining security may be more than only recovering contact with a bonding figure: for a guinea pig pup, the presence of a conspecific adult may be an indication about nearness of the group and may be interpreted as a signal of safety. Thus, it is plausible to suppose that the pup has already installed the mechanism of searching company as an anti-predator defense: for such a vulnerable individual, with no body arms, that trusts only in hiding under high grass as protection against predator, but that needs to forage in more open areas (Cassini and Galante, 1992), being in group may be an important way of staying alive.

Coherent with such interpretation is the fact that whistling duration was less affected by the presence of siblings, which may not offer the same security basis as adults. The focal animal and its young companion sometimes vocalized together in the isolation cage (seen in the wild species as well, Monticelli and Ades, 2013). The role of siblings in tranquilizing induced vocalization is not the same as adult companions. Pups tested with a sibling vocalized more than those tested with adults (although not statistically significant) and spent more time pausing. Functionally, siblings may be not as important as adults in security value. It can offer no care and cannot be a guide to the pup as an adult may be.

An interesting result was the pups discriminating father and strange male, but directing more active contact to strange males than to their fathers. This might be explained as social exploration and it could be a function of familiarity between pup and companions. Active contact includes smelling companion, a behavior that is known to decrease as familiarity increases (Beauchamp and Wellington, 1984; Martin and Beauchamp, 1982; Ruddy, 1980). Fathers from both conditions are familiar to the pup, but the strange male is not. However, withdraw decreased from strange male to father from temporary absence condition to father from constant presence condition. However, this could be related to how much these males smelled pups. A qualitative analyzes indicated that withdrawal occurred when pup smelling was prolonged and fathers from the temporary absence condition seem to smell more than other males. This could be due to its isolation period as occurs in rats (Niesink and Van Ree, 1982). Alternatively, the behavior of the pup directed to the strange male could be interpreted as a submission and auto-defensive reaction: to avoid being attacked by an adult male, it should show itself as a completely submissive partner.

Although it is argued that all conspecifics tested here had a role in decreasing isolation induced behavior independently of attachment bonds, Hennessy et al. (1995) and Sachser (1998) showed that only the presence of individuals to which guinea pig pups and adult males were attached to, did decrease their plasma cortisol levels when isolated for half an hour or more. It would be interesting to see (1) if a short isolation period, such as the one employed here, would have any effects over the adrenomedullary system, and (2) the role of the different conspecifics tested here over this same system.

Differences in gender found here were also found in other studies. Pettijohn (1979b) found that females vocalized more than males in their first week of life and Hennessy and Sharp (1991) found that female pups spent more time in contact with mother than males. These authors did not discuss these results. A possible explanation could be based on the social organization of these animals. If dispersion is done by males (as some works indicate – Greenwood, 1980; King, 1956; Sachser, 1986) cohesion behavior may be more pronounced in females.

Pups showed discrimination between different environments. In the alone period they spent more time pausing and less time in locomotion when tested in unfamiliar cages. This pattern was also found for adult guinea pigs tested in 'open fields' (Tobach and Gold, 1966). Although discrimination was not reflected in decreased vocalization rate in the familiar cage. When compared to Pettijohn's study (1979b), the mean vocalization rate of our subjects, independently of environment, was the same as his subjects tested in a familiar cage. It seems that our subjects' discrimination between environments was not enough to arouse vocalization. This could be suggestive that different defensive behavior as vocalization and immobility are activated according to different degrees of familiarity.

In the accompanied period, pups spent more time following and in contact with companion when tested in the familiar cage. In Porter et al.'s work (1973a) pups moved more in the presence of the mother. In the present study all companions had the same effect over pup. Again, the presence of a conspecific, despite attachment links, could represent security, causing the pup to move more and hence facilitate contact with companion.

Many differences were found between pups from prior presence and prior absence of father. Pups from prior presence of father condition, vocalized more in the alone period and with sibling, spent more time in active contact and ate or drank more when accompanied. Pups from prior absence of father spent more time pausing without contact. Other authors have reported behavioral and physiological differences in a separation test according to different conditions of maintenance (Hennessy et al., 1991; Hennessy and Moorman, 1989; Hennessy and Sharp, 1991). According to these works vocalization and time in contact decreased as the level of plasma cortisol increased. These effects were seen in conditions of continued stress.

In the procedure used here the only difference between conditions was the withdrawal of father from home cage after the first week of life of pups in the condition of prior absence of father. One cannot state if the absence of the male or the action of withdrawing could be the factors increasing stress in this condition. Two works that investigated the effects of the presence or absence of males over the development of pups found some differences in sexual and aggressive behavior due to this factor (Beisiegel, 1993; Levinson et al., 1979). However, it is noteworthy that in these works males were removed from home cage after just 24 h of pups' birth. One of Beisiegel's results was that pups in the presence of father ate more, just as occurred here, and male pups grew more than pups in the absence of father. Those pups were not tested in a separation test so we could not compare vocalization data. There are no reports on conspecifics' withdrawing effects over group members of guinea pigs. In this particular case it was just done once when pups were relatively new. It is difficult to suppose that this single action of withdrawing could have had a stressing effect per se over pups. This would implicate in memory processes beyond the scope of this work.

The distinction between the social support provided by any conspecific during short periods of isolation and the support provided by attachment figures, which includes decreasing in cortisol levels during longer periods of isolation, can help to understand the apparent contradictory results in the literature and can set a new perspective in the study of the distress responses to isolation. Differences can be expected both in the mechanisms that control these responses and in the function that they present. According to Corat et al. (2012) the behavioral responses of immobility and vocalizations are triggered by conditions of mild stress, as short periods of isolation. It is proposed here that the social support given by any conspecific in this condition is due to the interaction between the animals which, in turn, dependent on familiarity, age, and sex of the interacting animals. It can be hypothesized that when animals are attached, the interaction will include more passive contact and will lead to a decrease in cortisol levels even if the isolation period is prolonged. If animals are not attached or are unfamiliar a more active interaction will occur, which will, consequently, suppress the distress responses. If the interaction is not aggressive, and interactions between adults and pups usually are not, the companion can function as a security source, ultimately guiding the pup back to the group or protecting the pup against predation.

5. Conclusions

We propose that separation induced behavior is not strictly a measure of attachment in guinea-pigs. It may also be seen as a reaction to a new and potentially dangerous situation. Behavior displayed to a conspecific after reunion must be viewed in relation to the social structure of the particular animal under study.

Conflict of interest

None declared.

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Capítulo 2.

Comunicação acústica e socialidade: qual a relação?

> *"The consensus, at least for the primate family, is that we have our big brains to enable us to cope with the complexities of our social world. " (p.12) "language is just a form of grooming" (....) "to spend hours stroking, touching, leafing through hair. Physical contact, in short, is an essential part of the rhythm of social life. To this, we humans add language (...) a kind of grooming at a distance" (Dunbar, 2010, pp. 73-74).*

Neste capítulo, vou precisar caminhar por um cenário filogenético maior. As comparações entre cobaias e as espécies brasileiras de preás permitiram discussões interessantes sobre os processos de domesticação, de vinculação afetiva e a "constelação de respostas em cadeia" do sistema que integra motivações e respostas de enfrentamento (Müller e Shair, 2016). Neste capítulo, meu foco é a relação da comunicação acústica, em termos distais (Tinbergen, 1963), com a vida social. Vou começar com um olhar comparativo para as espécies do grupo que eu mais estudei, os roedores da família Caviidae, especificamente com 2 das 3 subfamilias: Caviinae (dos preás) e Hidrochoerinae (mocós e capivaras). Dolichotinae, a terceira subfamília, não ocorre no Brasil (Wilson e Reeder, 2005) e ainda não tive a oportunidade de gravar. Mas vou usar as informações da literatura sobre *Dolichotis patagonum*. Na Tabela 2.1 apresento a posição taxonômica das espécies, sem apontar a relação filogenética entre os grupos.

Rood (1970-72), Eisenberg (1974) e Lacher (1981), biólogos evolutivos que estudaram, independentemente e de forma comparativa (*sensu* Lorenz 1995), espécies de Caviinae (para Eisenberg, Hystricomorpha, que inclui Caviidae e Echimyidae, e outras espécies do Novo Mundo), notaram o uso extensivo do canal acústico, em maior ou menor grau, em todos os táxons. Rood criou grupos de *C. aperea, C. porcellus, Galea musteloides* e *Microcavia australis* em cercados amplos ("cativeiro semi-natural"; Rood 1970, 1972); e Lacher (1981), sob condições semelhantes, criou mocós *Kerodon rupestris* e preás-de-dente-amarelo *Galea spixii*, ambos da Caatinga. Em conjunto, esses autores oferecem descrições detalhadas e comparativas dos repertórios comportamentais e acústicos de cinco espécies, de 4 gêneros (três deles de Caviinae e um de Hidrochoerinae: *K. rupestris*, indicadas em negrito na Tabela 2.1), mas usaram por vezes termos diferentes para o que podem ser sinais homólogos. É preciso olhar para todo esse conteúdo e buscar o que é comum e o que são particularidades das espécies. Espero poder dar uma contribuição com isso neste texto.

Tabela 2.1. Relação de espécies atuais de Caviidae: Rodentia, por gênero e subfamilia, segundo Wilson e Reeder (2005), com alterações apontadas por (*). Negritos indicam as espécies que eu estudei ou que pude observar.

Subfamília	Gênero	Espécies
Caviinae	Cavia	**aperea** fulgida **intermedia** **magna** **porcellus** tschudii
	Galea	flavidens **musteloides** **spixii** monasteriensis* comes**
	Microcavia	australis niata shiptoni
Hydrochoerinae	Hydrochoeris	**hydrochaeris** isthmius
	Kerodon	**acrobata** rupestris
Dolichotinae	Dolichotis	patagonum salinicola

*Solmsdorff, K., Kock, D., Hohoff, C., & Sachser, N. (2004). Comments on the genus Galea Meyen 1833 with description of *Galea monasteriensis* n. sp. from Bolivia (Mammalia, Rodentia, Caviidae). Senckenbergiana biologica, 84(1/2), 137-156.
** Kerber (2017)

A impressão de Rood (1972) foi de que *Microcavia* era o táxon mais basal, por ter um repertório menor de comportamentos, que poderia indicar uma semelhança ao protótipo ancestral: com poucos padrões agressivos, nenhum deles ritualizado como o de *Cavia porcellus*, predominância de comportamentos de contato e apenas três vocalizações (Rood, 1972; Lacher, 1981). O gênero *Cavia*, como bem notou o autor, tem um repertório mais rico em formas variadas e com rituais de corte e agonísticos, mistos de padrões visuais, acústicos e químicos (Rood, 1972, Eisenberg, 1974, Monticelli, 2000). As galeas (visualmente parecidas com preás, mas com dentes amarelos e um anel branco em torno do olho), têm repertórios acústicos semelhantes entre si, um pouco menor do que *C. aperea*. Lacher (1981) propôs, então, que a comunicação vocal tenha se desenvolvido em *Caviinae (na época, Kerodon fazia parte de Caviinae, não de Hidrochoerinae, como na Tabela 2.1)* favorecida pelo habitat _ os ambientes teriam colocado mais obstáculos à transmissão integral do som à mocós do que aos preás, por isso o repertório teria se tornado mais elaborado em um do que no outro; ainda, propôs o autor, as espécies teriam passado por diferentes pressões sócio-reprodutivas _ a poliginia de mocós e capivaras teria pressionado a extensão do repertório dessas espécies, por causa da competição pelo acesso às fêmeas, o que não teria acontecido em *Cavia* e *Galea*, consideradas promíscuas. Hoje se sabe, contudo, que as espécies de *Cavia* e a *Galea monasteriensis* não são promíscuas: uma tende à poliginia, e outra à monogamia (Adrian, Kaiser, Sachser, Jandewerth, Löttker *et al.*, 2008; Adrian e Sachser, 2011; Verzola-Olivio, 2017). Também o ambiente não parece ser uma boa explicação para a ampliação do repertório, já que muitas espécies, inclusive *Galea spixii* e *Kerodon rupestris*, são simpátricos hoje e talvez desde o Mioceno (Upham e Patterson, 2015).

Ao longo dos meus estudos, primeiro como aluna, depois orientando alunos, eu tive condições de fazer uma comparação baseada em um número maior de informações, do que os autores tinham na época. A variedade de formas que compõe o repertório das espécies desses dois gêneros, e que são compartilhadas com os outros gêneros de Caviinae (obs. pess., ainda não publicada, em parte discutida em Monticelli, 2005), requer outras explicações funcionais e o conhecimento da história evolutiva desses táxons (Tinbergen, 1963).

Uma breve história evolutiva de Caviidae

Os fósseis mais antigos de Caviidae, na revisão recente de Madozzo-Jaén, Pérez, Montalvo e Tomassini (2018), datam do Mioceno (fase Mayoan, de SALMA: South American Land Mammal Age, há quase 12 ma). A origem do grupo pode ter sido anterior a isso, ao final do Oligoceno, ao que indicam dois estudos filogenéticos moleculares (Upham e Patterson, 2015 e Àlvarez, Arévalo e Verzi, 2017; Figura 2.1). Caviidae poderia ter divergido de Cuniculidae, pela analise mais abrangente de Alvarez *et al.* (2017), ou compartilhar um ancestral com o grupo Cuniculidae+Dasyproctidae, como propunha a visão tradicional (e.g., Uphan e Patterson, 2015). *Galea* parece ter sido o primeiro táxon de Caviinae a divergir, ao contrário do que previa Rood (1970, 1972), e Microcavia e Cavia, teriam se separado numa mesma época em que *Kerodon* e *Hydrochoerus*, antes do Mioceno Médio (Figura 2.1). As espécies atuais de preás, mocós e capivaras parecem ter a mesma idade (Alvarez *et al.*, 2017).

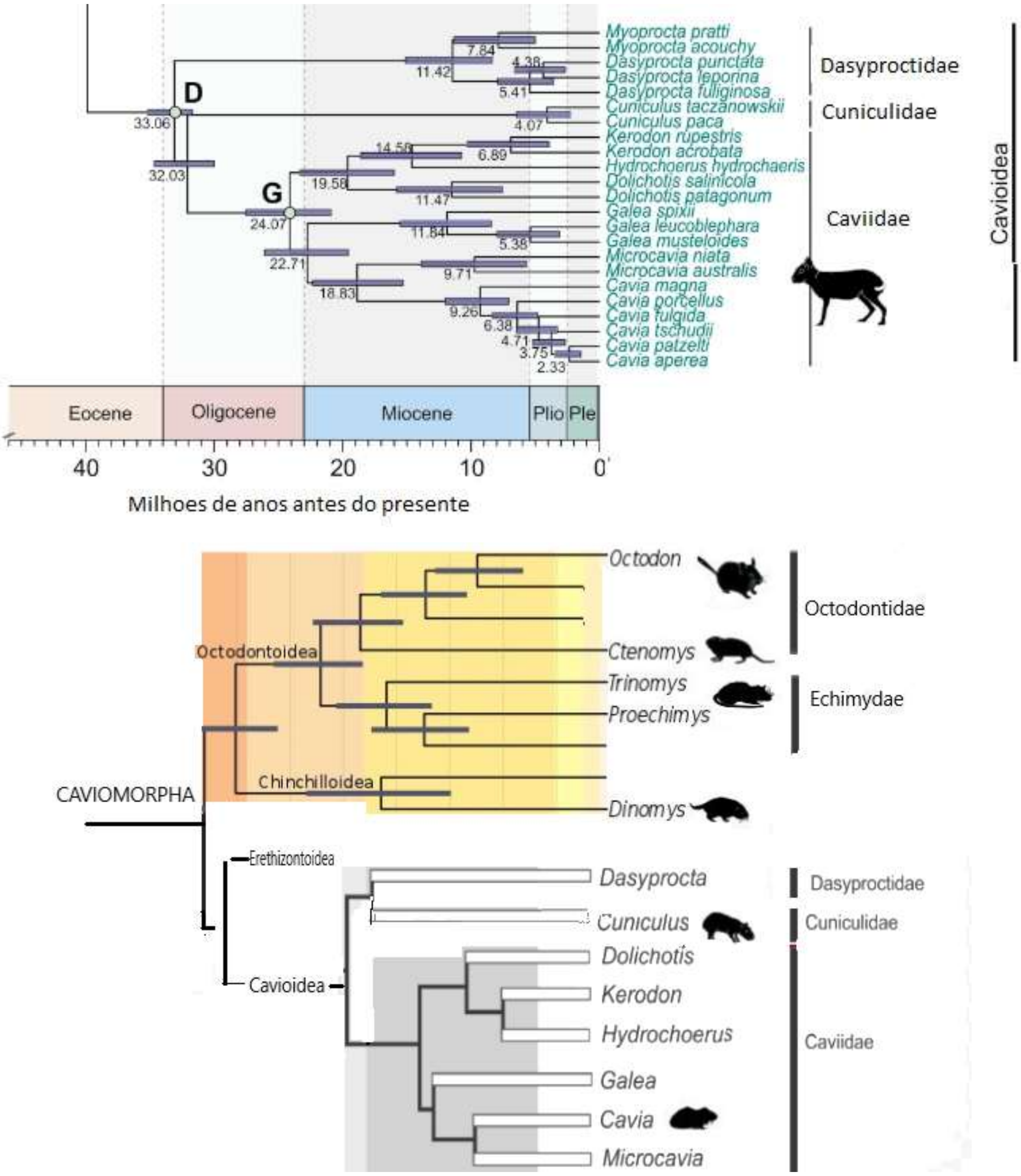

Figura 2.1. Relações filogenéticas de Caviodea (acima e abaixo) e de Caviomorpha, em uma visão mais ampla (abaixo), indicando a relação com Octodontoidea. As duas hipóteses filogenéticas usaram dados moleculares (acima: Àlvarez, Arévalo e Verzi, 2017; abaixo: Uphan e Patterson, 2015). Acima, tem-se as idades estimadas indicadas nos nós em milhares de anos e com indicação do intervalo de confiança de 95%. Fonte: https://www.researchgate.net/publication/314078736_Diversification_patterns_and_size_evolution_in_caviomorph_rodents, recuperado em 22/05/2019. A figura do meio, em tons de laranja foi adaptada de Vilela (2012), e a inferior, de Upham e Patterson (2015).

Caviidae era um grupo taxonômico muito maior do que hoje, especialmente Hydrochoerinae. Essa subfamilia que hoje é representada só por 2 espécies de mocós e 2 de capivaras, já foi composta por 12 espécies de "Cardiomyines" (grupo já extinto de animais que eu descreveria como mocós grandes, de registros fósseis do Mioceno até o Plioceno; Pérez, Deschamps e Vucetich 2017); 4 de Cardiatherium (capivaras do final do Mioceno), do qual divergiram duas linhagens, uma que deu origem à capivara pleistocênica gigante (*Neochoerus*, uma só espécie surge e some dos registros fósseis no Pleistoceno) e outra linhagem de dois gêneros de 2 e 4 espécies (*Hydrochoeropsis* e *Phugatherium*, respectivamente; Kerber, 2017). Em Caviinae, pelos registros fósseis, considera-se ter havido outras duas espécies de *Cavia* no Pleistoceno Quaternário (*C. vates* Winge, 1887; *C. lamingnae* Locks & Montenegro, 1985; Kerber, 2017), duas de *Galea* (*G. ortodonta* do Pleistoceno do Uruguai e Bolívia; *G. tixiensis* do Pleistoceno-Holoceno de Buenos Aires; Kerber, 2017; Madozzo-Jaén et al., 2018) e quatro de *Microcavia* (*M. chapalmalensis, M. reigi, M. robusta*, *M. criolloensis*; Madozzo-Jaén et al., 2018). Durante o Quaternário, que engloba o Plio e o Pleistoceno, os caviomorfos assemelham-se às atuais, segundo Kerber (2017), com diferenças principalmente na faixa de distribuição geográfica (e.g., entre os dois extremos de distribuição atual de *Galea*, a caatinga nordestina e o sul do continente, encontraram-se fósseis; Kerber, 2017).

Os repertórios acústicos dos cavioideos atuais

Após estudar a comunicação acústica em diferentes espécies de Cavioidea (Fig. 2.1: Caviidae+Cuniculidae+Dasyproctidae; Wilson & Reeder 2005), duas de Octodontoidea (Echimydae) e de fazer observações livres de chinchilas (Chinchiloidea), vejo algumas semelhanças em termos de forma e uso dos sinais (Figura 2.3; Tabela 2.2). Parece haver compartilhamento de um "núcleo duro" do repertório dessas espécies, sugestivo de uma origem filogenética em Caviidae, e alguns até anterior em Mammalia (grito de agonia). Vou discorrer sobre isso partindo da hipótese filogenética de Àlvarez *et al.* (2017) e de sua estimativa temporal da diversificação das espécies. Que os biólogos entendam que não tenho pretensão de oferecer uma análise filogenética comparada. Fiz isso para o chamado de côrte, em 2012, como vou expor mais adiante no texto, mas só para ele. Minha pretensão é simplesmente contribuir com a visualização integrada do que podem ser sinais comuns entre as espécies, difíceis de serem identificados pelo leitor ou pesquisador que estudou as espécies separadamente. A análise filogenética comparada poderá ser feita, facilitada por esta tentativa de unificação de formas e usos semelhantes, que podem indicar uma origem comum.

Em 1974, John Eisenberg[2], um proeminente mastozoólogo com especial interesse em Rodentia, comparou os repertórios acústicos de 17 caviomorfos aos quais ele tinha acesso no National Zoo Park, em Washington. Ele descreveu os repertórios dos caviomorfos seguindo um sistema de classificação baseado na forma das sílabas (ou notas). De acordo com o padrão de distribuição de energia nas notas, Eisenberg distinguiu dois tipos: se em frequências discretas (tipo I) ou de forma dispersa (tipo II, ruidosa ou caótica; um clique gutural que soa como "có" ou "*cluck*", em inglês; Figura 2.2). O tipo II pode modificar-se em três direções: (a) a cliques mais longos, que vão gradualmente tornando-se harmônicos até chegar ao tipo III (o assobio ou "*wheet*" do filhote isolado da mãe); (b) ao purr da cobaia (diversos cliques emitidos em sequência e rapidamente), ou (c) a um agrupamento menor de unidades que na cobaia, que era a única *Cavia* que Eisenberg tinha, correspondem ao *double chut* de Berryman (1976) e aos *subsounds* de Arvola (1974). No preá, o chutter da cobaia ("tut-tut") soa mais assoprado, como "pru-pru" (mais parecido com o purr e o chamado de alerta de curta distância drrr, figura 2.3) repetidos em um padrão temporal que, como na cobaia, parece acompanhar a locomoção e excitação do emissor (um animal aproximando-se de outro ou farejando um local novo; Monticelli & Ades, 2013; Verzola-Olivio & Monticelli, 2017; Apêndices E e F do cap. 1).

A teoria de Eisenberg sobre a origem dos sinais nos caviomorfos me parece válida, e corresponde à de Andrew (1963) para primatas. Nessa teoria, o cluck seria uma unidade básica, a partir da qual os outros sinais teriam originado-se. Roedores caviomorfos das famílias Caviidae, Dasyproctidae, Cuniculidae, Octodontidae, Echimyidae, Dinomyidae (volte à Fig. 2.1, se precisar, ou veja a Tabela 2.2; Fenerich, Gasco, Magrini, Freitas, Oliveira *et al.*, 2012; Takata, Monticelli & Luchesi, 2012; Fenerich, 2013), e até na ordem Carnivora, o furão *Mustela* (obs. pess.), a lontra *Lontra longicaudis* (Prado *et al.*, 2010: Apêndice D) e o quati *Nasua nasua*, emitem vocalizações semelhantes ao cluck em forma, que usam como chamado de contato. Em quatis, o sinal semelhante ao cluck é usado em contato próximo também, mas comumente na defesa de um item alimentar entre membros do grupo social (Gasco *et al.*, 2016; Apêndice A). Mas, esses clucks não devem ser todos homólogos. Como discute Eisenberg (1974) ao olhar para Caviomorpha, um sinal como o cluck pode ser

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[2] É interessante a forma como ele é descrito por um aluno de pós-graduação já formado, em notícia sobre seu falecimento, em 2003: "*He was constantly sorting, sifting and parsing bits and pieces of information about the life histories of mammals and putting it all together*", Dr. Chris Wemmer, diretor do setor de Conservação do National Zoological Park, em Washington, EUA. É de J. Eisenberg a obra "*The Mammalian Radiations: An Analysis of Trends in Evolution, Adaptation and Behavior* (1981)". Fonte: Nagourney, E. (2003, 20 de julho). *John F. Eisenberg, 68, Dies; Leading Expert on Mammals*. Recuperado de https://www.nytimes.com/2003/07/20/us/john-f-eisenberg-68-dies-leading-expert-on-mammals.html. Recuperado em 21 de maio de 2019.

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produzido por mecanismos e tratos diferentes, ou selecionados por forças comuns que favoreceram a transmissão do sinal com o menor gasto energético. Eu também não me precipito em dizer que os chamados de contato dos caviomorfos têm a mesma origem (não antes de estudá-los pessoalmente).

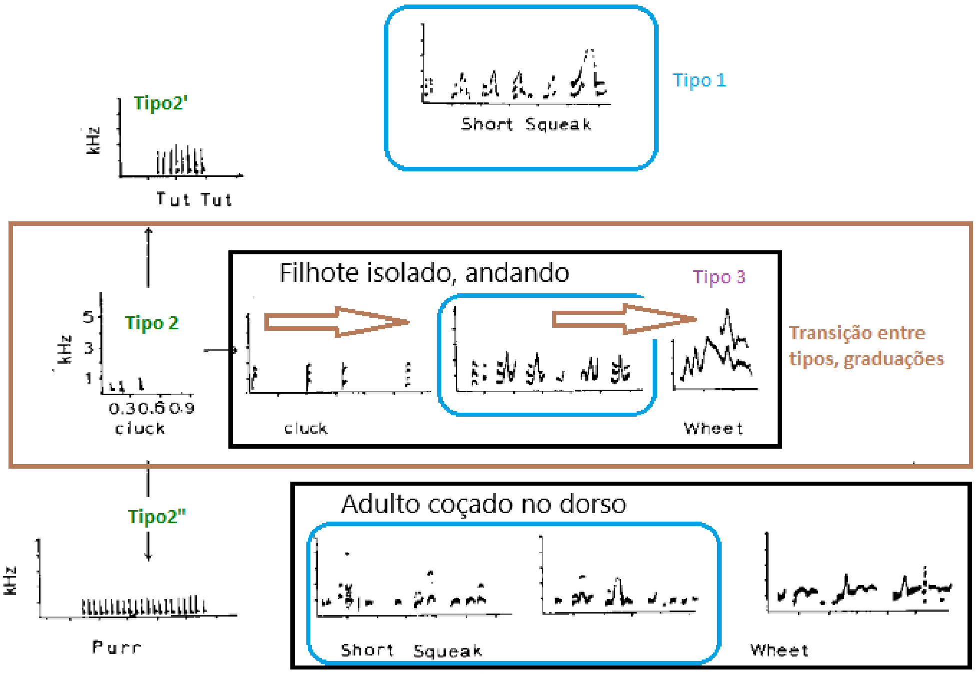


Figura 2.2. Esquema de Eisenberg (1974) de sua classificação das vocalizações de caviomorfos, com base na forma das unidades. Quando a energia se distribui em frequências discretas, o autor as classifica como tipo 1 (em azul; repare na variação na modulação de frequência das notas); se a distribuição é dispersa, é o tipo II, em verde (ruidosa ou caótica; um clique gutural que soa como “coô” ou “*cluck*”). O tipo II pode modificar-se em três direções: a cliques mais longos, que vão gradualmente tornando-se harmônicos até chegar ao tipo III (um assobio ou “*wheet*”, em lilás), ao purr da cobaia (diversos cliques emitidos em sequência e rapidamente) ou a um agrupamento menor de unidades (*double chut* de Berryman, 1976, e aos *subsounds* de Arvola, 1974). Em marrom, as setas indicam transições naturais entre categorias discretas, que também foram descritas como característica compartilhada entre as 17 espécies de caviomorfos de Eisenberg (1974).

Caviomorpha é um grupo muito especioso e diverso de formas, que ocupou quase que todos os continentes (e.g., Alvarez *et al.*, 2017). Mas considero a semelhança entre os “clucks” em Caviinae (talvez também em Dasyproctinae e Cuniculinae, mas eu não os analisei pessoalmente, ainda) e a proximidade entre os repertórios gerais dessas espécies, suficiente para se argumentar a favor disso (Figura 2.3). O cluck é um sinal muito simples em termos de estrutura e forma de produção (é emitido com a boca fechada, é basicamente nasal -

Arvola, 1974 - tem baixa intensidade, portanto supõe-se menor custo energético, e não tem uma estrutura harmônica); aparece desde cedo na vida dos animais (desde o dia do nascimento em *Cavia*) e é emitido pelos dois sexos. O cluck pode ser uma derivação ritualizada de um som naturalmente produzido como resultado ou subproduto da respiração, como sugerido por Tembrock (1963; chamados de "*noises generated by air flow stream current*"). Um argumento a favor dessa hipótese é a de que há uma notável sincronicidade, apontada por autores independentes, na emissão de notas de cluck e na locomoção (Arvola, 1974; Coulon, 1982; Barros *et al.*, 2011).

O cluck é o chamado de contato de curta distância de todas essas espécies (em inglês, *contact calls*) e está apresentado para a comparação estrutural na Figura 2.3. Diferentes nomes foram dados a esses sinais de cada espécie, entre onomatopéias (có, chut e cluck: Eisenberg, 1974; Berryman, 1976; Monticelli, 2005; e estalido ou *click*, para Hydrochoerinae e Cuniculidae: Barros *et al.*, 2011, por causa do ritmo mais rápido), ou descrições morfo-funcionais (*subsounds*: Arvola 1974; Monticelli & Ades, 2013; Verzola-Olívio & Monticelli, 2017; *cri de cohésion sociale*: Coulon, 1982; e estalido para as capivaras: Barros 2009). Para o leitor não acostumado a ver representações visuais de som, as vocalizações da Fig. 2.3 podem parecer diferentes. Mas, como sugerem as onomatopéias adotadas independentemente pelos autores, todos aqueles sons são muito parecidos ao ouvido. Além disso, as descrições das situações de emissão dessas vocalizações são praticamente as mesmas entre os diferentes autores: produzidos em situação social de exploração ou forrageamento, por filhotes que se separam do grupo, ou antes da entrega de alimento em cativeiro (neste caso, apenas descrito para cobaias, capivaras e pacas: Barros *et al.*, 2011; Lima *et al.*, 2018; Ades *et al.*, 1993).

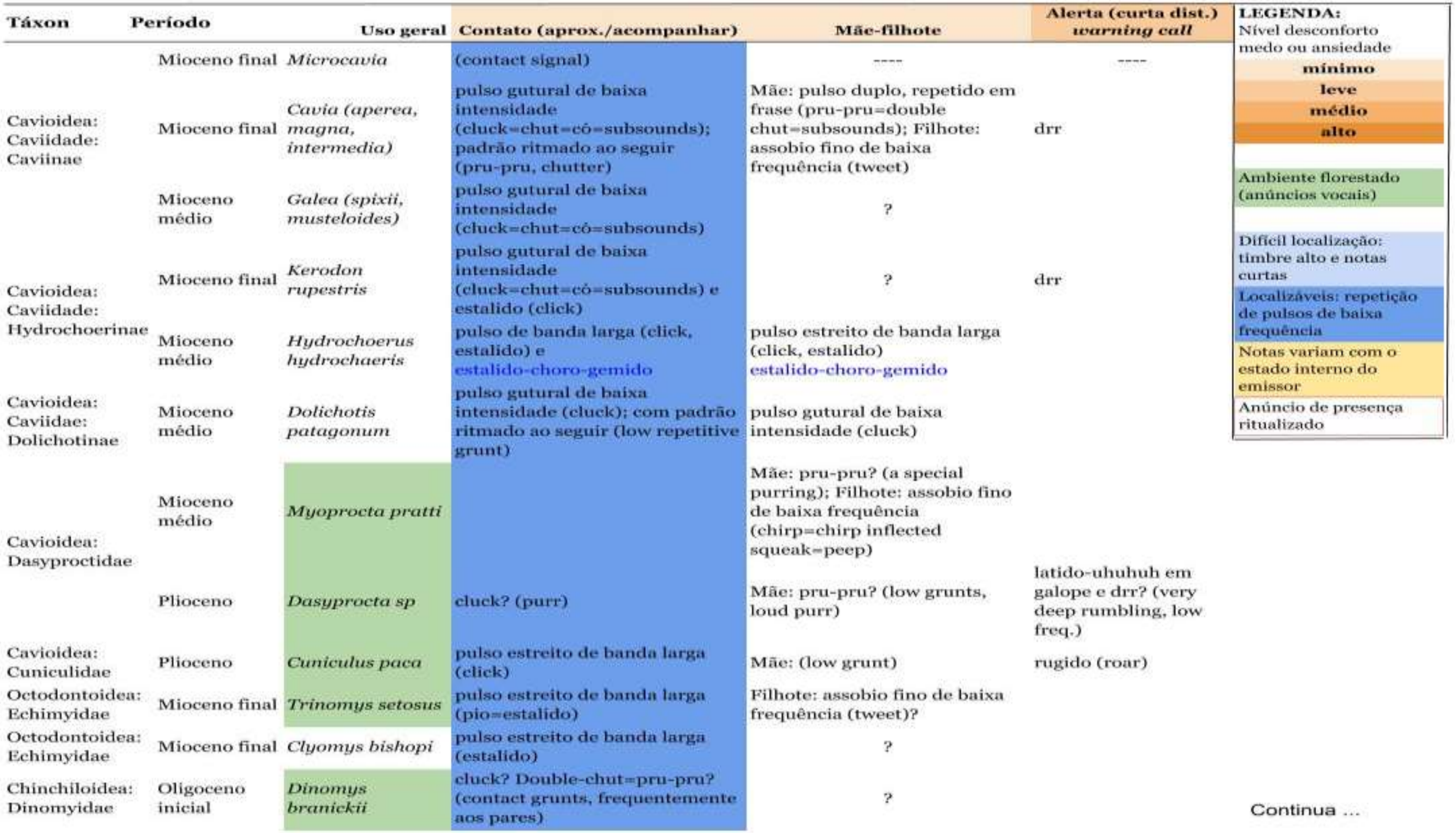
A partir desse sinal simples, outros poderiam ter-se originado por mudanças no padrão temporal de emissão (como o drr, o purr e o double-chut ou pru-pru, Tabela 2.2.) ou na produção de frequências harmônicas, como se vê nos repertórios das espécies que mais estudei: os preás (*C. aperea pamparum*, *C. aperea* de Itu e de Jaboticabal, *C. intermedia* e *C. magna*: Apêndices E a G do Cap. 1); o mocó (*Kerodon rupestris*, Alencar-Jr, 2011: Apêndice E); as capivaras (*Hydrochoerus hydrochaeris*, Geraldi, Olivio, Suzuki, Barros, Tokumaru *et al.*, 2011; Suzuki, 2016; Oliveira-Ueno, 2017; Nievas, 2019; Tabela 2.2).

O seguimento (*following*) parece ser um comportamento importante para as espécies de mais alto nível de socialidade, como pacarana (*Dinomys*), pacas (*Dasy-* e *Myoprocta*), capivaras, mocós e preas. Em preás, o comportamento de adultos durante atividade de exploração é muito característico. Parece que seguem em patrulha, verificando a área e mantêm-se em contato durante isso usando o pru-pru-pru (o mesmo que a fêmea emite quando recebe os filhotes para mamar; Apêndice C). Em cobaias esse comportamento não é tão marcante. Cobaias emitem o chut nessa situação, menos ruidoso do que o pru-pru (ambos correspondem ao cluck, como categoria geral e para facilitar nossa discussão). Os preás, usam o cluck para aproximações um do outro, mas na patrulha usam o pru-pru, uma espécie de cluck ritmado, pulsado onde cada pulso é uma sílaba dupla ou um trio de clucks. Um filhote separado começa emitindo cluck, qdo se move, e depois, se não encontrar a mãe, vai gradualmente virando assobio (conforme aumenta o nível de excitação/ansiedade), como mostra o esquema de Eisenberg na Fig 2.2 (Eisenberg, 1974).

A batida de dentes sim, deve ser uma forma ancestral de Caviomorpha, já que aparece em espécies de famílias diferentes e está bem distribuído entre as espécies. Por exemplo, foi registrado em todas as espécies de Eisenberg (1974), parte delas apresentada na Tabela 2.2. Quatis batem dentes, mas por outros mecanismos (Gasco *et al.*, 2016). A produção do som da batida de dentes foi detalhadamente descrita em Arvola (1974) e poderia ter-se originado por ritualização a partir de respostas autônomas que acompanham ou decorrem de um estado interno alterado de medo ou conflito entre bater ou fugir (Eibl-Eibesfeldt, 1979). Autores notaram de forma independente a relação entre o ritmo (aceleração) e intensidade do som e o aparente nível de perturbação do emissor (e.g., Arvola, 1974; Coulon, 1982; Barros *et al.*, 2011). E essa forma de produção de som foi relatada em roedores caviomorfos (Eisenberg, 1974; Lima *et al.*, 2018) e no myomorfo *Cricetus*, e em canídeos e primatas (Tembrock, 1963).

Outros sinais mecânicos são produzidos pelos caviomorfos e por outros mamíferos, como o tamborilar (Tembrock, 1963). Mas em alguns táxons da tabela 2.2, por exemplo, são feitos com as patas dianteiras, outros pelas traseiras, alguns em situação de excitação sexual, outros em interações agonísticas.

Tabela 2.2. O repertório acústico de espécies de Caviomorfos das superfamílias Cavioidea (Família: subfamília), Octodontoidea e Chinchiloidea a partir da minha interpretação. Os chamados foram separados por categorias de contextos (colunas). Nas células, a descrição física dos chamados é apresentada (entre parênteses: nomes e caracterizações dos autores consultados). "=" indica sinônimos, "?" indica dúvida (ver texto). Tamborilar indica a produção de sons pela batida das patas dianteiras (D) ou traseiras (T). Período refere-se à divergência evolutiva (Oligoceno-Plioceno). À direita é dada a legenda das cores usadas nas células.



Táxon	**Período**	**Uso geral**	**Contato (aprox./acompanhar)**	**Mãe-filhote**	**Alerta (curta dist.) *warning call***
	Mioceno final	*Microcavia*	(contact signal)	----	----
Cavioidea: Caviidade: Caviinae	Mioceno final	*Cavia (aperea, magna, intermedia)*	pulso gutural de baixa intensidade (cluck=chut=có=subsounds); padrão ritmado ao seguir (pru-pru, chutter)	Mãe: pulso duplo, repetido em frase (pru-pru=double chut=subsounds); Filhote: assobio fino de baixa frequência (tweet)	drr
	Mioceno médio	*Galea (spixii, musteloides)*	pulso gutural de baixa intensidade (cluck=chut=có=subsounds)	?	
Cavioidea: Caviidade: Hydrochoerinae	Mioceno final	*Kerodon rupestris*	pulso gutural de baixa intensidade (cluck=chut=có=subsounds) e estalido (click)	?	drr
	Mioceno médio	*Hydrochoerus hydrochaeris*	pulso de banda larga (click, estalido) e estalido-choro-gemido	pulso estreito de banda larga (click, estalido) estalido-choro-gemido	
Cavioidea: Caviidae: Dolichotinae	Mioceno médio	*Dolichotis patagonum*	pulso gutural de baixa intensidade (cluck); com padrão ritmado ao seguir (low repetitive grunt)	pulso gutural de baixa intensidade (cluck)	
Cavioidea: Dasyproctidae	Mioceno médio	*Myoprocta pratti*		Mãe: pru-pru? (a special purring); Filhote: assobio fino de baixa frequência (chirp=chirp inflected squeak=peep)	
	Plioceno	*Dasyprocta sp*	cluck? (purr)	Mãe: pru-pru? (low grunts, loud purr)	latido-uhuhuh em galope e drr? (very deep rumbling, low freq.)
Cavioidea: Cuniculidae	Plioceno	*Cuniculus paca*	pulso estreito de banda larga (click)	Mãe: (low grunt)	rugido (roar)
Octodontoidea: Echimyidae	Mioceno final	*Trinomys setosus*	pulso estreito de banda larga (pio=estalido)	Filhote: assobio fino de baixa frequência (tweet)?	
Octodontoidea: Echimyidae	Mioceno final	*Clyomys bishopi*	pulso estreito de banda larga (estalido)	?	
Chinchiloidea: Dinomyidae	Oligoceno inicial	*Dinomys branickii*	cluck? Double-chut=pru-pru? (contact grunts, frequentemente aos pares)	?	

LEGENDA:
Nível desconforto medo ou ansiedade
mínimo
leve
médio
alto

Ambiente florestado (anúncios vocais)

Difícil localização: timbre alto e notas curtas
Localizáveis: repetição de pulsos de baixa frequência
Notas variam com o estado interno do emissor
Anúncio de presença ritualizado

Continua ...

Tabela 2.2. Continua

		Uso geral	**Alarme/intimidação** ***alarm call***	**Agonístico (ofensivo/intimidatório)**	**Autodefesa, submissão, afiliação**
Superfam.: Fam.: Subfam.	**Origem**	**Táxon**			
	Mioceno final	*Microcavia*	piado (tsit=bird song)	batida de dentes	choro (twitter)
Cavioidea: Caviidade: Caviinae	Mioceno final	*Cavia (aperea, magna, intermedia)*	piado (song=chirrup)	grunhido (grunt=pru-pru) e batida de dentes	cluck até choro whine e choro gritado (slow wheet-wheet=bubbling squeaks=chutter=after shriek=sociable squeal)
	Mioceno médio	*Galea (spixii, musteloides)*	piado (bark)	grunhido (stutter) Relincho, tamborilar T, batida de dentes	Choro whine e choro gritado (slow wheet-wheet=bubbling squeaks=chutter=after shriek=sociable squeal)
Cavioidea: Caviidade: Hydrochoerinae	Mioceno final	*Kerodon rupestris*	piado (alarm whistle=bark)	grunhido (grunt), bufada (arfar, nasal hiss), batida de dentes	cluck até whine (click, whine=churr), choro gritado (ganido=yelp) e ronco (snore)
	Mioceno médio	*Hydrochoerus hydrochaeris*	latido ruidoso de banda larga (latido=bark)	grunhido de três notas (cacarejo) e batida de dentes	cluck até whine (click, whine), assobio modulado e cacarejo
Cavioidea: Caviidae: Dolichotinae	Mioceno médio	*Dolichotis patagonum*	piado? (sharp squeak) com saltitar (stotting)	grunhido (low grunt) e batida de dentes	Choro whine e choro gritado (prolonged wheet dropping to a terminal grunt of low intensity)
Cavioidea: Dasyproctidae	Mioceno médio	*Myoprocta pratti*	tamborilar (T)?	bufada (snort=coughing sound) e batida de dentes	Choro e assobio (whine=mewing e inflected squeaks)
	Plioceno	*Dasyprocta sp*	(sequência de grunhidos curtos, alarm bark; high-pitched bark)?	grunhido ou latido (short growl=fight grunt) e batida de dentes	(appeasement squeaks, creak-squeak, low grunts, purr loud)
Cavioidea: Cuniculidae	Plioceno	*Cuniculus paca*	latido ruidoso de banda larga (latido=bark)	grunhido (ronco, snore), rugido (rosnado, prolonged growl, growl) e batida de dentes	grunhido (ronco, snore), rugido (rosnado, prolonged growl, growl) e batida de dentes
Octodontoidea: Echimyidae	Mioceno final	*Trinomys setosus*	tamborilar (T)	grunhido (grunt) e batida de dentes	choro-trinado e tamborilar (T) e choro harmônico modulado (low whine)
Octodontoidea: Echimyidae	Mioceno final	*Clyomys bishopi*	piado	grunhido (grunt), grasnado (em U-invertido) e batida de dentes	choro de banda larga, choro-trinado e grito curto
Chinchiloidea: Dinomiidae	Oligoceno inicial	*Dinomys branicki*	chiado (hiss) com tamborilar (D) e assobio (wha whee)	rugido (growl of considerable amplitude) e batida de dentes	Choro e choro-trinado (tremulus whine pulsed or fused; low inflected squeaks=staccato whimper), chiado+tamborilar D (hiss+stamping)

Tabela 2.2. Continua

		Uso geral	**Agitação social *(arousal/annoyance)***	**Separação (filhotes)**	**Longa distância *loud call***	**Grito de dor ou agonia**
Cavioidea: Caviidade: Caviinae	Mioceno final	*Microcavia*	twitter, assobio ao tratador (expectativa de item apreciado)	?	----	(shriek)
	Mioceno final	*Cavia (aperea, magna, intermedia)*	choro gritado com final ascendente, notas variáveis (agitating squeak)	Assobio naso-oral (assobio de separação=ao tratador=isolation=high whistle)	*C. intermedia* e *C. magna*: pio (peep)	quack ou grito de agonia (scream=shriek)
	Mioceno médio	*Galea (spixii, musteloides)*	choro gritado com final ascendente, notas variáveis (whine+squeak)	?	Tamborilar T	(shriek=squeak)
Cavioidea: Caviidade: Hydrochoerinae	Mioceno final	*Kerodon rupestris*	choro gritado com final ascendente, notas variáveis (whine+squeak, ganido),	Assobio naso-oral (assobio de separação)	silvo (slow Whistle)	tsick ou scream
	Mioceno médio	*Hydrochoerus hydrochaeris*	choro gritado com final ascendente, notas variáveis (whine/squeal)	Assobio-v, piado, choro-gemido, choro-modulado e notas compostas estalido-assobio e estalido-choro-modulado	assobio, cry (choro-assobiado)	grito-de-agonia, grito-arfar, grito-estalido.
Cavioidea: Caviidae: Dolichotinae	Mioceno médio	*Dolichotis patagonum*	choro gritado com final ascendente, notas variáveis (agitating squeak)	Assobio naso-oral (isolation whistle=high whistle)	piado? (sharp squeak=wheet)	----
Cavioidea: Dasyproctidae	Mioceno médio	*Myoprocta pratti*	(courting squeak)	(squeak; chirp)	choro com tamborilar T	(sharp squeal)
	Plioceno	*Dasyprocta sp*	----	Assobio oral-nasal (high squeak ending in a soft grunt; creak-squeak=a high-pitched squeak followed by a definite staccato creak)	sequencia de pequenos grunhidos (alarm bark)?	grito (distress scream)
Cavioidea: Cuniculidae	Plioceno	*Cuniculus paca*	choro harmonico não modulado	Filhote: cry; Mãe: bark ou batida de dentes com tamborilar T		gemido-rugido (groan+roar)
Octodontoidea: Echimyidae	Mioceno final	*Trinomys setosus*	choro-trinado+tamborilar (T)	----	assobio longo descendente?	----
Octodontoidea: Echimyidae	Mioceno final	*Clyomys bishopi*	grasnado + choro de banda larga ou choro-trinado?	----		quack ou grasnado estridente (growl very loudly)
Chinchiloidea: Dinomiidae	Oligoceno inicial	*Dinomys branicki*	(staccato whimper= tremulous whine)	assobio (wha whee)	(song; nyak-nyak em Chinchilla)	----

As estimativas temporais e relações filogenéticas foram feitas com base em Álvarez et al (2017). Fontes: Rood (1970, 1972); Eisenberg (1974); Smythe (1978); Lacher (1981); Marquet et al. (1993); Alencar-Jr (2009); Kessler et al. (2009); Suzuki (2016)

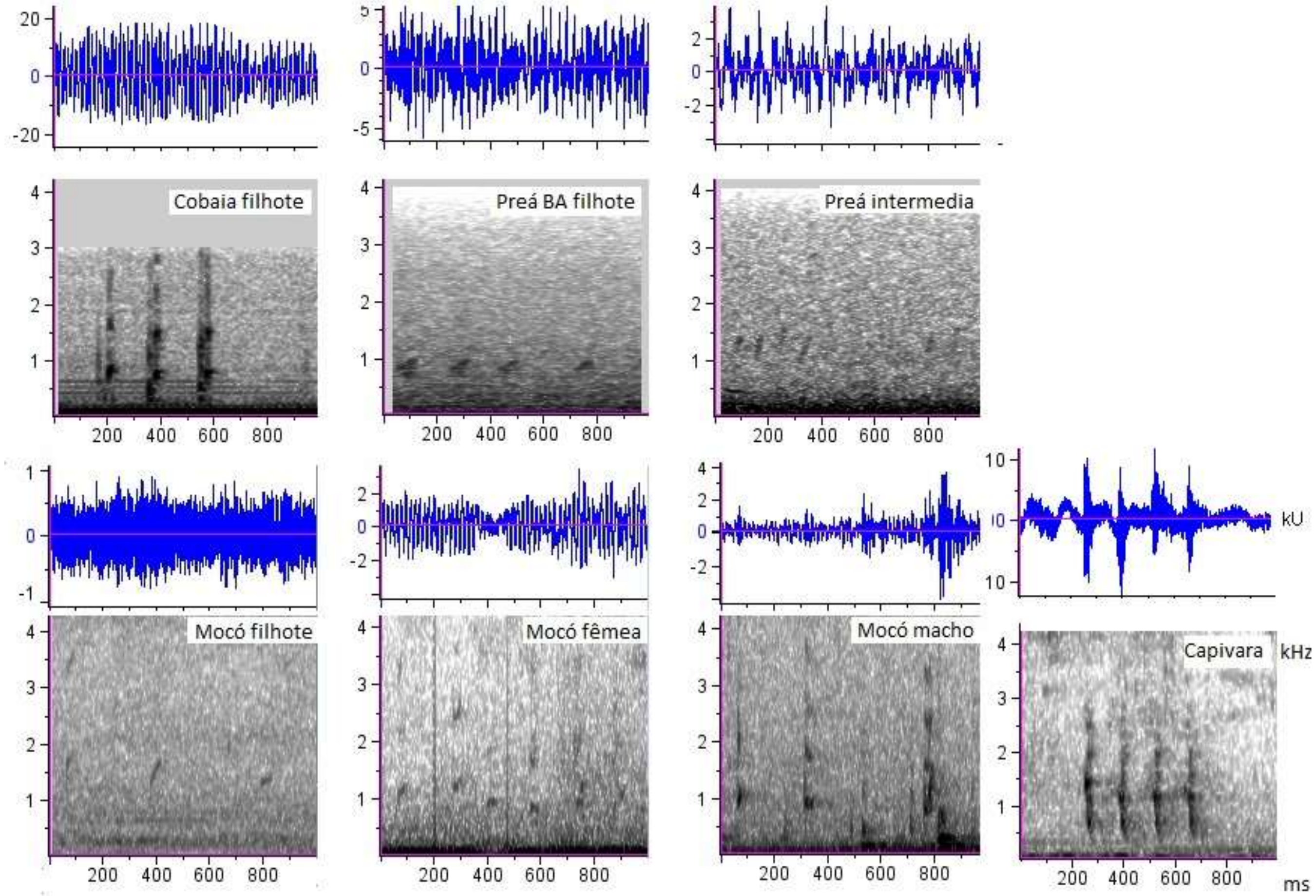


Figura 2.3. Trechos típicos dos chamados de contato dos cavíneos dos gêneros *Cavia* (cobaia e preás *C. aperea pamparum* originários de Buenos Aires, BA, e *C. intermedia*), *Hydrochoerus* (capivara) e Kerodon (mocó). Compare a estrutura geral dessas unidades com o esquema e a descrição de Eisenberg (1974, Fig. 2.2 e texto). Todas as imagens estão na mesma escala de tempo (ms, eixo horizontal) e frequência (kHz, eixo vertical), mas variam em termos de intensidade sonora (kU) e razão sinal/ruído (diferença entre tons de cinza). Ao ouvido, todos, exceto a última vocalização de cobaia (abaixo à direita), soam como secuencias de "tu" (e.g., tu-tu-tu em cobaias na primeira imagem, tu-tu-tu-tu nas três imagens seguintes), com diferenças no ritmo de emissão (e.g., 3,12 notas/s em cobaia e 7 n/s em capivara, na primeira linha). Todos os registros que usei para a produção dos espectogramas foram retirados do acervo de sons de mamíferos terrestres neotropicais da Fonoteca César Ades (FOCA), criada e mantida pelo Laboratório de Etologia e Bioacústica (EBAC).

O repertório de comportamentos reprodutivos dessas espécies também é interessante, pela elaboração. Roedores caviomorfos produzem filhotes precoces e têm cio pós-parto. O macho começa uma guarda intensiva da fêmea já alguns dias antes do parto. E algumas horas depois, copula com ela, se tiver conseguido afastar os outros machos e se ela aceitar. Nessa guarda e exibição à fêmea, todas as espécies da tabela 2.2, de Caviidae, Octodontidae e Chinchillidae, usam algum comportamento estereotipado, típico desse contexto: em *Cavia*, a rumba e o movimento em 8 ao redor da fêmea, que também é feito pelos outros preás - *Microcavia, Galea* - e pela mara *Dolichotis*. Em Hydrochoerinae (do mocó e da capivara) que compartilham boa parte dos padrões comportamentais com Caviinae, a estratégia sócio-reprodutiva parece ser outra: os machos não guardam as fêmeas diretamente, eles mantêm um território com itens essenciais à sobrevivência (o microclima úmido dos rochedos na Caatinga, para os mocós, e a água, para as capivaras). Não há um *display*, i.e., uma exibição visual ritualizada específica. Em um registro que fizemos do que pareceu ser um episódio reprodutivo em mocós de vida-livre, a fêmea movia-se pela parte externa do rochedo e o macho a seguia; quando ela parava, ele fazia contato naso-anal, ela emitia um quack e saia, e ele ia atrás novamente (Alencar Jr, 2009). Se eles estivessem vocalizando em baixa intensidade (como é o purr de *Cavia*), não teríamos conseguido ouvir. Em capivaras, vimos algo semelhante, mas o macho seguia a fêmea dentro da água, e ela emitia um trinado, que só registramos nesse episódio (Suzuki, 2016). Em cutias e pacas (Dasyproctidae e Cuniculidae), os machos usam um tremor das patas dianteiras ou traseiras (de onde a rumba de *Cavia* poderia ter-se derivado) enquanto emitem um assobio trinado.

Em Monticelli e Magrini (2012) fizemos o mapeamento filogenético dos *displays* de corte e dos sinais (sem vocalização, com purr ou com whimper) em Rodentia, a partir de 37 táxons, usando uma espécie de Lagomorfa como grupo externo. A reconstrução do estado ancestral do purr sobre a filogenia de Uphan e Patterson (2015), resultou em ao menos quatro origens independentes, pelo método da parcimônia: duas em Sciurognathi (no rato-toupeira e nos esquilos arborícolas *Sciurus*), uma em Octodontoidea (degus, tuco-tucos e ratos-de-espinho), outra em Cavioidea (exceto *Cuniculus*), e ao menos quatro perdas. O estado ancestral ficou ambíguo (ausente ou purr) para todos os ancestrais imediatos de Octodontoidea (exceto para o ancestral de Octodontinae, que teria o purr) - e para Cavioidea (exceto *Cuniculus*) e Caviinae. Dentro de Cavioidea, o purr foi reconstruído inequivocamente apenas para o ancestral de Cavia+Microcavia. O purr foi o estado ancestral mais provável em Caviinae (61%) e Octodontinae (71%), e a ausência dele foi o estado mais provável do ancestral de Caviinae+Dolichotinae+Hydrochoerinae (68%). O whimper apareceu em *Dinomys*, *Erethizon* e *Myoprocta,* que são clados independentes. Esse mapeamento precisa ser refeito sobre a filogenia mais atual de Álvarez *et al.* (2017), após a inclusão de dados de novos táxons.

A semelhança entre estrutura e contexto de uso de sinais logicamente é maior quando o nível de análise é Caviidae, que também é onde posso dar maior contribuição. Encontramos (eu e meus alunos) um repertório acústico composto de pelo menos 10 sinais distintos em *Cavia aperea*, *C. intermedia* e *C. magna*, mais uma categoria (*peep*) nas espécies do Sul (Monticelli e Ades, 2013: Apêndice E do Cap 1; Verzola, 2014 e Verzola-Olivio e Monticelli, 2017: Apêndices F e G, Cap 1); há pelo menos 11 sinais no repertório do mocó *Kerodon rupestris*, e pelo menos 14 no da capivara (Fig. 2.2 e 2.3; Barros *et al.*, 2011; Suzuki, 2016; Oliveira-Ueno, 2017).

O tamanho do repertório de todas essas espécies pode estar subestimado: as descrições foram feitas em um primeiro nível de análise, pelo agrupamento dos tipos mais distintos em categorias que consideramos ser unidades básicas; mas, algumas dessas categorias, como a dos chamado de contato de *Cavia*, os latidos de mocós (Alencar-Jr, 2011) e capivaras (Oliveira-Ueno, 2017) e os gritos e choros de todas essas espécies, possuem variações internas e tipos intermediários (como se viu em outros histricomorfos: Eisenberg, 1974). Essas variações, em uma segunda análise, podem vir a ser separadas em um número maior de categorias, se forem encontrados padrões distintos, aumentando o numero de unidades básicas. Estimo que em *Cavia* a variação nos "*subsounds*" (Fig 1 do Apêndice E e Fig. 3 do Apêndice F) represente 2 ou 3 categorias distintas em forma e uso (ou tipo de respostas), e que haja ao menos dois choros diferentes, um comumente emitido pela fêmea na interação com um macho e outro por animais de ambos os sexos frente a um animal dominante (algo como um choro de subordinação). Eisenberg (1974) parece separar esses choros nas situações "*avoiding while being approached*", "*when defeated*" e "*when being groomed*". Eu concordo que possam mesmo haver distinções mais discretas nesses choros e ainda no choro que o agressor faz durante a exibição agonística, como sugerido por Coulon (1982) para *Cavia porcellus*.

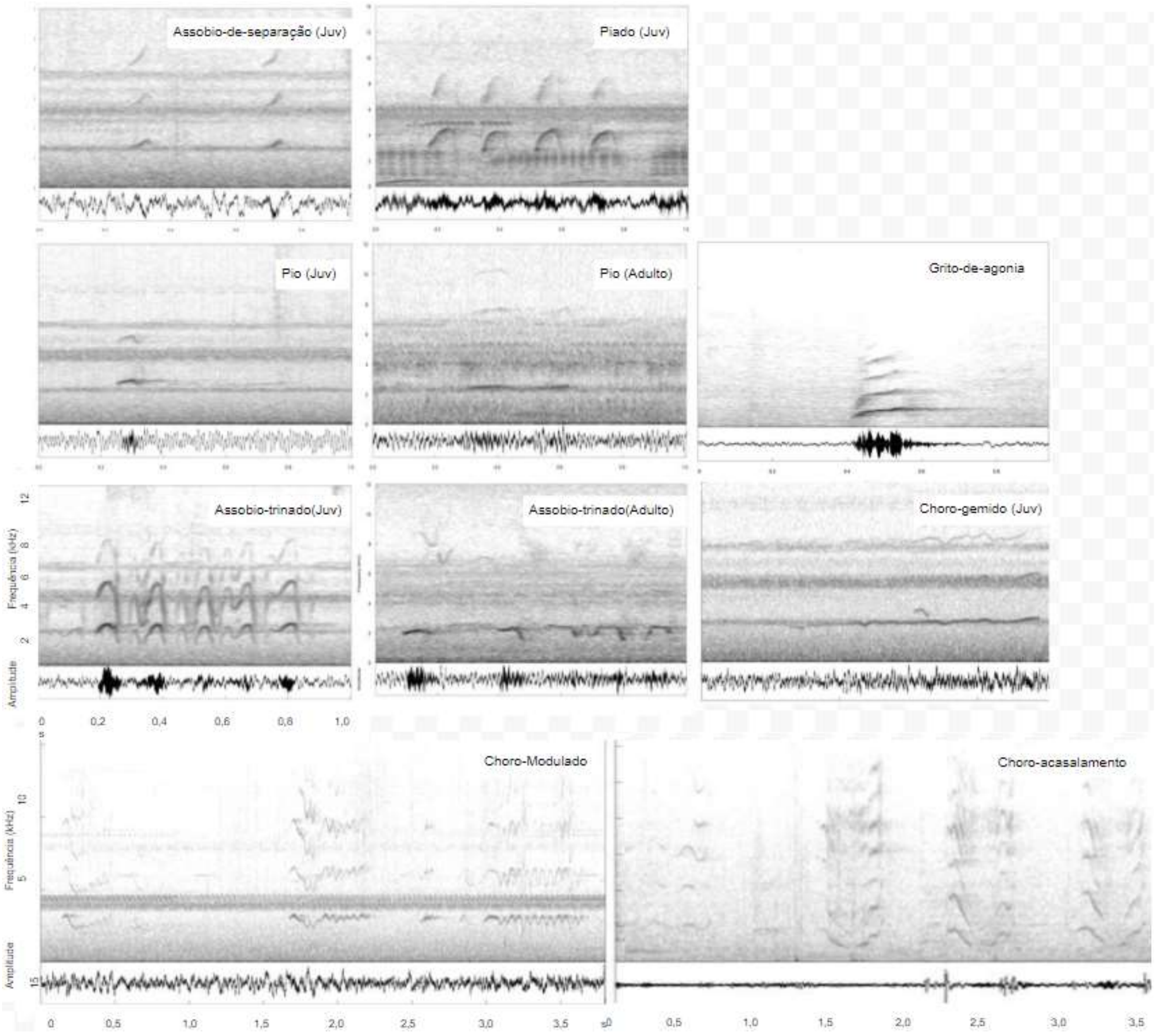


Figura 2.2. Repertório de sinais harmônicos de capivaras, descrito Suzuki (2016). Estão representadas 8 unidades básicas: assobio-de-separação, assobio-trinado e piado e pio (em duas fases ontogenéticas: juvenil e adulto), emitidos durante separação; grito-de-agonia, de animal contido em puçá; choro-gemido, de juvenil apressando o passo na direção do grupo que iniciou o deslocamento; choro-modulado, emitido por juvenil, quando a amamentação foi interrompida pela saída da fêmea), e choro-ascendente, registrado de uma fêmea durante ritual de acasalamento dentro da água. Duração em segundo (abscissas), frequência sonora em kHz (ordenadas) e onda sonora (amplitude x tempo) abaixo de cada figura. As imagens foram extraídas e editadas de Suzuki (2016).

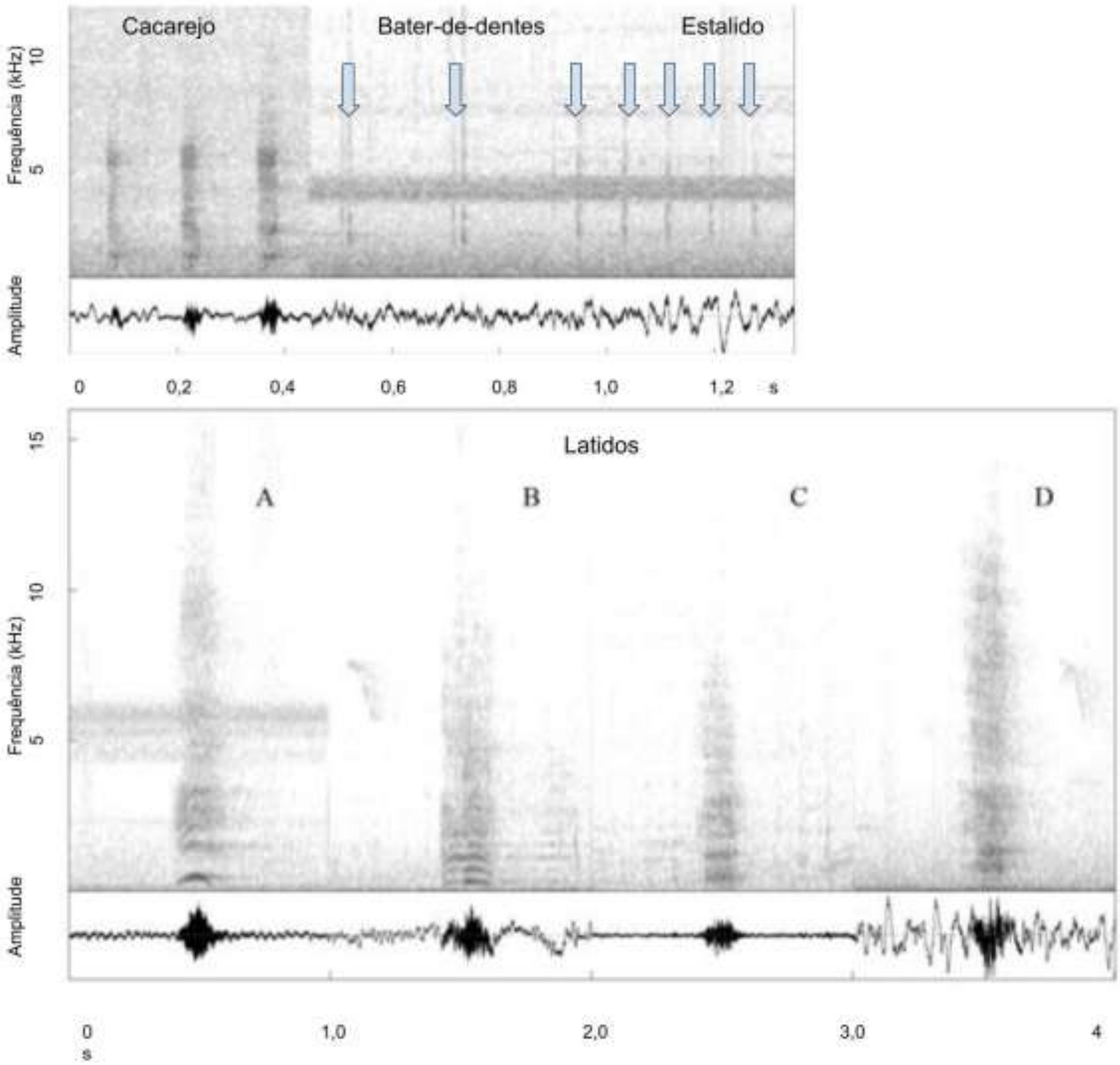


Figura 2.3. Repertório de sinais ruidosos de capivaras, descrito na dissertação de mestrado de Cintia Tomoe Suzuki. Estão representadas 4 unidades básicas: cacarejo, batida de dentes, estalido e latidos, na mesma escala temporal e de frequência acústica. Abaixo, representam-se os latidos que parecem variar em estrutura de acordo com a idade ou contexto: (A) frente à aproximação de perigo (pessoas ou automóveis), (B) durante o nado, (C) frente à oferta de alimento (em zoológico), e (D) animais mantido presos em curral para o manejo. Nas abscissas, representa-se o tempo em segundos, e nas ordenadas, a frequência sonora em kHz. Imagens extraídas e editadas de Suzuki (2016).

Existe uma relação difícil de se fazer entre as classes gerais de contextos de emissão (na Tabela 2.2, agonístico, apaziguamento, socialização, por exemplo) e os estados internos (subjetivos) do emissor: conflito motivacional, medo, determinação em obter os recursos disputado, por exemplo, podem estar presentes em qualquer um desses contextos, e ainda em outros. E por isso, presumivelmente, que alguns sinais são registrados em mais de um contexto. Mas alguns padrões podem ser notados. Por exemplo, na classe dos *warning signals*, normalmente traduzido para sinais de alerta (melhor seria aviso mesmo), o estado do emissor seria de um nível de "contraste médio" entre a situação geral do ambiente antes e durante um estímulo (Andrew, 1963, Eisenberg, 1974, Coulon, 1982), decorrente da aproximação de um predador (quando o ataque não é iminente), ou qualquer perturbação no meio social. Nessa condição de perturbação "média" (mild), pode-se registrar o tamborilar com as patas dianteiras (D) em cutias e nas pacaranas *Dinomys*, e com as patas de trás (T) em pacas Cuniculus e em um Chinchillidae, o Lagostomus (Eisenberg, 1974). Smythe (1978) descreveu uma sequência de latidos graves como parte de um display de intimidação de predador em Dasyprocta, como eu vejo que seja o chirrup de *Cavia* e assobio/latido de alarme do mocó, *Kerodon*. Preás *C. aperea* em situação natural, emitem o chirrup em meio à plantação de cana, por exemplo. É muito difícil apontar de onde vem o sinal (eu tentei!); e mocós dão seu alerta de alta intensidade de algum lugar onde estão muito visíveis: no descampado ou no topo de um rochedo, ao lado de ou de costas para outros mocós (como em *Lagidium peruanum*). Na mara *Dolichotis*, há uma "marcha saltada" (*stotting gait*) que, como nas gazelas de thomson (*Gazella thomsonii*), parecem sinalizar ao predador - e não a coespecíficos - que o emissor não será facilmente pego (pela sua capacidade física e por já ter notado a presença do predador; Zahavi & Zahavi, 1998). *Dolichotis* está em área aberta, como a gazela, e ao contrário de *Dasyprocta*, que vive em áreas florestadas (Eisenberg, 1974).

Repertórios acústicos em relação com a socialidade

Há uma previsão de que o tamanho de grupo de uma sociedade animal, seja de aves ou mamíferos, está relacionado ao nível da complexidade do sistema de comunicação intraespecífico (Hipótese da Complexidade Social, HCS; Freeberg, Dunbar e Ord, 2012). Robin Ian M. Dunbar (Univ Oxford), um dos proponentes da HCS, é um nome consagrado na primatologia, antropologia, psicologia evolucionista, autor de títulos como "*Primate social systems*" (1988), "*Grooming, gossip and the evolution of language*" (1996), "*How Many Friends Does One Person Need?* " (2010). Ele também é o proponente da Hipótese do cérebro social (ou da "Inteligência Maquiavélica" de Byrne e Whitten, 1990). Sociedades primatas são complexas, no sentido de que impõe dificuldades adicionais àquelas que qualquer indivíduo já enfrenta na vida. Pense em todas as habilidades que usamos ao tentar manter uma boa relação com alguém (prestar e dar atenção, elogiar, acolher, evitar conflitos, por exemplo, evitando generalizações negativas). Indivíduos de uma sociedade não-primata também enfrentaram os desafios da vida grupo (Lee, 1994) e relacionam-se com indivíduos de perfis diferentes que estão acima ou abaixo do seu posto hierárquico, que são egoístas ou não, que agem como adultos ou como filhotes (Blumstein, 2007a, para marmotas; Oliveira-Ueno, 2017, para capivaras; Lima *et al*, 2018, para pacas). Essa "expertise" pode ter sido selecionada ao longo da evolução dessas espécies pressionada pela complexidade da vida em grupo que requer reconhecimento individual, a memorização das interações passadas com cada membro e a capacidade de usar isso para decidir como agir no futuro com cada um (Dunbar, 2010).

O mocó, *Kerodon sp*, e a capivara, *Hydrochoerus sp.* (subfamília Hydrochaerinae; Rowe and Honeycutt 2002) são semelhantes em muitos aspectos, inclusive no tamanho do repertório acústico básico (Lacher, 1981; Alencar, 2012; Barros, *et al.*, 2011; Suzuki, 2016). Mas o fato é que as capivaras usam uma diversidade maior de sinais a partir do repertório básico, recombinando os tipos, como já havia sido descrito em aves (e.g., Schaedler, Ribeiro, Guaraldo & Manica, 2019). Ao estudar seu sistema de comunicação a partir de uma população de vida-livre por dois anos, notamos um repertório "extra" de sinais constituído a partir da junção de duas ou mais categorias básicas (Fig. 2.4). Chamamos de sílabas combinatórias o agrupamento de unidades com intervalo entre elas, e de notas combinadas, a junção sem intervalo interno (Suzuki, 2016).O conjunto de unidades básicas, que parece ser boa parte compartilhado pelas espécies de Caviidae (Tab. 2.2), pode gerar diversidade de sinais através da combinação de tipos. Também vimos isso em quatis (Carnivora: Procyonidae; Gasco, Ferro e Monticelli, 2018, Apêndice A deste capítulo). Essas unidades básicas (ou "*single units*", Bregman e Gentner, 2010) que se combinam de forma específica e, nessa nova forma, "transmitem diferentes informações" (ou simplesmente geram reações distintas; Rendall, Owren e Ryan, 2009), representam sistemas de comunicação "sintaticamente complexos" (Bregman e Gentner, 2010).

Esses sistemas tem as vantagens da redundância e da variabilidade de formas (Bregman e Gentner, 2010). A redundância é especialmente relevante em ambientes ruidosos: como as unidades são combinadas de forma não-aleatória, o reconhecimento de um elemento dessa nova unidade favorece a previsão da sequência (Bregman e Gentner, 2010); e a possibilidade teoricamente infinita de combinações possíveis, permite que se aumente o repertório de sinais a partir de estruturas já existentes. Essa "eficiência combinatória" é justamente uma das características da linguagem humana (Bregman e Gentner, 2010). Nós descrevemos o sistema de recombinação de unidades básicas na composição de novos sinais em quatis, inclusive registramos o uso regras "gramaticais" na composição de sílabas (ordem não aleatória de unidades em uma sequência vocal; Gasco, Ferro & Monticelli, 2016, Apêndice A), como havia sido descrito em macacos neotropicais mono-carvoeiros (Demolin, Ades & Mendes, 2010).

Os efeitos não-lineares em vocalizações de mamíferos

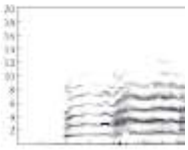
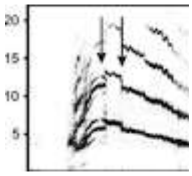
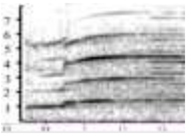
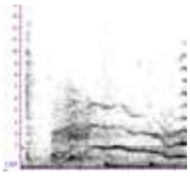
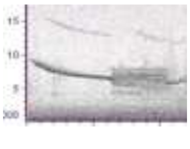
O trato vocal é um sistema dinâmico que em alguns momentos produz episódios intermitentes (ou fases) não-lineares, particularmente em vocalizações que atingem frequências mais altas, como gritos e choros (Wilden, Herzel, Peters e Tembrock, 1998; Suthers, Narins, Lin, Schnitzler, Denzinger, Xu & Feng, 2006). A presença, tipo e distribuição desses fenômenos nas vocalizações precisam ser descritas a partir da terminologia da dinâmica não-linear da matemática (Tokuda Riede, Neubauer, Owren & Herzel, 2002). Como dito no capítulo anterior, os choros estridentes de bebês humanos (que seriam melhor chamados de gritos) são especialmente bons em chamar a atenção e evocar respostas em que os ouça. É possível que essa capacidade de provocar reações decorra da presença de efeitos não-lineares que tornam o som irritante e difícil de ignorar ou habituar-se a ele, pela sua imprevisibilidade, como sugerem Fitch e Hauser (2002). Determinado grito pode apresentar-se como um "ciclo limite" ("*limit cycle*"), no qual o espectro da onda sonora compõe-se de uma frequência fundamental (F0) com harmônicos, que são séries de multiplos inteiros dessa freqüência, ou pode apresentar um ou mais dos efeitos não-lineares (ENL) descritos abaixo e ilustrados na figura 2.4:

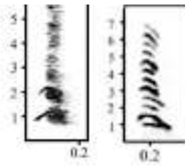
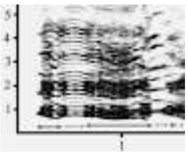
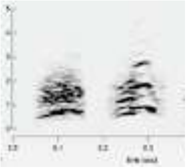
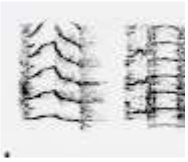
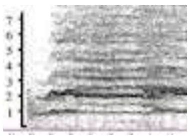
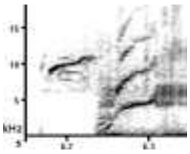
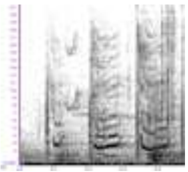
a. Regime sub-harmônico (ou simplesmente sub-harmônicos, SH): são componentes espectrais vistos entre a série harmônica, em múltiplos de 1/2 ou 1/3 de F0 (Fig. 2.5, seta indicada por A, abaixo à direita)
b. Bifonação: a ocorrência simultânea de duas frequências fundamentais independentes, por convenção chamadas de F0 e G0.
c. Caos: um segmento de banda larga sem harmônicos no espectro. Estocástico (um ruído Gaussiano randômico, cujo estado atual não permite prever seu estado futuro) ou previsível, denominado de caos determinístico (CD), gerado por um sistema não-linear (Figura 2.4).
d. Bifurcação: transição entre estados dinâmicos não-lineares (SH e CD, por exemplo). Também chamadas de "saltos de frequência" (SF, "*frequency jumps*", Wilden, *et al.*, 1998; Fig 2.5, seta indicada por E)
e. Ondulação ou vibrato (warble para Blumstein *et al* ., 2008; articulation effect flutter, Pokrovskaya 2013): são modulações de frequência rápidas e de baixa amplitude, que se vêem como ondas baixas e repetidas no espectrograma.

Transições entre as diferentes fases não-lineares decorrem de propriedades intrínsecas dos sistemas de vibração da laringe, como foi bem descrito por Fitch, Neubauer & Herzel (2002), e sem a necessidade de um sistema nervoso complexo de controle. Os ENL são gerados pelo acoplamento ou oscilação assincrônica das pregas vocais, por variação na pressão do ar que vem dos pulmões ou por tensionamento assimétrico dos músculos horizontais e verticais de cada prega (Wilden *et al.*, 1998), ou ainda, pela presença de "lábios" ou remanescentes de membranas vocais nas pregas, como observado em alguns mamíferos (Riede, Herzel, Mehwald, Seidner, Trumler, Böhme, e Tembrock, 2000). Mesmo uma pequena assimetria na tensão das pregas pode produzir uma G0 (Wilden et al., 1998), comumente nas frequências mais altas do som e sob condições muito particulares de produção sonora (Mergell & Herzel, 1997: passagem alargada de ar pelas pregas, fechamento incompleto da glote e quando há formação de formantes pela vibração coincidente da frequência fundamental com a frequência de ressonância do tubo que produz o som).

A presença e descrição de ENLs em vocalizações tem sido documentada apenas recentemente, primeiro em mamíferos, mas também em aves e peixes. Também foram descritos em vocalizações de mamíferos marinhos, como as baleias orca (*Orcinus orca*) e franca (*Eubalaena glacialis*; Tyson, Nowacek e Miller, 2007) e no manatin (peixe-boi, Mann, O'shea e Nowacek, 2006). A prevalência em mamíferos pode sugerir um papel comunicativo dos ENL, que vale a pena ser investigado (Wilden *et al.*, 1998).

O casal Volodin e Volodina do Zoológico de Moscou, vêm estudando há mais de duas décadas os ENLs, sua forma, função e produção (em uma variedade de ungulados e de canídeos e em roedores Sciurognathi, as marmotas e esquilos). Em Volodin, Volodina & Isaeva (2001), eles apresentam uma ampla revisão da literatura sobre o aparecimento de ENLs e notam a prevalência em humanos e outros mamíferos ainda em desenvolvimento (Herzel & Reuter, 1997) ou com distúrbios fisiológicos diversos (e.g., hérnia de disco, doenças laríngeas, traumas cerebrais). Nesses casos, o surgimento de ENL em choros, gritos e latidos não parecem ter vantagem ao emissor, uma vez que soam aversivos à coespecíficos e podem atrair a atenção de predadores. Mas, sendo um subproduto comum do mecanismo de produção de som nesse grupo animal, é possível que alguns tenho sido incorporados como sinais de comunicação intraespecíficos, pelo processo de ritualização de sinais, por conferir vantagens ao emissor. Por exemplo, aumentando a variabilidade sonora de um grito, produzindo assim marcadores extras para o reconhecimento individual mais apurado, produzindo dicas sobre o tamanho do emissor à longas distâncias (as vezes de forma enganosa, como notaram os autores com base na teoria da estrutura dos sinais, de Morton 1977: sub-harmônicos tornam o som mais grave, mesmo de animais pequenos, soando como som de animais maiores), favorecendo a estimativa da distância do emissor e da direção de seu movimento; (Volodin *et al* ., 2001). Ainda, a constante variação que os ENLs (caos e sub-harmônicos) produzem nos chamados, tornaria difícil habituar-se a eles e ignorá-los, como foi sugerido em Owren e Rendall (1997), mantendo a mãe atenta e reativa a gritos dos filhotes (Fitch *et al.*, 2002).

Situação de emissão	Amostra obtida de	Ordem: Fam/Superf.	Táxon	Tipo de ENL	Fonte	Imagem*
Risco extremo: aproximação humana/predador à animal preso ou seguro em mão/boca humano/predador	filhote	Rodentia: Sciurognathi	marmota de barriga amarela (*Marmota flaviventris*)	CD, SH, BF, ON	Blumstein *et al.* 2008	
	filhote	Rodentia: Sciurognathi	esquilos terrestres (Spermophilus sp)	SH, FJ, SB	Matrosova *et al* . 2012	
	filhote	Artiodactyla: Suidae	porco doméstico	CD, SH	Tokuda *et al* ., 2002	
	adulto	Rodentia: Hystricognathi	Capivara	CD, SH, BF, ON	Suzuki 2016	
	adulto	Rodentia: Hystricognathi	Moco	CD, SH, BF, ON	Alencar Jr 2010	
Risco médio: aproximação/avistamento humano/predador	adulto	Rodentia: Hystricognathi	Rato-de-espinho Trinomys setosus	CD, FJ?	Takata *et al* ., 2012	
	adulto	Carnivora: Herpestidae	suricata (*Suricata suricatta*)	SH	Townsend and Manser 2010	

Defensive scream appealing for defense	filhote	Carnivora: Ursidae	urso negro asiático (*Ursus thibetanus*)	CD, SH	Pokrovskaya 2013	
Choro "whine", " begging for or defending food"	filhote	Carnivora: Ursidae	urso negro asiático (*Ursus thibetanus*)	CD, SH, SB, FJ, "flutter"	Pokrovskaya 2013	
Agonístico?	adulto	Carnivora: Canidae		CD, FJ	Riede *et al* ., 2005	
Subordinação, medo?	adulto	Primata: *Macaca*	rhesus *Macaca mulatta*	CD, SH	Owren e Rendall 2003	
	adulto	Carnivora: Mustelidae	Lontra	CD, SH	Prado, Ferreira & Monticelli, 2010; Prado, 2013	
	adulto	Primata: *Macaca*	rhesus *Macaca mulatta*	CD, SH	Owren e Rendall 2003	
Agonístico?	adulto	Carnivora: Procyonidae	quati Nasua nasua	CD, SH, SB, FJ, "flutter"	Gasco, Ferro e Monticelli (2018)	
	adulto	Rodentia: Hystricognathi	Rato-de-espinho *Trinomys setosus*	CD, SH	Takata *et al* ., 2012	

Townsend e Manser (2010) acreditam ter mostrado pela primeira vez em animais humanos ou não, que os ENLs têm função adaptativa. Eles reproduziram para suricatas de vida livre gravações de “chamados de alarme de média urgência”, emitidos na presença de predadores aéreos, que tinham e que não tinham ENL. Os animais responderam, como descrevem os autores, com mais intensidade e forragearam menos ao ouvirem chamadas de alarme não lineares do que os lineares, o que suportaria a "hipótese da imprevisibilidade" dos ENLs. This adaptive function would be particularly plausible for calls that influence the survival of individuals, such as screams or alarm calls and indeed it has been shown that baby and animal screams containing NLP are more evocative to human listeners than vocalizations without [9,10]

Choro, grito ou chamado de alarme?

A literatura da bioacústica de mamíferos ainda precisa entrar em consenso sobre o que são choros, assobios, gritos ou, em inglês, whine, squeal, squeak, cry, scream, whistle, yep, etc. São dados nomes diferentes para estruturas espectro-temporais semelhantes, e isso dificulta comparações entre autores e espécies. As bibliotecas digitais podem ajudar nisso, a medida que amostras de chamados já caracterizados forem consultadas antes do uso de um novo rótulo a algo que se assemelhe. Mas algumas relações podem ser feitas e os ENL podem ajudar, dada sua relação com o estado interno do emissor.

1. Adultos e filhotes em perigo produzem gritos de agonia (ou chamado de agonia ou *distress call*, ou simplesmente *scream*, e.g., Blumstein *et al* ., 2008). O estado interno de um mamífero nessa situação, de alta excitação (*high arousal*) ou em perigo iminente, pouco varia entre as espécies (Darwin, 2003). O som produzido neste estado tem características próprias e acredita-se servir à função de atrair a atenção de possíveis ajudantes, ou de atrapalhar o predador. Gritos de agonia não são a mesma coisa que chamados de alarme, nem em função nem em estrutura. Por exemplo, filhotes de marmota (um roedor da subordem dos esquilos) “gritam” quando em perigo e esse grito é melhor do que o chamado de alarme da espécie em evocar reação em animais adultos (interrupção de forrageamento, aproximação ao filhote e/ou emissão de chamado de alarme; chamados de alarme, ao contrário, não evocaram aproximação e/ou emissão de chamado de alarme, Blumstein *et al.*, 2008). Em termos estruturais, os gritos dos filhotes são mais longos e frequentemente contém ENL: 55% dos gritos de filhotes emitidos na mão do pesquisador tinham SH, 13% tinham bifonação, 74% tinham ondulações e muitos (não foram contados) tinham CD (Blumstein *et al.*, 2008).
2. Chamados de alarme ou *alarm calls* (em mocós e preás soam como assobios e em capivaras e pacas como latidos graves e ruidosos; por isso foram chamados de *song*,

em referência ao canto de pássaros, ou pelas onomatopéias chirrup ou peep, ou de latido, em referência ao som dos cães). São comuns em espécies sociais terrestres, que não são topo de cadeia alimentar, durante forrageamento ou movimentação, como em preás, mocós e capivaras, marmotas (Blumstein *et al.*, 2008), suricatos (Manser, Seyfarth e Cheney, 2002) e tantos outros já descritos e ate apresentados em canais de televisão sobre vida animal. Ao contrário dos gritos de agonia, o chamado de alarme não contém ao menos não para marmotas, ENL (Blumstein *et al.*, 2008, Blumstein & Recapet 2009). Também não notei ENLs no latido de mocó e no chirrup de preás. Mas os latidos das capivaras são ruidosos. Essa categoria precisa ser revista, ao meu ver, à luz da teoria de Zahavi (Zahavi e Zahavi, 1999), dos sinais honestos ou *handicaps*. Em alguns casos os sinais funcionalmente identificados como alarme podem ser primordialmente dirigidos a predadores e não a coespecíficos (ainda que possam fazer uso dele), i.e., de servirem à intimidação, ainda que possam ser usados como pistas da presença de perigo, para outros membros do grupo social.

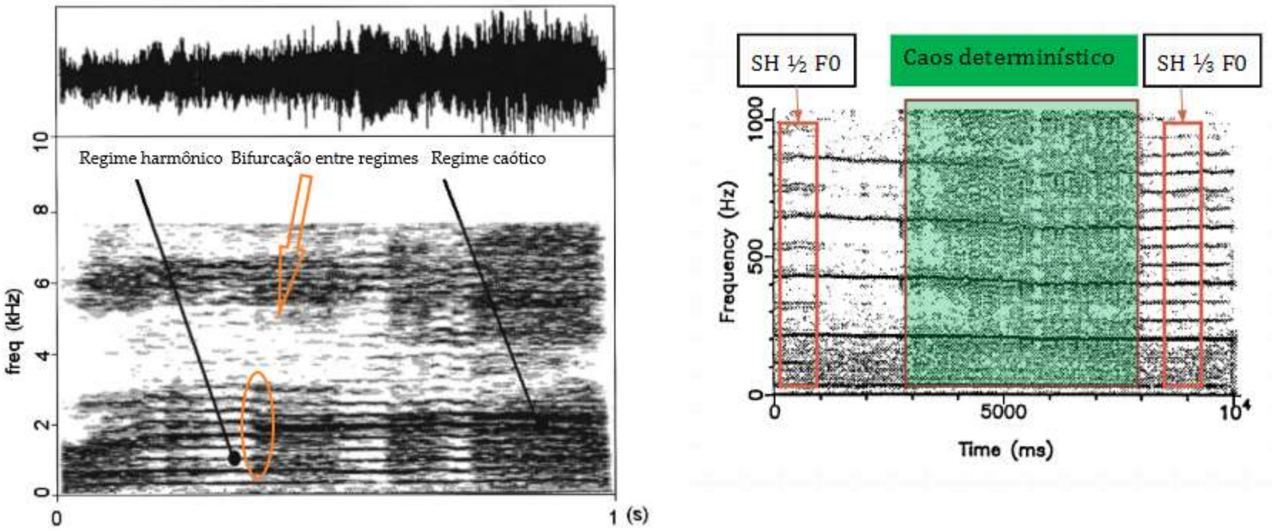


Figura 2.4. Identificação dos efeitos não-lineares descritos por Tokuda et al. (2002) (à esquerda), sobre figura de um grito de leitão dos autores, por mim adaptada. O gráfico de cima representa o envelope de onda, e abaixo está o espectrograma, um gráfico de frequência acústica por tempo (janela de 1 segundo). A nota inicia-se e termina em fases de caos determinístico, há fases harmônicas, algumas sobrepostas a caos, por exemplo, após a bifurcação entre a primeira fase harmônica para a segunda. À direita, representação de sub-harmônicos (SH) em múltiplos de 1/2 ou 1/3 da frequência fundamental (F0), adaptada de Widden et al. (2002).

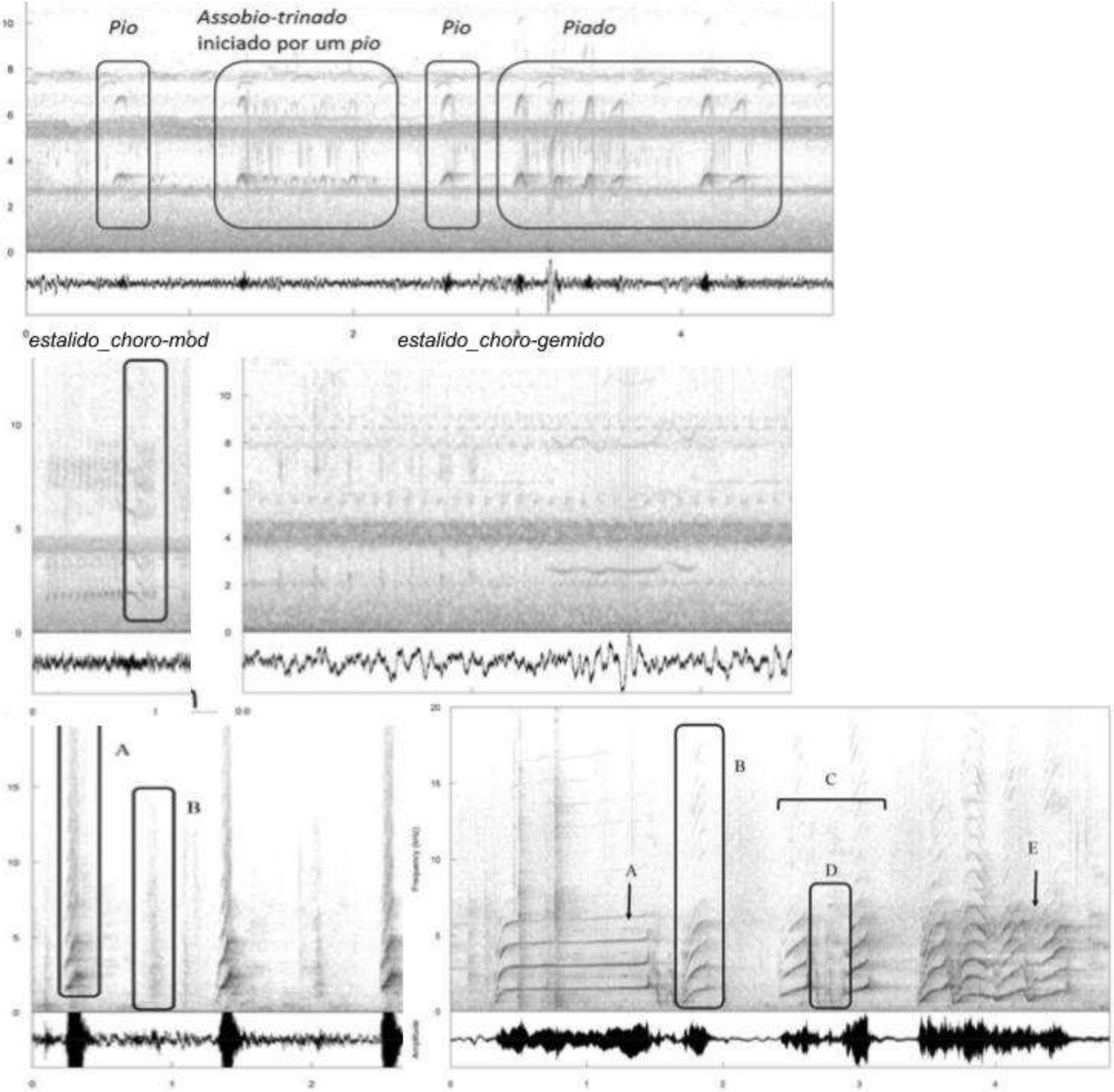


Figura 2.5. Repertório “extra” de sinais de capivaras, constituído a partir da junção de unidades básicas, formando notas combinadas (pio_assobio-trinado, acima; estalido_choro-modulado, no meio da figura) ou sílabas combinatórias (quando há intervalo entre as unidades: estalido_choro-gemido, no meio). A frase de assobios com alternância dos tipos pio, assobio-trinado e piado, representada na figura superior, foi obtida de um juvenil afastado do grupo. Na parte central da figura, a nota combinada de estalido_choro-modulado foi emitida por um adulto separado do restante do grupo por um alambrado. Na mesma escala temporal, a sílaba estalido_choro-gemido foi gravado de um adulto que se movia junto com o grupo durante o forrageamento. Abaixo à esquerda, a sílaba composta de grito (A) intercalado com arfar (B) foi registrada de adulto contido em puçá durante sexagem, marcação ou tricotomia; a nota de grito atingiu frequências máximas superiores a 20 kHz. À direita, a sequência combinada de gritos, estalido e com fases de não-linearidade acústica foi obtida de sub-adulto de zoológico durante a captura em puçá: inicia-se com um grito longo com subharmônicos (A), seguida de grito (B) e de uma nota combinatória (C) de grito_estalido_grito (fase de estalido em D) e salto de frequência na última nota (E). Imagens extraídas e editadas de Suzuki (2016).

Encontramos em capivaras, como vimos em quatis, três dos ENLs _ episódios sub-harmônicos, caos e saltos de frequência. Se ampliarmos o olhar para outras espécies que gravamos, notamos que esses ENLs são comuns em gritos de agonia (Fig. 2.6). Gritos de agonia são aqueles emitidos na situação máxima de estresse, quando o animal está contido por um predador, por um coespecífico durante conflitos severos ou mais perigosos, ou frente à humanos. Eu arrisco dizer que sejam emitidos pela maioria dos mamíferos capazes de produzir som, uma vez que os encontrei em grupos distintos de Mammalia. Parece um traço antigo na história filogenética do grupo (provavelmente anterior, já que aves e até sapos produzem grito de agonia), gerado em consequência da condição fisiológica dos músculos e do fluxo de ar que agem sobre o sistema fonador de animais em estados internos de intensa valência negativa (Briefer *et al.*, 2015). Há na literatura hipóteses sobre vantagens de se gritar na boca do predador (tempo para fuga ao se assustar ou distrair o predador, ou conseguir ajuda; Owren e Rendall, 1997). As exclamações que emitidos de forma não- intencional quando sentimos dor ou grande desconforto, por exemplo, produzem analgesia (Swee & Schirmer, 2015).

Por outro lado, a bifonação parece ser um ENL mais restrito. Encontramos apenas fora de Caviidae, no aulido do lobo-guará e no assobio de contato do quati (Gasco, 2013; Gasco, Ferro e Monticelli, 2018: Apêndice A Fig 1 A e B, pg. 5). Corresponde à produção simultânea de duas ondas sonoras de contornos distintos, pela vibração simultânea de diferentes fontes de som. Interessante que nesses dois casos, assobios de quati e aulido do lobo, o fenômeno foi encontrado em uma vocalização de contato à distância. Em quatis, os assobios acompanham o movimento do grupo pela mata, às vezes um contato próximo, mas visualmente deficiente, que precise ser coordenado por outro canal de comunicação. Em lobos, o sinal é usado à longas distâncias, supostamente para restabelecer o contato entre o casal reprodutivo, ao mesmo tempo que sinalizaria a presença em dado território. A bifonação não parece criar a variabilidade sonora dos gritos compostos de subharmônicos, saltos de frequência e caos, que podem se distribuir de maneira bem variada ao longo de cada nota. Mas, por outro lado, esses sinais bifonados de quati e lobo são individualmente distintos (Balieiro, 2016; Balieiro e Monticelli, 2018, Apêndice B). A variação estaria no nível individual, imune a efeitos do estado interno dos animais, como parece ser o caso dos choros e gritos.

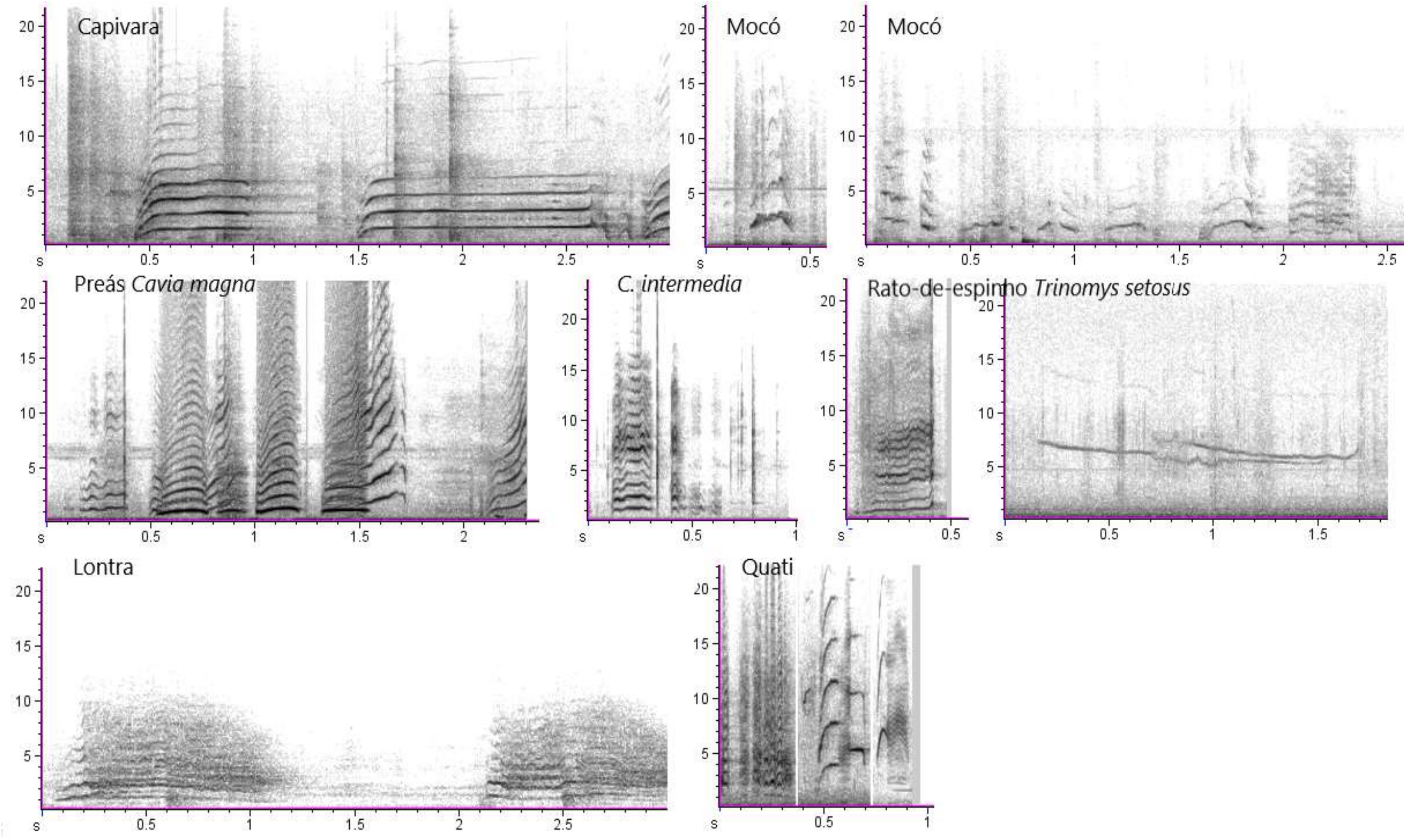


Figura 2.6. Efeitos não lineares em choros e gritos de diferentes espécies de mamíferos: capivara *Hydrochoerus hydrochaeris*, mocó *Kerodon rupestris*, preás do sul (*Cavia magna* e *C. intermedia*), rato-de-espinho *Trinomys setosus* e dos carnívoros lontra *Lontra longicaudis* e quati *Nasua nasua*.

Apêndice A _ *The communicative life of a social carnivore: acoustic repertoire of the ring-tailed coati* (Nasua nasua)

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The communicative life of a social carnivore: acoustic repertoire of the ring-tailed coati (*Nasua nasua*)

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ABSTRACT

The coati is a highly social mammal that features sophisticated cognitive and social abilities. We hypothesized that the ring-tailed coati, *Nasua nasua*, uses an extensive acoustic repertoire that correlates to their diverse range of social interactions. We tested this hypothesis by observing and recording a free-ranging managed population of *N. nasua* in Tietê Ecological Park (PET), in the municipality of São Paulo, State of São Paulo, Brazil. Of 404 h of sampling, 47 h of coati vocalizations were recorded over 3 years. Additional records were obtained opportunistically on other free-living populations at PET by using passive acoustic monitoring. We describe here an acoustic repertoire composed of 15 calls (12 basic calls, 2 rhythmic calls and the non-random complex calls composed of three or four different units). This diverse repertoire of signals was used in contact/cohesion regulation, foraging activities, alert or potential threat situations, playing and fighting interactions and during social isolation and acute distress. The contact call (chirp) is produced through biphonation, and other non-linear phenomena are present. Our study found a complex vocal repertoire that encourages further studies to describe the evolution of the cognitive characteristics and social abilities of ring-tailed coatis.

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Acoustic repertoire; chirp; non-linear effects; procyonids; vocal complexity; acoustic complex sequences

Introduction

Primates are classic examples of social and acoustic complexity (e.g. Cheney and Seyfarth 1990; Arnold and Zuberbühler 2006) among the terrestrial mammals, with some species equipped with especially large acoustic repertoires (e.g. 25 signals for the Formosan rock macaque *Macaca cyclopis*: Hsu et al. 2005; 27 for the white-headed capuchin *Cebus capucinus*: Gros-Louis et al. 2008). For these species, acoustic complexity is not only a matter of repertoire size, but also structure, since these repertoires are made of well-defined vocal units (with marked differences in their spectro-temporal compositions) and continuous gradations related to varying motivational states and varying social interactions (Robinson 1984). Different call-types can be combined into a 'higher-order sequence' that has a different

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Supplemental data for this article can be accessed https://doi.org/10.1080/09524622.2018.1477618 meaning (Arnold and Zuberbühler 2006; Ouattara et al. 2009) or increases individuality (Jansen et al. 2012; Déaux et al. 2016).

The acoustic repertoire is related to the level of complexity of social life (McComb and Semple 2005; Freeberg 2006), with signal variability reflecting the nature and nuances of intragroup interactions (in cotton-top tamarin *Saguinus oedipus oedipus*: Cleveland and Snowdon 1982; ring-tailed lemur *Lemur catta*: Macedonia 1993; Bolt and Tennenhouse 2017; barbary macaque *Macaca sylvanus*: Fischer and Hammerschmidt 2002; putty-nosed monkeys *Cercopithecus nictitans*: Arnold and Zuberbühler 2006; capuchin monkey *Cebus capucinus*: Digweed et al. 2007; Gros-Louis et al. 2008).

The order Carnivora also has remarkably social taxa, such as the coatis (Procyonidae: *Nasua* sp.), which stand out from other procyonids in terms of sociality (Hirsch 2007) and socio-cognitive abilities (Arsznov and Sakai 2012). Coatis live in large female-bonded social groups organized in linear dominance hierarchies (Hirsch et al. 2012) that may include up to 40 individuals. While some populations have little or no male parental care (e.g. Hirsch 2011), in our population of ring-tailed coatis, *Nasua nasua*, males regularly visit particular groups of females and care for the cubs (grooming and staying in contact, and sharing a nest; A. Gasco, pers. obs.). At the end of the annual reproductive season, the females depart from the group, individually or in pairs, and build nests in the trees (Olifiers et al. 2009). For more than two months, the lactating female lives apart from the group, feeding and protecting her cubs that are subject to predation by monkeys, snakes and birds of prey (Newcomer and De Farcy 1985; de Resende et al. 2004).

The social complexity of the coatis can be measured by their tendency to interact with each other in different contexts and repeatedly build networks over time. Kaufmann (1962) noticed the complexity of the coatis' social life, and Compton (1998) showed the gregarious behaviour of the females, the role of grooming, care and huddling in maintaining social stability, female philopatry and the systematic movement of adult males caused by aggression or acceptance of adult females. However, no work has related the coatis' social behaviour to their communication skills, nor has their vocal repertoire been thoroughly described.

Acoustic communication in coatis includes individualized contact calls (Trudgian 1995; Compton 1998; Maurello et al. 2000), warning signalization, dynamic coordination of social activities through vocalizations (Romero and Aureli 2008; Hirsch et al. 2012) and the use of the ultrasound frequency band (Maurello et al. 2000). The species uses large, forested foraging areas (Costa et al. 2009) and depends on short distance communication signals to coordinate group activities. Since the potential information that acoustic signals convey has presumably been selected by its adaptive value (Mendes et al. 2009), we suspected that the evolution of the social complexity in this species shaped its acoustic repertoire. Thus, we conducted a long-term study to develop the first complete description of the acoustic repertoire of ring-tailed coatis.

Materials and methods

Study site

The study site was the Tietê Ecological Park (PET), which is part of an environmental preservation area that aims to preserve the biodiversity of the Tietê River floodplain, but also provides cultural, educational and recreational activities to more than 330,000 visitors each month. Our particular study area in PET was the Núcleo Engenheiro Goulart, that is 1400 hectares (140 km^2) in size (23.4919°S, 46.5211°W), at 746 m in altitude, in the State of São Paulo (Brazil), among the municipalities of São Paulo, Guarulhos, Barueri and Santana do Parnaíba (Pinheiro and Sígolo 2006). It includes some exotic vegetation (e.g. *Pinnus* sp.), artificial lakes and Atlantic Forest native plant nurseries. It is one area in the city for releasing illegally captured animals that have been confiscated (Ottoni and Mannu 2001), and the population of ring-tailed coati there derived from such animals. Ring-tailed coatis occupy almost all the PET area, but our study focused primarily on the wooded area, which includes artificial ponds and the Reception Center of Wild Animals (CRAS). Visitors were not allowed in this area. In 2013, we extended our study area to the 'Jardim das Primaveras' in a public leisure area of PET. The population density of ring-tailed coatis in PET was estimated as being 125 ring-tailed coatis/km^2 (Souza and Beisiegel 2002), much higher than the one recorded for natural areas (3.7–7.7 individuals/km^2 at Carlos Botelho State Park, State of São Paulo: Beisiegel and Mantovani 2006; 33.7 ind./km^2 at Prosa State Park, State of Mato Grosso do Sul, Brazil: Costa 2009). Throughout the study period, a coati sterilization programme was in progress to reduce population, which was conducted by CRAS veterinarians. There was also a trough where fruits and other vegetables were served, close to CRAS.

Animals and procedures for sound collection

Five groups (48 animals in total) were monitored weekly from July 2010 to December 2013. Two groups were chosen opportunistically (whichever was found first) to be followed on each observation day, one in the morning and the other after midday. Those groups were designated as follows:

(1) Ruiva (RV), with three females, one male and two cubs;
(2) Black (BK), with seven adults of indeterminate sex, a female, two juveniles, two cubs;
(3) Berta (BE), with six adults of indeterminate sex, a male and a lactating female, two juveniles, three cubs;
(4) Jardim das Primaveras (JD), met only in 2013, initially composed of four pregnant females, one male, two subadult males, two juvenile females, three juveniles male);
(5) Pedalinho (PE), with two pregnant females, one male, two juvenile females, six juvenile males.

RV, BK and BE were followed from 2010 to 2012. In 2013, we followed just JD and PE. The groups were stable throughout the study period, changing only after births of cubs, indicated above.

Subjects were identified from natural marks and size (Figure S1, Supplemental Material), after familiarizing with A. Gasco's presence. The age of the animals was estimated by comparing and ranking the body size of the individuals in ascending order (Costa 2009; Hirsch et al. 2012).

Vocalizations were opportunistically collected and were recorded manually as they occurred. We documented in paper notes in the field the general behaviour of the focal group (e.g. travelling in group, resting, foraging on the ground), sex and age class of the animals involved in each episode. We also noted the stimuli and background behaviours that preceded or succeeded the sounds. Additional recordings taken during animal handling are also presented here: the threat call of an adult female inside a Tomahawk Live Trap (Model ATW900) at Cantareira State Park; and distress calls uttered by PE individuals held captive for two days during a neutering procedure (for which we used a remote and automated system, TMSongmeter SM2 + of Wildlife Acoustics).

Recording and acoustic analyses

Most sounds were recorded using a Marantz PMD660 recorder (sampling rate: 44.1 kHz, 16 bits of resolution, with phantom off) coupled to a Sennheiser ME67/K6 unidirectional microphone embedded in a Rode boom pole approximately 1 m from the vocalizing animal. Spectrograms of the recordings and their physical descriptions were made using Raven Pro 1.4 (Charif et al. 2010) (Table S1, Supplemental Material). We used the instant activation measuring tools of Raven to measure low frequency (set on F0 lower limit), high frequency (the last visible harmonic or frequency element on the 22 kHz spectrograms), begin frequency, end frequency, delta frequency, max frequency (Figure S2, Supplemental Material), delta time and internal intervals [time elapsed between the beginning of one event (i.e. onset) and the beginning of the next event]. The settings used for the measurements were: DFT 512 Hz, 256 points frame length, 1.16–2.31 ms grid resolution, 60.2–80.1% overlap, 43–86.1 Hz frequency res. (to narrow and wideband analysis, respectively), and Hamming window. The number of visible harmonics in the spectrogram was recorded. The pictures of the spectrograms of this work were created with Avisoft SASLab (Specht 2001).

Acoustic terminology and ethogram

Whenever possible, classification of calls was based on the terminology already adopted for *N. narica* and other procyonids (Table S2, Supplemental Material), although those works are generally qualitative (i.e. onomatopoeic) or based on sonographic analyses that are outdated for current technological standards (Kaufmann 1962; Trudgian 1995; Emmons et al. 1997). In other cases, calls were classified based on existing mammalian acoustic terminologies (Table S2, Supplemental Material) (Wong et al. 1999; Hsu et al. 2005; Pistorio et al. 2006; Gros-Louis et al. 2008; Reháková-Petru et al. 2012; Lingle and Riede 2014). The ethograms of the coatis (Trudgian 1995; Costa 2009) were used only to recognize the general context of the vocalizations in an observational (and thus correlational) manner, rather than experimentally through playback tests of causal effects. *Ad libitum* sampling of Altmann (1974) was adopted to infer general behaviour during vocalizations.

Statistical analysis and hypotheses

Valid sampling procedures to gather statistically representative vocalizations may consist of either a large population of individuals in a small timeframe or a few individuals for a long time. In this work, as noted above, we followed both approaches, gathering sounds from a relatively large population (48 individuals) for a long period of time (3 years). Such a large sampling effort resulted in a massive collection of raw data, from which we isolated 1,457 sound samples that were then carefully catalogued and classified into 15 call-types (Figures 1 and 2).

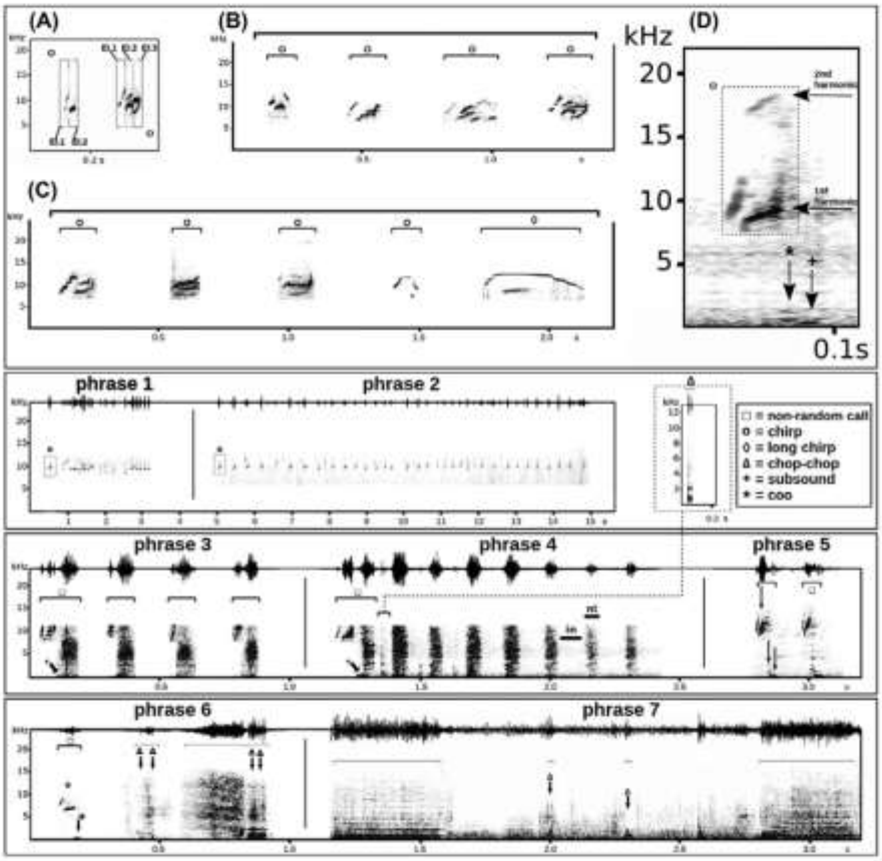

Figure 1. Call-types of a population of *Nasua nasua* at Tietê Ecological Park. *Chirp* (A–C, set individually by circles) is the *contact call* of the species, made of the subunits El.1, El.2, and El.3 (A). *Long-chirp* unique emission is shown in C (lozenge). In phrases 1 and 2 are soft-chitter and chirrup, respectively, uttered in *contact contexts*. *Non-random complex calls* (D, and square at phrase 3 and phrase 4 are ordered sequences of individual calls, starting with a *chirp* (detailed in the dotted square) and usually followed by a *coo* (*) and a *subsound* (+). *Non-random complex calls* are composed as *chirp* + *subsound* + *bark* in *alarm contexts* (phrase 3 and first syllable of phrase 4, that follows with a bark sequence, where 'Nt' indicates a unit of bark, and 'in', the interval), as *chirp* + *coo* + *subsound* during *food-competition* on the ground (phrase 5); or as *chirp* + *subsound* followed by *chop-chop* (triangles) in *threatening contexts* (phrase 6). In phrase 7 (triangles) *chop-chop* were uttered on either side of a strong exhalation by a live-trapped adult female. *The distress call* (phrase 7) is formed by *long roars* (line over) and chop-chops.

Calls were described in relation to the central tendencies of the measured acoustic variables and their ranges (minimum and maximum). After exploratory analysis of the calls, we tested the following statistical hypotheses: (1) the calls can be classified according to multilevel temporal criteria into *single notes*, *double notes*, *syllables*, *phrases* and *bouts* (Table S3, Supplemental Material provide the numerical boundaries of them), which is revealed by comparing their durations with a Kruskal–Wallis test followed by Games–Howell post hoc

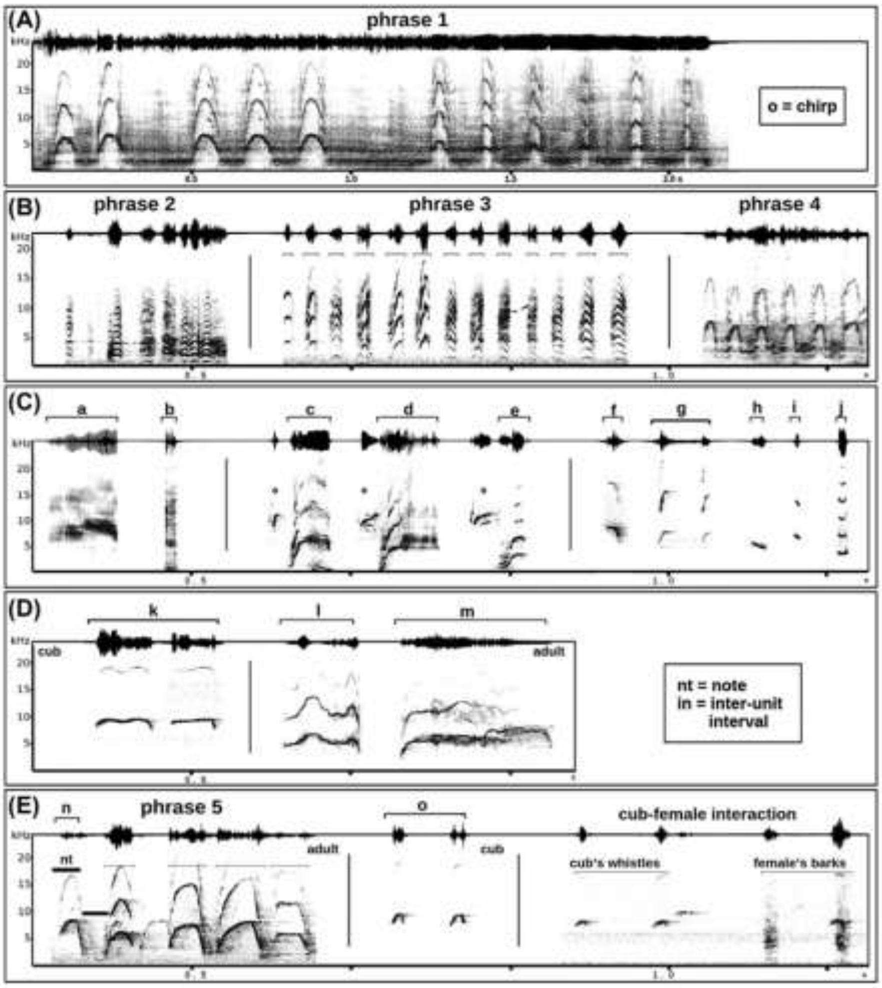

Figure 2. Call-types of a population of *Nasua nasua* at Tietê Ecological Park. A: *twitter* uttered in *distress contexts* (phrase 1). B: *short roar* (phrase 2) and *squeak* (phrase 3) in *play contexts*, and the trill *U-shaped whistles* (phrase 4) of *play-fighting and agonistic contexts*. In C, the *long vibrate* (a) of *agonistic contexts*, and *short vibrate* (b) of *play and distress contexts*; the *ascending arc shaped whistles* of *agonistic contexts* (c–e, preceded by a chirp (o); the *short whistles _ descending arc shaped whistle* (f) and *ascending arc shaped whistle of cubs* (g) of *isolation contexts*, *pure whistle* (h) of *play contexts*, *peep* (i) and *peep-like* (j) of *agonistic contexts*. D: the *whine* of cubs (k, used in *isolation contexts*) and of adults, the *whine NLP_nonlinear phenomenon present* – (l, without nonlinearity; m, with nonlinearity) uttered in *agonistic contexts*. E: the *U-shaped whistle* of adults (n), of *agonistic contexts*, and of cubs (p) of *isolation contexts*, and a cub-female vocal interaction during *isolation*. 'Nt' indicates a note, and 'in', the internote interval.

tests (Table 2; Tables S3 and S4, Supplemental Material); (2) the position of the notes that constitute *complex calls* is not random as suggested by a probabilistic tree analysis (Figure 3); (3) there is a difference between rhythmic calls (*chirrup* and *soft chitter*) as determined by ANOVA (Table S5, Supplemental Material). All analyses were performed using SPSS (IBM SPSS Statistics Desktop 22.0).

In this work, we omitted statistical comparisons between the components of the call-types generated by the ring-tailed coatis' complex call systems and the corresponding basic call-types. Our goal was to reveal major categories of call-types (lexicon repertoire), without digging into the minor distinctions among the chirps when uttered alone or in each of the situations of emission of the complex call.

Ethical note

This study was approved by the Animal Use Ethics Committee (CEUA) of the Psychology Institute of São Paulo University (number 004-2009).

Results

Twelve basic call-types were recorded and quantified (sound units singly emitted; Figures 1 and 2, and Table 1), as well as three additional complex vocalizations: two formed by the repetition of a single call-type and one formed by the concatenation of four call-types. The sounds vary widely in their acoustic structures, which are briefly described for ease of reference as follows (comprehensive descriptions are provided later in this Section):

(1) high-pitched sounds, such as the (1) chirp, previously described by Maurello et al. (2000) as the individually distinctive contact call of coatis and the long chirp, a longer pattern of chirp uttered in a phrase only once by a single male adult moving alone (described in 'Incidental or Non-Consistent Calls' section);
(2) low-pitched and narrowband sounds as the (2) coo and the (3) subsound;
(3) noisy and broadband sounds, as (4) bark and (5) roar;
(4) screaming whistled sounds as the (6) short whistle, (7) U-shaped and ascending arc shaped whistles and (8) twitter, the distress call;
(5) a varied repertoire of whine and squeak sounds as the (9) squeak, a juvenile affiliative call, (10) whine and (11) vibrate (an extensive ripple note of short amplitude);
(6) a non-vocal sounds apparently produced by two fast teeth chattering, previously named (12) chop-chop (Smith 1980).

U-shaped whistles and chirps were the most common calls uttered by the ring-tailed coatis from PET (respectively, 28.41 and 26.97% of the 1457 sound samples analysed in this study, see Figure 4A). Barks were the third most frequent (13.73% of the call-types). The rest of call-types accounted for less than 8% of the recorded call-types. From the 260 call-types registered from cubs, 78% were whines and U-shaped whistles uttered in isolation contexts. The remaining 22% of call-types sampled for cubs were chirps, coos, subsounds and barks.

Besides those 12 basic calls, we also identified certain calls made of multiple elements that are calls in their own right. Often, such vocalizations are referred to simply as *complex calls* in species like the chickadee (Freeberg and Lucas 2002), whose repertoire exhibits a single structural complexity system. In turn, we determined that ring-tailed coatis feature *two*

Table 1. Physical structure of 12 basic call-types and the two rhythmic calls (chirrup and soft-chitter) collected from a population of ring-tailed coatis (*Nasua nasua*) living in Tietê Ecological Park between 2011 and 2013. The calls were emitted by adults, sub-adults, juveniles and cubs of both of sexes in general situations of contact, food-association, threat, alarm, play, agonistic, isolation and distress. Detailed measurements of single notes, non-random complex calls and phrases are represented by mean values ±SD, range, *N* (number of samples of a call-type recorded from 3–5 different individuals for each studied group).

Context of emission	Call-type	*N*	Deltatime (s)	Lowfreq (Hz)	Highfreq (Hz)	Deltafreq (Hz)	Maxfreq (Hz)	N Harmonic	N ultrasonic harmonic	Interchirp interval duration (ID)
Contact	Chirp	393	0.053 (±0.026) *0.01–0.265*	8048.81 (±968.44) *81.1–10689.6*	12110.05 (±2730.55) *8445.5–21381.8*	4061.24 (±3032.08) *813.1–20022.5*	9311.50 (±748.16) *7278.2–12230.9*	0.1 (±0.4) *0–2*	0 –	– –
Contact	Chirrup	5	9.716 (±0.271) *9.265–9.997*	– –	– –	– –	– –	– –	– –	0.185 (±0.135) *0.014 – 0.858*
Contact	Soft chitter	4	5.233 (±3.252) *2.602–9.903*	– –	– –	– –	– –	– –	– –	0.178 (±0.210) *0.014–0.912*
Food	Coo	40	0.015 (±0.006) *0.006–0.028*	587.14 (±322.56) *76–1865.9*	1961.07 (±1292.54) *814.6–8362.6*	1373.93 (±1070.81) *372–6496.7*	938.85 (±1020.78) *86.1–6804.5*	1.1 (±1.1) *0–5*	0 –	– –
Food/alarm	Subsound	53	0.022 (±0.013) *0.008–0.077*	78.37 (±44.14) *20.7–271.1*	556.23 (±99.94) *335.6–873.6*	478.98 (±97.47) *288.2–729.30*	125.12 (±74.74) *86.1–430.7*	0.03 (±0.2) *0–1*	0 –	– –
Alarm	Bark	200	0.048 (±0.018) *0.016–0.140*	1379.07 (±1582.34) *27–8912*	14171.45 (±4479.53) *5001.1–22050*	12792.38 (±4725.26) *2487–20980.4*	3897.29 (±2441.9) *43.1–10594.3*	0 –	0 –	– –
Alarm/threat	Chop-chop	34	0.012 (±0.008) *0.006–0.040*	318.76 (±157.82) *108.1–784*	2539.41 (±878.57) *1324.6–5881.7*	2220.65 (±869.83) *1135.4–5678.9*	936.87 (±358.67) *215.3–1875*	0 –	0 –	– –
Play/alarm	Roar	15	0.125 (±0.132) *0.015–0.420*	967.07 (±458.21) *278.2–1924.9*	16039.73 (±5998.93) *5777.8–23749.3*	15072.02 (±6012.86) *5333.4–23122.7*	2128.9 (±905.3) *904.4–4134.4*	5.9 (±8.9) *0–21*	0.07 (±0.3) *0–1*	– –
Play/isolation	Short whistle	84	0.032 (±0.016) *0.008–0.082*	3875.29 (±1548.88) *973.8–10984.8*	15119.32 (±5367.83) *2086.8–22050*	11244.03 (±5423.59) *486.9–19761.7*	5661.21 (±2027.62) *1722.7–12575.4*	3.0 (±1.4) *0–7*	0.06 (±0.2) *0–1*	– –
Play	Squeak	33	0.041 (±0.015) *0.017–0.106*	1883.6 (±1227.73) *19.1–4135.5*	19594.32 (±3295.3) *10937.3–22050*	17710.74 (±3545.49) *9070.2–21227.9*	6997.64 (±3305.48) *86.1–12446.2*	8.3 (±5.4) *0–22*	0.1 (±0.3) *0–1*	– –
Agonistic/ isolation	Whine	116	0.199 (±0.126) *0.05–0.698*	4413.22 (±1773.30) *941.7–8153.4*	19456.94 (±2831.71) *7913.3–22050*	15043.72 (±3805.75) *2086.9–20352.9*	7058.81 (±1359.09) *3746.8–11627.9*	2.9 (±0.81) *1–6*	0.1 (±0.4) *0–2*	– –

(Continued)

Table 1. (*Continued*).

Context of emission	Call-type	*N*	Deltatime (s)	Lowfreq (Hz)	Highfreq (Hz)	Deltafreq (Hz)	Maxfreq (Hz)	N Harmonic	N ultrasonic harmonic	Interchirp interval duration (ID)
Agonistic/play	Vibrate	60	0.076 (±0.103)	2462.36 (±1775.67)	20262.03 (±3004.03)	17799.66 (±3957.36)	5391.97 (±3424.68)	0.7 (±1.5)	0.03 (±0.2)	–
			0.003–0.475	*65.4–5486*	*6747.2–24000*	*6566.5–23498.7*	*86.1–12446.2*	*0–5*	*0–1*	–
Agonistic/play	U-shaped whistle	414	0.066 (±0.059)	4746.58 (±2261.01)	17375.65 (±4612.05)	12629.08 (±5690.86)	6996.11 (±1680.29)	2.3 (±1.3)	0.2 (±0.5)	–
			0.011–0.496	*69.6–9792.3*	*5218.4–22050*	*719–21569.4*	*3617.6–12273.9*	*1–7*	*0–3*	–
Distress	Twitter	15	1.106 (±0.711)	282.1 (±315.91)	23733.09 (±474.98)	23450.99 (±762.9)	4141.83 (±2018.43)	0.3 (±1.03)	0.07 (±0.3)	–
			0.129–2.127	*62.7–1252.1*	*22050–24000*	*20797.9–23874.7*	*1500–8250*	*0–4*	*0–1*	–

Table 2. Mean difference among five temporal categories *single notes, double notes, syllables, phrases* and *bouts* based on their median durations (in seconds) by a Games–Howell post hoc tests (Table S3, Supplemental material).

Games–Howell post hoc			
Temporal patterns		Mean difference (s)	Sig.
Bout	Double	4.0412*	0.000
	Note	2.6217*	0.000
	Phrase	2.6217*	0.001
	Syllable	3.7670*	0.000
Double	Note	0.0550*	0.000
	Phrase	1.4194*	0.000
	Syllable	0.2741*	0.002
Note	Phrase	1.3644*	0.000
	Syllable	0.2191*	0.026
Phrase	Syllable	1.1453*	0.000

*The mean difference is significant at the 0.05 level.

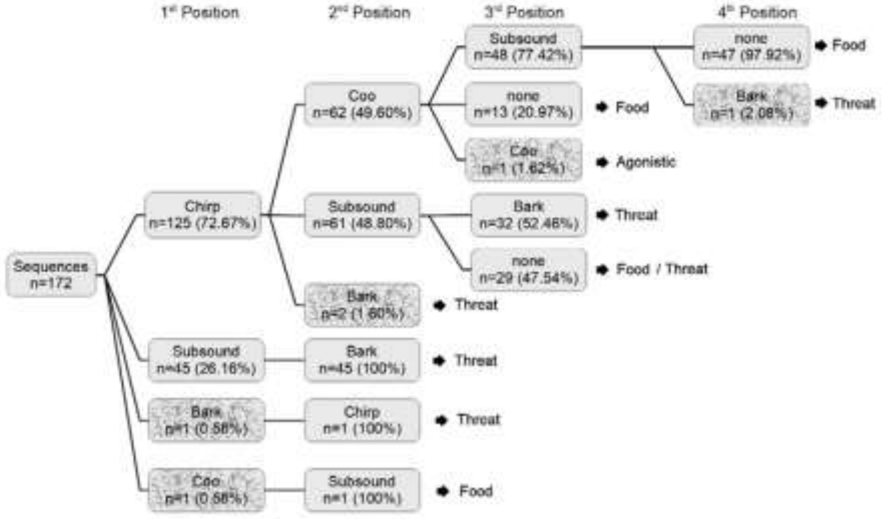

Figure 3. Tree of probabilities depicting how the components of the non-random complex calls distribute in a probability space.

Notes: By analysing the tree, it is possible to identify from all 172 sequences gathered six random sequences that rarely occurred (scratched cells; note that the chirp + bark sequence occurred two times, as indicated by '*n* = 2'). The other 166 were considered non-random complex calls. A component named 'none' is used to denote the end of a sequence when necessary, so that the probabilities always add up to 100% in every ramification of the tree.

complex systems, that we named *rhythmic calls* and *non-random complex calls*. The rhythmic calls system is made up of identical sounds repeated rhythmically, referred to as *soft chitter* (Trudgian 1995) and *chirrup*. On the other hand, non-random complex calls were always made of a permutation with different notes, which can lead to several unique call-types as seen in the chickadee's complex system (Clucas et al. 2004; Lucas and Freeberg 2007).

In order to properly describe all calls within the scope of this work, we resorted to the definition of some distinct temporal patterns (Table 3, Supplemental Material), namely:

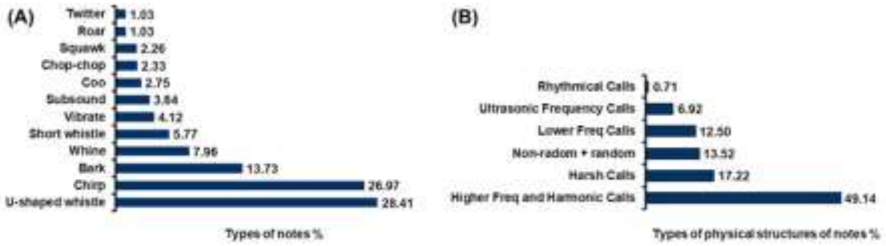

Figure 4. Types of calls (%) collected from a population of ring-tailed coatis (*Nasua nasua*) living in Tietê Ecological Park between 2010 and 2013. (A) Call-types within a sample of 1457 analysed calls. (B) Types of physical structures of calls and temporal variations within a sample of 1272 analysed calls (the label 'Non-random complex calls + random' refers to both non-random complex calls and random sequences. See Section *Non-random complex calls*).

(1) note, a basic unit normally singly emitted, but also pairs (doubled note) in chirps and in the chop-chop;
(2) syllable, group of 3–5 notes uttered in short sequences that are recognized as a single sound compound. Syllables are separated from each other by intervals that are shorter than those found in phrases.
(3) phrase, sequence of only one type of note, as in chirrup and soft chitter (Figure 1E), or a mix of types uttered in short time interval, e.g. threat call (Figure 1G, phrase 6).
(4) bout, sequences of phrases, as complex call + barks repeated until the evocative stimulus ends (Figure 1F, phrase 4).

We certified that such patterns are indeed distinct by comparing the duration of all basic and rhythmic units measured, categorized into one of the 4 temporal categories (Kruskal Wallis test χ^2 (4) = 638.416, p = 0.0001; Table 2; Table S4 of Supplemental Material). All *post hoc* comparisons were significant with $p < 0.05$. Below, a description of the 12 basic calls and their associated contexts are provided. Subsequently, the two rhythmic calls and the non-random complex call are described.

Chirp (Gilbert 1973 as cited in Trudgian 1995; Maurello et al. 2000)

Use and emission context

Chirps are the contact call of the species, as they are for *N. narica*, since they were uttered by coatis when they were moving from one location to another, both when alone (solitary males and pregnant females) and in groups, on the ground or climbing fences and trees. They were also heard by A. Gasco from animals that were building nests together, and during affiliative social interactions between juvenile and adult males, as is characteristic of contact calls.

Structure and acoustic analysis

Chirps are short and high-frequency contact calls, uttered alone (mean interval > 10 s of silence between chirps, n = 88) or in pairs, forming double notes (mean interval < 0.05 s, n = 15 call-types). Chirp structure varied significantly among individuals (Figure 1B–C). Chirps are biphonated (Figure 1B), featuring two independent fundamental frequencies that are distinct. In this work, we describe the effects of the biphonation by dividing the notes up to 3 structural elements, referred to as El.1, El.2 and El.3 (Figure 1A). Strictly speaking, such elements cannot be considered as notes since they were not uttered alone, existing only within the structure of a chirp. Moreover, the literature is unclear regarding their source of production (nasal, oral, both or a mere consequence of some instability in the vocal cords), so that it is possible that all elements are parts of a single sound unit.

We noted that 79 out of 94 chirp samples (i.e. 84%) were composed of two elements (El.1 and El.2; Figure 1A), always in the same order (i.e. El.2 was never before El.1). In 15 of 79 call-types, only one element was present. We also observed the existence of a third element El.3 (never before El.2), which is very uncommon though. In the two-element chirps, El.1 was always the shortest (El.1, mean ± SD = 0.019 ± 0.01 s, range = 0.004–0.05; El.2, mean ± SD = 0.044 ± 0.018 s, range = 0.014–0.153), had the narrower frequency band (El.1, mean ± SD = 3.0 ± 0.91 kHz, range = 0.66–4.81; El.2, mean ± SD = 6.89 ± 4.42 kHz, range = 0.66–13.64), and rarely had harmonics above the fundamental frequency (i.e. was a pure tone). El.1 rarely showed non-linear phenomena (only in 9 out the 79 call-types). Sidebands and frequency jumps were more common in El.2 (in almost 38% of the call-types).

El.2 had considerably less frequency modulation than El.1 and commonly had one harmonic above f0, resulting in a wider band (Figure 1D). Chirp elements seemed to be uttered by distinct sound sources, that is, two-element chirps are in fact biphonations as indicated by the presence of two fundamental frequencies (f0 and g0) and their interactions (Figure S3, Supplemental Material).

Coo

Use and emission context

Coos were uttered in the exploratory phase of foraging on the ground, when a food item was found. In the foraging behaviour on the ground, an individual moved slowly with the head down, apparently searching for invertebrates, and chirping. When a coati sensed a potential food item in the soil, it stopped, pushed away any debris (e.g. leaves) with its snout and dug with its forepaws. Although the group members foraged next to each other, each animal seemed to be involved in its own digging task. When two animals were digging almost in contact and one apparently found something to eat, it uttered a multicomponent call made of chirp + coo + subsound (labelled non-random complex call of food contexts in phrase 5 of Figure 1F). Generally, when an individual was digging a hole and was approached by others, they disputed that spot. Sometimes, the first individual ended up sharing the spot if the other animal approached walking backwards (a behaviour that has not been described yet and that we named here as 'walk-backwards strategy'). When this strategy is not employed by the approaching individual, a fight was started with mutual exchange of kicks to the head, struggle between the two animals or attack-and-run, and vocalizations of the U-shaped whistles (Figure 2n) with nonlinear effects (labelled agonistic whistles SH and CH in Figure 2c–d, and described later).

Structure and acoustic analysis

The coo was the second lowest call-type of the acoustic repertoire of PET coatis (Table 1), and the 2nd position of the non-random complex call (Figure 3). The number of harmonics in coo ranged from 1 to 5 (just one unmodulated coo in frequency with 0.02 s was identified;

minimum frequencies at 0.25 kHz, maximum at 1.37 kHz and dominant at 0.52 kHz, two visible harmonics). It was rarely uttered outside the non-random complex call. When it happened, it was a single note with 1–3 harmonics within the bandwidth supported by our recording equipment (0–22 kHz).

Subsound

Use and emission context

The term subsound was chosen based on Arvola's (1974) description of guttural sounds, and because of their atonal structure and frequency below 100 Hz. It was almost exclusively produced in non-random complex call, after a coo, in three situations: (1) uttered in aggressive disputes for food, as described above (i.e. in the following order: chirp + coo + subsound, as in the phrase 5 of Figure 1F, non-random complex call); (2) after the approach of the researcher during foraging activities (i.e. in the following order: chirp + subsound + bark, as in the phrases 3 and 4 of Figure 1F, non-random complex call); and (3) composing a phrase of threat call in the following order: chirp + subsound (phrase 6 of Figure 1G) uttered by animals under restraint. It was also uttered by solitary males commonly before a bark, in a phrase of subsound followed by a series of barks (alarm call, phrase 4 of Figure 1F). In such cases, it was a long version of subsound that occurred in few agonistic contexts, uttered mostly by subadult males. All subsounds uttered in syllable or phrase containing a coo were uttered just after it, except in two cases in which cubs uttered the subsound before a coo.

Structure and acoustic analysis

The subsound was the lowest acoustic frequency call-type of the repertoire (Table 1). The average duration of short subsounds uttered by solitary males or in syllables by females during foraging contexts was quite similar (Table 1). In the agonistic context, the longer subsound lasted 0.076 s (± 0.002 s, range = 0.74–0.077, $N = 2$ call-types) and it was likely recorded from the same individual.

Bark (Kaufmann 1962)

Use and emission context

The bark was uttered in the presence of people, dogs, cats and capuchin monkeys. Even during the approach of another group of coatis through the treetops or the extreme agitation of the branches or litter by the action of the wind or animals, served as a triggering stimulus for the emission of barks. Commonly, when the emitter was on the ground and barked, it ran in the opposite direction to the triggering stimulus, quickly climbing a tree, stopping and looking back, barking a few more times.

Barks would be followed by fleeing to the trees or freezing by those individuals close to the emitter. When the bark was uttered on a tree, it came with a visual display of moving the body and head side-to-side, apparently an intimidator display. For example, one animal that barked on the trees went up and down on the same trunk, jumped from a trunk to another, stopping in a branch from where it moved the shoulders and head from one side to the other, opening and closing his mouth quickly and successively, staring at the observer with a lateral-frontal look. In a situation where an individual was alone, the entire display looked so intense that a strong expiration caused a trickle of water through the nose, followed by defecation and urination, and the concomitant emission of a soft chitter. The climbing, jumping and moving side-to-side were repeated until the individual could supposedly find a line of escape through the trees or going down, or after the potential threat went away.

Structure and acoustic analysis

The bark is a broadband noisy call-type (phrases 3 and 4 of Figure 1F), relatively short in duration (Table 1), singly uttered or in mixed phrases (e.g. barks uttered with non-random complex call; phrase 4 of Figure 1F). It may last as long as the time of exposure to the stimulus: in one case, the animal barked for almost 30 min. Barks intensity seemed to vary according to the substrate in which the individual was (on the ground or up in trees) and the distance from the stimulus. The lower intensity barks were registered in bouts of average duration of 0.087 s (± 0.119 s, range = 0.028–0.629, $N = 48$ samples). After that, the more intense barks were uttered in bouts of non-random complex call + bark, whose average duration was 0.825 s (±0.496 s, range = 0.237–2.46, $N = 34$ samples).

In non-random complex calls, barks were registered in alarm contexts in the absence of coo, immediately preceding the subsound; there was no time interval between them. The presence of subsound (Figure 1F) seemed to increase the length of the bark (mean ± SD = 0.016 ± 0.055 s, range = 0.036–0.14, $N = 44$ samples), extend its frequency range (mean ± SD = 13,085 kHz ± 4875, range = 5873–20,644, $N = 44$ samples), which started varying on average between 0.094 kHz (±0.066 kHz, range = 0.027–0.324, $N = 44$ samples) and 13,178 kHz (±4875 kHz, range = 6117–20,725, $N = 44$ samples), lowering the dominant frequency (mean ± SD = 2885 kHz ± 2284, range = 0.043–7063, $N = 44$ samples). The highest amplitude of barks was registered in phrases, composed of barks and chop-chop (phrase 4 of Figure 1F). Lower amplitude barks were registered in bouts of barks that lasted, on average, 0.087 s (±0.119, range = 0.028–0.629, $N = 48$ samples).

Roar

Use and emission context

Roars were recorded (1) in a situation of imminent danger, uttered by BE adults foraging on the ground with cubs, after howls of a hidden domestic cat were heard; (2) during contention and handling of a PE juvenile, performed by the veterinarian team, uttered in phrases just before the animal was captured in a net (phrase 7 of Figure 1G); and (3) during social play between a juvenile and an adult female (phrase 2 of Figure 2B). In the distress situation of contention, the phrase of roars were intensified with the concomitant emissions of chop-chop, and ceased after the total immobilization for the anaesthesia application.

Structure and acoustic analysis

Roars along with barks were the noisiest calls of the repertoire. They were filled with chaos and showed decreasing loudness. In a single episode of social play between juvenile and adult female, roars had a harmonic phase (10–21 harmonics) interleaved with chaos phases (phrase 2 of Figure 2B). Roar also occurred along with strong expirations from the PE juvenile captured in a net. A sentence that lasted 11.68 s, consisted of 21 roars, 9 chop-chops, and internal intervals of mean ± SD = 0.166 s ± 0.109 s (range = 0.016–0.738, $N = 30$ samples; phrase 7 of Figure 1G). There was 30 s of strong expirations that totalled 0.082 s.

Short whistle

Use and emission context

The short whistles had four structurally different subclasses and were uttered by cubs and juveniles that were away from the group, in apparent difficulty: trying to cross an obstacle (a large branch over the ground), during playful interactions and at the end of them. The short whistle 1 (a pure whistle, Figure 2H) was recorded in a playful interaction between a cub and a juvenile female from BE, interspersed with short vibrates (Figure 2b), when the animals were out of sight. Before that, the juvenile moved chasing the cub, which was hanging in the branches of demoted trees. The juvenile stood upright and used its forelegs to pull a stick that got in the cub's direction knocking him in the face, and after that, jumped over the cub, making the cub roll on the ground. The short whistle 2 (a peep, Figure 2i) and 3 (peep-like, Figure 2j) were uttered with the U-shaped whistles (Figure 2n) in agonistic contexts between the cub and adult females also from BE. Adult females were chasing the cub, running in circles, rolling on the floor and jumping in playful interactions. When the females collectively jumped on the cub, it uttered agonistic call-types, stopped, and looked straight at them. Another adult female approached and moved the others away from the cub, which ran in the opposite direction from the group.

The short whistles 4 and 5 (Figure 2f–g) were uttered several times by the cub that got separated from the group and once by a JD juvenile. Whenever a cub was left behind or left in front of the group (e.g. at the end of the resting time, when adults start moving, or during movement) they whistled types 4 and 5. Females used to come up to them barking (e.g. cub-female interaction, Figure 2E), suggesting that this call attracts females' attention and help.

The short whistles 4 and 5 (Figure 2k) gradually turn into whines as the urgency of cub seemed to increase. The situation involving the separation of a JD juvenile uttering the short whistles 4 and 5 occurred in the fission time of the groups when pregnant females often quarrelled with juveniles if they followed them. After observing JD group once for 1 h 30 min moving around, three JD juveniles were left behind and one of them kept uttering short whistles with chirps. No adult female came towards the emitter, which continued vocalizing until it found the group.

Structure and acoustic analysis

The short whistles were shorter than U-shaped whistles (Table 1), and varied in the number of harmonics and the outline of the fundamental as follows: (1) it was a pure tonal note modulated in frequency (pure whistle); (2) it had about two harmonics modulated in frequency and sounded like a pipe (peep); (3) it also sounded like a pipe, had broader delta frequency, and longer duration than a peep; it differs from a peep due to the higher number of harmonics between 2 and 7, and by the presence of ascending frequency modulation: the beginning frequency is lower than the ending frequency. The short whistles 4 and 5 were longer than the others, and they were only found in the cub's audio recordings. Short whistle 4 had a descending arc shape with some harmonics (Figure 2f); and (5) had an ascending harmonic arc shape at the beginning of the note (ascending arc shaped whistle) (Figure 2g).

U-shaped and ascending arc shaped whistles

Use and context of emission

A varied class of squeak-like whistles, longer than the peep-like short ones previously described, were uttered in syllables and phrases. They were shaped like an inverted U and ascending arc notes and were uttered by an animal that was interacting with others and was suddenly surprised or injured, for example in situations of physical separation, foraging and play-fighting. For example, the U-shaped whistle was uttered by cubs (Figure 2p) and by a pair of juvenile females (BE and RV) during playing. It was also heard during an event of play fight involving a couple of subadult males and other dyadic play event of a juvenile male and an adult female. Yet, a more complex structure of U-shaped whistle, as a fast repetition of notes without interval between them, was uttered as a trill (phrase 4 of Figure 2B) when animals started a real fight (note the presence of non-linear effects that are normally associated with negative and intense motivational states). This trill was uttered by males competing for a female, chasing each other for short distances, kicking the head and rolling on the ground. In food competition situations, the animals uttered phrases of whines and ascending arc shaped whistles. When the animals defended their food items, these ascending arc whistles appeared to represent their motivational state, ranging from the least aggressive state (whistle SQ, Figure 2e) to the most aggressive state (whistles SH and CH, Figure 2c–d). Most of the time, the squeaked-whistle (whistles SQ, Figure 2e) appeared to be enough to repel an intruder away.

Structure and acoustic analysis

The U-shaped whistles were longer than the short whistles (Table 1) and occasionally featured nonlinear effects and had 3–5 harmonics, the 5th harmonic just above 22,050 Hz. The whistles SH and CH had several harmonics, and even extended over the 22,050 Hz range in some cases. The squeaked-whistle had the highest number of harmonics in its elongated S-form.

Twitter (Figure 2A)

Use and context of emission

Twitter was uttered only by a PE juvenile several times after being captured in a hand pole net. It must be the distress call of the species since it was uttered in agony related contexts. It sounded like a higher pitched and urgent trill easily recognized. Immediately after its emission, the other individuals that were inside and outside the cage approached him.

Structure and acoustic analysis

Twitters are seen as repeated waves on the spectrogram with no internal interval; they feature sub-harmonics, frequency jumps and deterministic chaos. They were long in duration and extended over the 22 kHz range. Strong expirations, short whistles (Figure 2i–j), long roars (Figure 1G) and short vibrates (Figure 2b) were also uttered right before or after the twitter.

Squeak

Use and context of emission

Squeaks were produced in interactions associated with playing in foraging areas or nests. The squeaks occurred during play fight between juveniles during the birth season. Squeaks were also uttered in social play between a cub and an older individual, or when several individuals huddled together in arboreal nests and stayed overnight. During passive acoustic and continuous monitoring of JD nests, a concentration of squeaks was recorded early in the morning at 5AM.

Structure and acoustic analysis

Squeaks were emitted in syllables or phrases, partially harmonic (up to 22 harmonics, which rarely extended the 22 kHz range) (Table 1) and were partially filled with sub-harmonics (phrase 3 of Figure 2B).

Whine

Use and context of emission

Adults' whines (Figure 2l–m) were uttered by males that had lost agonistic interactions. Cubs' whines (Figure 2k) were interspersed with short whistles 4 and 5 (Figure 2f–g) when they separated from the adult females. Taking into consideration only the cub's utterances sampled in this present study, among the 260 calls registered for cubs (chirp, coo, bark, subsound, whine, U-shaped whistle, short whistles 4 and 5), 78% were whines (Figure 2k) and U-shaped whistles (Figure 2p) entirely related to isolation contexts of the cubs. It is noteworthy that chirps, coos, barks and subsounds were not as representative as the isolation calls (U-shaped whistles and whines), that are characteristic of the age. As cubs grow up those proportions are expected to gradually change to chirps and represent the vast majority of calls emitted in adulthood.

Structure and acoustic analysis

Whines were uttered in syllables and phrases and classified into adults' and cubs' whines (Figure 2D). The whines of adults were filled with sub-harmonics and frequency jumping (whine NLP, Figure 2m). Whines of cubs had no more than 2 harmonics and had higher fundamental frequency than adult's whines. Nevertheless, the duration (mean ± SD = 0.037 s ± 0.044, range = 0.003–0.196, N 436 = 24 call-types), modulation contour as seen in the spectrograms (with no quantitative measurements) and intervals between notes were similar for both cubs' and adults' whines.

Vibrate

Use and context of emission

Vibrate calls were uttered by young animals (cub and juvenile of BE) while play-fighting and by adult males when staring or fighting each other while competing for females in oestrus during the mating season.

Structure and acoustic analysis

Vibrates were both noisy and had two emphatic frequency elements (harmonics in Table 1). They were filled with chaos and flutter (a wavy outline). The maximum frequency was as high as in twitter (Figure 2A) and squeak (Figure 2B), representing 49.14% of the highest high frequency calls (Figure 4B). They varied in the duration according to the context. The long vibrates (Figure 2a) were uttered along with the U-shaped whistles (Figure 2n) and whines (Figure 2m) by adults and subadults in agonistic contexts. The short vibrates (Figure 2b) were uttered along with the short whistles (Figure 2h) and squeaks (phrase 3 of Figure 2B) by juveniles and cubs during social play.

Chop-Chop (Kaufmann 1962; Smith 1980)

Use and context of emission

The ring-tailed coati's teeth chattered in situations of high excitement inherent to alarm, threatening and distress situations (Figure 1F–G). The individual would tooth chatter while jumping through the branches, pushing the body up and climbing the trunk of the tree. The chop-chops were also recorded from a PE juvenile when restrained (phrase 7 of Figure 1G) and by an adult female from PEC (threat call, phrase 6 of Figure 1G), captured in a Tomahawk Live Trap. This female exhibited extreme motor restlessness, stepping back and forward rapidly with short pauses, gazing the threatening stimulus at the same time she quickly opened and closed her mouth. This sequence was repeated until the cage was covered.

Structure and acoustic analysis

Acoustic signals were produced by two to three beats of the teeth, which sounded like as a fast chop-chop (see narrative in Kaufmann 1962). They were present in phrases of alarm call, just after the first bark, or after a strong exhalation in threat call (Figure 1F–G). They were extremely short, in average 0.012 s (Table 1), with average intervals of mean ± SD = 0.033 s ± 0.048 s (range = 0.004–0.114, $N = 8$ call-types) between the chop-chops. Along with subsounds and coos, they were the lowest and noisiest calls from the acoustic repertoire, representing 12.50% of 1272 call-types (Figure 4B).

The rhythmic calls

Phrases exclusively composed of a type of very short chirps were observed in two different temporal patterns: chirrup and soft chitter (the former being named after previous qualitative descriptions of the *N. narica* calls). Below we present a description of these calls, characterizing them in relation to (1) rhythm of emission (note/second, NS), (2) number of notes present in each bout (NN), (3) average duration of notes (ND), (4) average duration of intervals between chirps (ID), and (5) duration of bout (PD). Statistically significant differences between chirrup and soft chitter were found, but we faced an issue in this analysis. Judging by the Levene test, the variances of the ND and NN variables are similar, whereas the variables of the ID, PD and NS variances are different. Thus, we used ANOVA to test ND and NN ($F_{(1,7)} = 5.756$ with $p = 0.048$ for NN and $F_{(1,294)} = 22.015$ with $p < 0.001$ for ND) and the Welch test for ID, PD and NS ($F_{(1,7)} = 0.393$ with $p = 0.551$ for NS, $F_{(1,92.158)} = 0.084$ with $p = 0.773$ for ID and $F_{(1,3.033)} = 7.562$ with $p = 0.070$ for PD). The results of these tests are presented in detail in Table S5 of Supplemental Material. Initially, we considered a level of significance equal to 0.05, which was corrected to 0.01 by the Bonferroni criterion. With the adjustment, we found statistically significant differences between the means of the ND variable, so we conclude that this variable may have discrimination power to differentiate the calls from each other, supporting our hypothesis that they effectively constitute different calls.

Chirrup

Use and context of emission

Chirrups are a group movement initiation call uttered in bouts by adult females or males while leading the group somewhere (e.g. from a resting area to a foraging area). In the mating season, the resident male uttered a chirrup after a solitary male approached and then the whole group went in the opposite direction. The circumstance when the most chirrups were uttered was when pregnant females left the group to give birth alone (fission phase), and a single female emitted a chirrup to move the remaining of the group between foraging areas.

Structure and acoustic analysis

Chirrups were made of chirp components and had no harmonics (phrase 2 of Figure 1E) within the bandwidth captured by our recording equipment (22 kHz). 60% of the chirp components (110 out of 181) had two elements (El.1 and El.2).

On average, chirrups lasted twice as long as soft chitter (PD; phrase 1 of Figure 1E; Table 1), although there was no significant difference. Chirrups' chirp components were shorter than those of soft chitters, had a narrower frequency range (mean ± SD = 2.59 kHz ± 0.54 kHz, range = 0.81–4.06, N = 181 call-types), and had a higher low frequency (mean ± SD = 8.31 kHz ± 0.51 kHz, range = 6.50–10.39, N = 181 call-types) and lower high frequency (mean ± SD = 502 10.90 kHz ± 0.63 kHz, range = 8.45–12.56, N = 181 call-types) compared to soft chitters.

Soft chitter (Trudgian 1995)

Use and context of emission

Soft chitters were also made of chirp components (phrase 1 of Figure 1E) and seem to function as approaching calls, uttered during grooming and allogrooming. These call-types were only observed at the time of group fissions, uttered by the males of the JD group. The passive acoustic monitoring of vocal activities in the nests recorded peaks of emissions of soft chitters around 12 PM and 6 PM. It was noted that after the resident male or sub-adult uttered soft chitters, juveniles approached and performed grooming and allogrooming before entering the nests.

Structure and acoustic analysis

The soft chitters did not have harmonics within the 0–22 kHz frequency range. 55% of the chirp components had only a single element (El.1). Compared to the chirrups (phrase 2 of Figure 1E), soft chitters sounded shorter, slightly faster (PD; Table 1) and had less chirp components that repeated at shorter intervals (ID, Table 1). Statistical differences between the stand-alone chirps and the soft chitters chirps will be presented in a further study.

Qualitatively, it is interesting to note that the soft chitters' chirp components were slightly shorter (mean ± SD = 0.055 s ± 0.012 s, range = 0.018–0.099, N = 72 call-types), had a narrower frequency range (mean ± SD = 3.16 kHz ± 0.94 kHz, range = 0.97–9.06, N = 72 call-types), a higher low frequency (mean ± SD = 8.18 ± 0.60, range = 6.84–10.69, N = 72 call-types) and a lower high frequency (mean ± SD = 11.33 kHz ± 0.72 kHz, range = 9.62–16.63, N = 72 call-types).

Non-random complex call

When disputing food items or in threatening situations, coatis often uttered orderly sequences made of 2 or 3 distinct component calls (e.g. coo + subsound + chirp; Figure 1D). Most of these sequences were used in a consistent fashion, denoting that they were specifically associated with certain circumstances or motivational states. Similar sequences are described for other species in the literature, where they are often classified as complex calls (e.g. Clucas et al. 2004; Krams et al. 2012). Here, such sequences were named non-random complex call to distinguish them from the rhythmic calls which, although *complex* in the sense they are also made of multiple components, clearly define a distinct structural complexity system since their components are identical. The non-random complex call accounted for 13.05% of all calls (i.e. 166 out of 1272 calls, Figure 4B) and 96.51% of all orderly sequences we recorded (166 out of 172) as, for instance, 'chirp + coo + subsound', 'subsound + bark' or 'bark + chirp' and others.

The tree of probabilities seen in Figure 3 provides a statistical perspective of the structures of the non-random complex call, showing how their component calls distribute in a probability space with n = 172. In order to make its interpretation more intuitive, the tree occasionally presents a 'none' component in some positions to indicate the end of a sequence, so that all the probabilities in a given ramification will always sum up to 100%. Also, Figure 3 gives useful insights about the sequences allowing, for example, to tell actual non-random complex call from constructs that, although orderly, are random, i.e. looked randomly formed, as observed through a very low number of occurrences. For instance, by analysing the tree, it can be seen that the 2-component calls that began with a bark or coo (i.e. coo + subsound, and bark + chirp) accounted for less than 1.2% of all analysed recording samples so, in a first analysis, it is reasonable to assume that they occurred merely by chance rather than design and therefore cannot be considered as non-random complex call (nor complex calls of any kind). In other words, within the scope of this study, a given sequence is considered random if its likelihood of occurrence is too low as told by its relative frequency; otherwise, it is classified as a non-random complex call. The main point of interest here – the *existence* of the non-random complex call – is unequivocal as the tree depicts, which is relevant as it indicates that the ring-tailed coatis developed a syntax-like natural communication system similar to the one Freeberg and Lucas (2002) described for the chick-a-dee (Hailman et al. 1985, 1987; Hailman 1994; Freeberg 2006; Hailman and Ficken 1986; Krams et al. 2012).

In consonance with the convention just stated, if the likelihood of every component of a given sequence is larger than t (an empirical threshold used to identify non-random complex call in the sequences shown in Figure 3), then it is classified as a non-random complex call. Since the likelihoods of all components in the tree are either very low (below 3%) or fairly high (above 20%), any intermediary value in this range is statistically justifiable for t. In the particular case of Figure 3, this analysis is not only simple but also numerically robust because it can change appreciably without modifying the final classification (meaning that the same non-random complex calls are identified for any 3% ≤ t ≤ 20%). Considering t = 10%, the tree unveils the existence of 5 non-random complex calls, as follows (Figure 3):

(1) chirp + coo + subsound – foraging context – 47 occurrences;
(2) subsound + bark – threatening context – 45 occurrences;
(3) chirp + subsound + bark – threatening context – 32 occurrences;
(4) chirp + subsound – foraging / threatening contexts – 29 occurrences;
(5) chirp + coo – foraging context – 13 occurrences.

Other sequences like, for example, chirp + coo + subsound + bark (a single occurrence out of 172 samples) are regarded as random or anecdotal. We have observed that in all the threatening situations we recorded, the coatis coughed, sneezed, exhaled forcefully or emitted a chop-chop after uttering non-random complex calls.

As discussed above, the tree modelling is self-explanatory and can be easily interpreted. For instance, based on sequences 2 and 3 above, it is clear that barks are likely to follow subsounds and that coos normally follow chirps, not subsounds. Also, the non-random complex call is likely to start with chirps (1st position, 72.67% of the cases) and is generally followed by coos (2nd position, 49.60%), subsounds (3rd position, 77.42%) and then finishes (4th position, 97.92%).

The non-random complex call varied in duration, being longer in the agonistic context (mean ± SD = 0.086 s ± 0.021 s, range = 0.054–0.113) than in foraging context (mean ± SD = 0.071 s ± 0.015 s, range = 0.024–0.100), followed by alarm/threat context (mean ± SD = 0.048 s ± 0.019 s, range = 0.013–0.081). Syllabic chirps varied in duration and number of harmonics in relation to the isolated chirps uttered in contact contexts. In contexts of alarm, syllabic chirps were slightly longer (mean ± SD = 0.073 s ± 0.019 s, range = 0.042–0.116) and showed no harmonics above the fundamental. In the agonistic and foraging contexts, the syllabic chirps lasted less than the isolated chirps (mean ± SD = 0.046 s ± 0.019 s, range = 0.024–0.098; and mean ± SD = 0.029 s ± 0.005 s, range = 0.023–0.04, respectively). Yet, in foraging contexts, chirps occupied a narrower frequency band (mea n ± SD = 5.22 kHz ± 4.91 kHz, range = 1.06–12.51) whose low frequency was higher (mea n ± SD = 8.24 kHz ± 0.66 kHz, range = 7.21–9.2), and the high frequency was lower (mea n ± SD = 13.46 kHz ± 4.64 kHz, range = 9.76–20.39) than in contact contexts.

Incidental or non-consistent call: the long chirp (Figure 1C)

There was a single record of a sound, supposedly a long-distance contact call, which did not appear frequently enough for us to fully characterize it. Considering the large variance that is characteristic of biological processes, anecdotal information can be valuable and should not be discarded carelessly. For this reason, we include here the description of this sound. This call sounded like a chirp stretched in time and was uttered in a series of five by a single young male walking alone. The design of the call-type is of a chirp formed by two interconnected elements, continuous and long (a long plateau, between up and down fast and short frequency modulations) which lasted 0.201 s. The long chirp occupied the frequency range from (±0.23 kHz, range = 7.66–8.29) to 12.42 kHz (±200 0.46 kHz, range = 11.65–12.81, N = 5 call-types) dominant at 10.9 kHz (±0.94 kHz, range 201 = 9.91–12.23) in 5 call-types.

Discussion

This study provides the first description of the acoustic repertoire of free-ranging ring-tailed coatis *Nasua nasua* based on quantitative measures of vocalizations. Concatenation of calls forming a multicomponent signalling had not been described for this species until now. We established that coatis use 15 communication sounds that frequently feature non-linear phenomena (NLP), including rhythmic calls (chirrup and soft chitter) and non-random complex calls (i.e. concatenation of calls) that enrich their communication system. We also found evidence that some of these sounds uttered in agonistic and play contexts have energy in the ultrasonic band harmonics were still considerably intense above the human-audible range (>20 kHz), as previously suggested (e.g. Maurello et al. 2000). The high vocal activity in social interactions supports social functions of the vocalizations.

We believe that the sociality of the ring-tailed coati is associated with its call repertoire size, an attribute of communicative complexity, as it was described for other carnivores, rodents, primates and birds (Blumstein and Armitage 1997; McComb and Semple 2005; Arnold and Zuberbühler 2006; Freeberg 2006; Ouattara et al. 2009; Jansen et al. 2012; Pollard and Blumstein 2012; Déaux et al. 2016). For now, this is the largest and most diverse acoustic repertoire among procyonids: thirteen call-types were described for the *Procyon lotor* (raccoon; Sieber 1986 as cited in Compton 1998) and nine for both the *Bassariscus astutus* (ringtail; Toweill and Toweill 1978 as cited in Compton 1998) and *Potos flavus* (kinkajou: Poglayen-Newall 1976 as cited in Compton 1998), and much less for the *Bassariscus* sp. (olingo), *B. sumichrasti* (cacomistle) (Poglayen-Newall 1976 as cited in Compton 1998; Trudgian 1995; Maurello et al. 2000; Compton et al. 2001). However, most of these reports, if not all, contain only qualitative and onomatopoeic descriptions (e.g. Trudgian 1995; Compton 1998; Maurello et al. 2000), hindering comparisons.

In the genus *Nasua*, between 9 and 21 acoustic signals had been reported for *N. narica* and 10 for *N. nasua* (Trudgian 1995; Compton 1998; Maurello et al. 2000; Compton et al. 2001). The coo and the subsound are also present in the repertoire of *N. narica* as we heard them in the recordings available in the literature (Emmons and Feer 1997) (see our sonographic analysis in Figure S4, Supplemental Material). None of the previous descriptions for the genus *Nasua* (Trudgian 1995; Compton 1998; Maurello et al. 2000) mentioned the presence of non-linear effects in call-types, nor the combinatorial system described here, labelled *non-random complex call*. Regarding the rhythmic calls, Trudgian (1995) might have seen them in *N. narica* and named them chitter, *a series of 7–8 chirps of short duration*, in two forms (loud and soft chitters) in grooming and social bonding. Our description of the soft chitter seems to match that report. The bark, was uttered both in the alarm and threat contexts for the *N. nasua*, but only attributed to warning situations, in *N. narica* (Trudgian 1995).

The whistles, squeaks, vibrates and twitters were not described before. On the other hand, growl, hiss, chuckling, squeal and grunt were onomatopoeias used to refer to the *N. narica* signals that were impossible to compare with our recordings. Again, the quality of the description and image did not allow us to identify what the authors called squawk (Compton et al. 2001), but it does not seem to correspond, neither in form nor in context of emission, to our description.

The nonlinear phenomena (NLP) we found in coati calls might be an evidence of the complex use of signals to convey rapid variations in internal states or identity, as proposed by Wilden et al. (1998), and then by Fitch et al. (2002), in mammals. Those phenomena were noted in calls recorded mainly during stressful situations as found in a varied range of mammalian species (e.g. in pig, dog and macaque: Tokuda et al. 2002; in chimpanzees: Riede et al. 2004; in aquatic mammals: Mann et al. 2006; Tyson et al. 2007; in carnivores: Volodina et al. 2006; Townsend and Manser 2011; in rodents: Blumstein and Recapet 2009; Verzola-Olivio and Monticelli 2017). More interesting is the first description of biphonation in coatis.

The infant isolation calls of the *N. nasua* recorded in PET were short whistles and whines that promptly attract caregiver's attention, which may have been described as loud chitter in *N. narica* (which may be composed of chirps), judging by the contextual description provided by Trudgian (1995). An interpretation supported by findings of Lingle and Riede (2014) in studies of the immediate response of mule deer caregivers to the fawn calls would relate to the behaviour of coati caregivers to assist cubs in distress. As suggested by Lingle and Riede (2014), the temporal and spectral variation of the infant's signals and the discrete transition between the contexts of contact and isolation may signal the rise of stressful circumstances, while the duration of the call-types (e.g. short whistles and whines of coati cubs) would emphasize an increase of urgency. For example, in the context of contact that is expected to be a little urgent, the cubs tend to chirp, while searching for, and approaching the group. As the arousal levels increase with the time of separation, there is a discrete transition of behaviours and contexts from contact to isolation (Lingle and Riede 2014) that leads the cubs to utter short whistles, and whines. In the most urgent level, a distress call (twitter) might be uttered; however, we have not yet observed utterances of twitter by cubs.

Chirps from *N. narica* had ultrasonic frequencies between 30 and 55 kHz (Maurello et al. 2000), which would not have been noticed here due the frequency range of our equipment (0–22 kHz), and two overlapping resonance bars (without harmonic relationship) instead of the vertical structures of El.1 + El.2 described by us. Biphonation has not been mentioned for *N. narica*, which needs reassessment, but it was certainly present in *N. nasua*, based on the temporal overlap of elements. Chirps of *N. narica* were shown to be individually distinctive (Maurello et al. 2000). As rhythmicity and non-linear effects should serve as a source of variation to be associated with different information (Mendes et al. 2009; Schneider and Anderson 2011), it is plausible to think that *N. nasua* chirps are also individually distinctive, favouring group coordination and modelling dyadic interactions through cues about the motivational state of the other individual (Da Cunha and Byrne 2009). Judging by its context and onomatopoeic description, the concluding plosive sound described in urgency situations after the chirp in the *N. narica* by Compton et al. (2001) might be analogous to the coo or subsound in the *N. nasua* (Figure S4, Supplemental Material). However, this is not the case as the sound described and depicted by Compton was a broadband sound ranging from at least 1–5 kHz. So, to the extent of our knowledge, both coo and subsound are exclusive to *N. nasua* (Figure S4, Supplemental Material).

The ability to combine the same set of call-types or calls into more complex structures in context-specific ways was reported before with chickadees (e.g. Freeberg and Lucas 2002 and Clucas et al. 2004). Freeberg and Lucas (2002) stated the presence of a single type of chickadee call, highly variable and possessing a simple Markovian rule regulating the order of the primarily notes (A, B, C and D). Those four notes may be omitted or repeated in a variable number of times, forming a fixed and stereotyped series for every call-type (Hailman et al. 1987; Freeberg and Lucas 2002; Bouchet et al. 2013). Nevertheless, the ring-tailed coatis have a larger acoustic repertoire with 15 different calls besides being capable of syntax-like combinations into the non-random complex calls, which may be related to their complex relationships and social networks (Hirsch et al. 2012).

The non-random complex calls are composed of an initial component described in this work as a chirp, which is suggested by Maurello et al. (2000) to convey cues to identify the sender. The second position of non-random complex calls is occupied very often with either coo or subsound according to the context. Because coos were uttered primarily in complex sequences immediately after an individual discovers food, they are supposed to convey the hunger level of the emitter. The acoustic structure of coos differs from the stereotyped subsounds and barks, which might mean coos function as affective components (Hauser 1998); therefore, they can reveal an enhanced ability to detect food resources (Szipl et al. 2015; Freeberg et al. 2017) in coatis. Nevertheless, further investigations are needed to understand the meaning of coos and subsounds in acoustic complex sequences. Barks are the last components of the non-random complex calls and we believe they convey information of predation risk. If those interpretations pointed non-random complex calls as the food-associated call of coatis, we believe that Hirsch et al. (2012) reported an expected female behaviour mediated with non-random complex calls, although they did not mention any call-type during their observations. According to the observations of Hirsch et al. (2012), female coatis favour juveniles foraging success, indicate food location and its quality (perhaps uttering non-random complex calls) and provide cues for their individual preferences.

Despite the literature existing on the social and communicative complexity of coatis (e.g. Romero and Aureli 2008; Arsznov and Sakai 2012; Hirsch et al. 2012), there are no studies that correlate the average group size to the vocal repertoire size. In other contexts, many studies have shown that repertoire complexity may match social structure complexity [e.g. chickadees (Freeberg and Lucas 2002); primates (Gustison et al. 2012); social rodents (Eisenberg 1974; Blumstein and Armitage 1998; Monticelli and Ades 2011; Pollard and Blumstein 2012)]. According to Pollard and Blumstein (2012), there are many ways to predict communicative complexity from a social complexity in which distinct attributes are evolutionarily or functionally linked. For the coatis, the most likely linked attributes of communicative complexity are individuality (through individual signatures), signaller reliability, repertoire size, urgency or affect, ordering or syntax and different response strategy to environmental stimuli.

This study opens new perspectives for comparative research on the evolution of communication in procyonid species. Despite our effort, it was not possible to precisely compare *N. nasua* and *N. narica* repertoires. In the future, the results presented here might help uncover the relative influence of distinct aspects of sociality, such as group size, social network complexity, strength of social bonds, in parallel with vocal complexity. For years, the literature has focused on social and cognitive abilities of the primates. Now, coatis may also be seen as a good model for such comparative studies about social aspects of behaviour (Romero and Aureli 2008; Gasco et al. 2016), offering insights about the selective pressures for the size and diversity of mammalian vocal repertoires.

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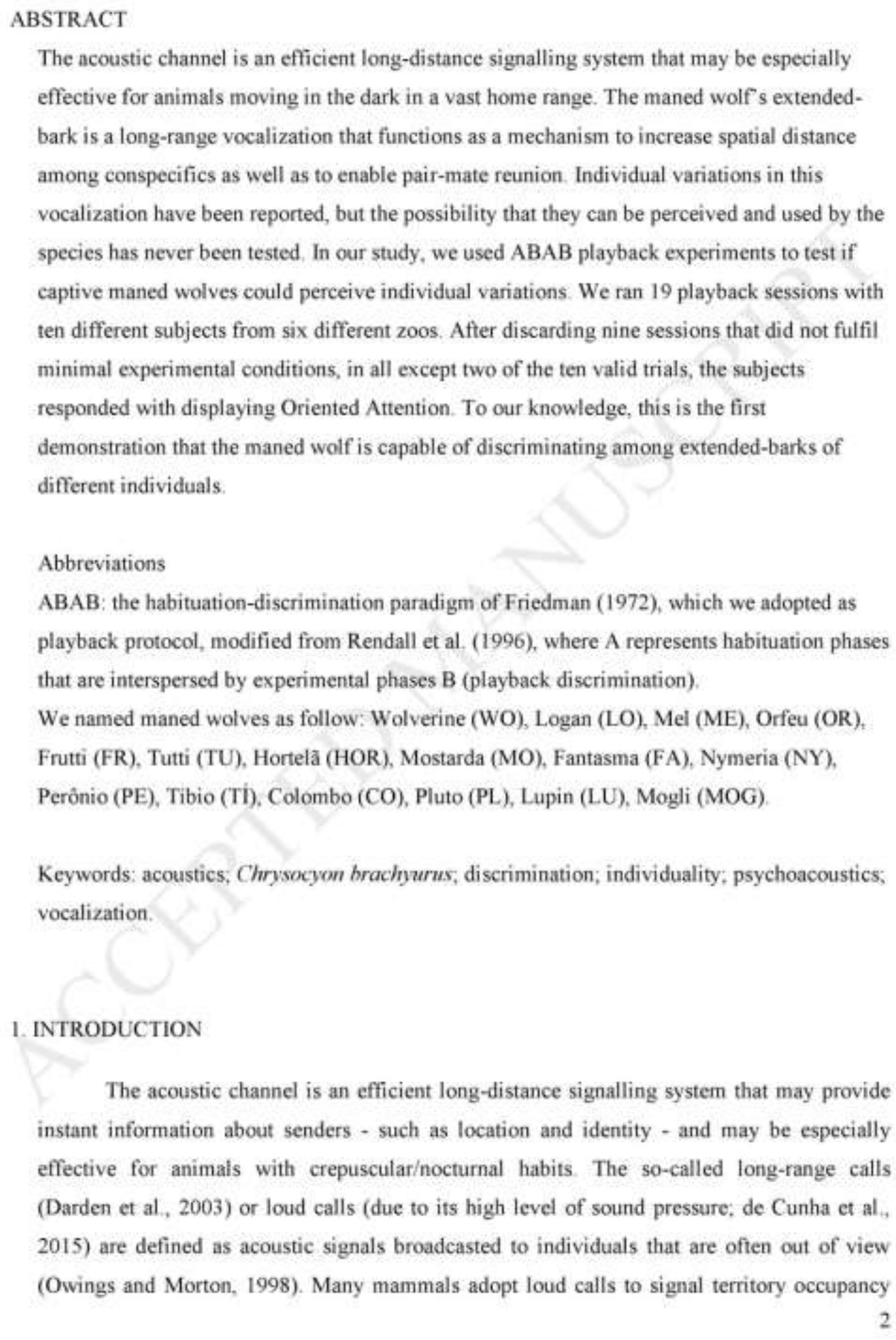
Apêndice B – *The evidence ou individual discrimination in the maned wolf long-*



ABSTRACT

The acoustic channel is an efficient long-distance signalling system that may be especially effective for animals moving in the dark in a vast home range. The maned wolf's extended-bark is a long-range vocalization that functions as a mechanism to increase spatial distance among conspecifics as well as to enable pair-mate reunion. Individual variations in this vocalization have been reported, but the possibility that they can be perceived and used by the species has never been tested. In our study, we used ABAB playback experiments to test if captive maned wolves could perceive individual variations. We ran 19 playback sessions with ten different subjects from six different zoos. After discarding nine sessions that did not fulfil minimal experimental conditions, in all except two of the ten valid trials, the subjects responded with displaying Oriented Attention. To our knowledge, this is the first demonstration that the maned wolf is capable of discriminating among extended-barks of different individuals.

Abbreviations

ABAB: the habituation-discrimination paradigm of Friedman (1972), which we adopted as playback protocol, modified from Rendall et al. (1996), where A represents habituation phases that are interspersed by experimental phases B (playback discrimination).

We named maned wolves as follow: Wolverine (WO), Logan (LO), Mel (ME), Orfeu (OR), Frutti (FR), Tutti (TU), Hortelã (HOR), Mostarda (MO), Fantasma (FA), Nymeria (NY), Perônio (PE), Tibio (TI), Colombo (CO), Pluto (PL), Lupin (LU), Mogli (MOG).

Keywords: acoustics; *Chrysocyon brachyurus*; discrimination; individuality; psychoacoustics; vocalization.

1. INTRODUCTION

The acoustic channel is an efficient long-distance signalling system that may provide instant information about senders - such as location and identity - and may be especially effective for animals with crepuscular/nocturnal habits. The so-called long-range calls (Darden et al., 2003) or loud calls (due to its high level of sound pressure; de Cunha et al., 2015) are defined as acoustic signals broadcasted to individuals that are often out of view (Owings and Morton, 1998). Many mammals adopt loud calls to signal territory occupancy (*e.g.*, lion *Panthera leo*: McComb et al., 1994, Grinnell and McComb, 2001; other great cats: Darden *et al.*, 2003; gray wolf *Canis lupus*: Harrington and Mech, 1983; chimpanzees *Pan troglodytes*: Mitani & Nishida, 1993), to attract mates and even to stimulate premature ovulation, as it indicates male quality (*i.e.* physical attributes or health condition; *e.g.*, "roar" in *Cervus elaphus*: Clutton-Brock and Albon, 1979; Reby and McComb, 2003; in *Panthera tigris*:, Ji et al., 2013; "songs" of the fin-whale, *Balaenoptera physalus*:, Croll et al., 2002). It has been demonstrated that individuals of some species are capable of distinguishing familiar vocalizations from unfamiliar ones, even when they are far away from the sender (*e.g.*, gray wolf *Canis lupus*: Tooze *et al.*, 1990; rhesus monkey *Macaca mulatta*: Rendall *et al.*, 1996; lion *Panthera leo*: Grinnell and McComb, 2001; African elephant *Loxodonta africana*: McComb *et al.*, 2000).

The maned wolf's extended-bark is a loud vocalization whose abrupt start and broadband frequency favour its perception and its location source (McGregor, 2005). If this vocalization is involved in the provision of clues about the sender's identity, either to permit reproductive partner recognition or to signal territory property, such vocalization might have recognisable individual variations.

The maned wolf (*Chrysocyon brachyurus*, Illiger, 1815) is a large canid inhabiting open vegetation areas, mainly savanna's phytophysiognomy (Batalha, 2011) of Cerrado (Dietz, 1985). The species has been described as having crepuscular/nocturnal habits and being solitary since the members of a reproductive pair are rarely found together during activity periods (Dietz, 1984). It would be more precise, however, to define the maned wolf as a level two or three of sociality species (*i.e.* aggregation for one or two activities, solitary to others: Lee, 1994), since the pair mates may share a vast territory (Kleiman, 1972; Dietz, 1984) from 20 to 115 km^2 (Dietz, 1984, 1985; Carvalho and Vasconcellos, 1995; Melo et al., 2007), may even rest and hunt together (Bandeira de Melo et al., 2007). The species tends to monogamy and both male and female invest in parental care (Bestelmeyer, 2000; Veado, 2005).

To the best of our knowledge, the only published studies that address the acoustic behaviour of the maned wolf were made in the 70's and 80's by Encke (1970, 1971 *apud* Kleiman, 1972), Kleiman (1972), Tembrock (1976), Brady (1981) and Dietz (1984). Since then, there is a gap on studies focusing on the maned wolf's acoustic repertoire. Brady (1981) has described ten different signals, including the loud "single-bark" and the "roar-bark" (called "single-bark" by Kleiman, 1972; and "extended-bark" by Dietz, 1984, which is the term we adopted in our work). The extended-bark resembles an extended variation of the bark of a large domestic dog (Dietz, 1984), and is emitted in sequences either by males and females or in duetting (Dietz, 1984; personal observations). Kleiman (1972) has suggested that the extended-barks function as a spacing mechanism among individuals, such as the roars of the great cats. After observing several exchanges of vocalizations between pairs and between pair-mates, Dietz also suggested that it occurs at all times of the year (most commonly during June through August, in that year of 1979), and may have a role in aiding the localization between pair-mates.

The maned wolf's body is large and requires a lot of energy supply. As its food is scarce and dispersed (mostly fruits and small vertebrates such as rodents and birds – De Arruda Bueno et al., 2003; occasionally, larger animals, Bestelmeyer and Westbrook, 1998) the best strategy for this species is to hunt alone and use mutually exclusive foraging areas. If a strict territoriality has increased the survival of individuals, then natural selection must have favoured characteristics that emphasize the property of an area (Dietz, 1984). In fact, the maned wolf exhibits scent-marking behaviours with urine and faeces and produces a long distance vocalization (the extended-bark), which are presumably related to territorial defence in close proximity and at longer range, respectively (Dietz, 1984; Kleiman, 1972).

Brady (1981) has reported individual variations in maned wolf extended-barks and stated that a human could distinguish individual differences in extended-barks at a distance of 1 km. Nevertheless, the possibility that this variability can be used by maned wolves for individual recognition has never been tested. One should expect these individual variations to be perceived by the species, for only in this scenario it would be plausible for the extended-bark to function as a mechanism to increase spatial distance among conspecifics and to enable pair-mate reunion. If this individual variability is not perceived by conspecifics, the efficiency of this vocalization at long distances would be compromised, as the receiver would not be able to identify the sender as its reproductive partner or a potential rival. We used playback experiments to test if individual variations in the extended-bark of maned wolves can be perceived by the species. To the best of our knowledge, the ability to discriminate individuals through the voice itself has never been demonstrated in this great carnivore of Latin-America.

2. METHODS

2.1. *Study sites and animals*

We carried out all acoustic and behavioural data collection in captivity, in seven different zoos of the State of São Paulo, Brazil. The total number of maned wolves participating in this study was 16 (Table 1). From this total, only 6 (four males and two females) produced extended-barks that could be used to create the soundtracks to be used in playback trials. Playback trials were conducted with 10 subjects (three pairs and seven solitary males). In all zoos, the enclosures permitted visitors to see the animals; hence, the maned wolves were accustomed to human presence.

Besides the captive maned wolves, we also had the chance to observe two wild individuals. The first (HOR) was a free-living animal from the ecological reserve bordering the zoo of São Carlos and that often entered the zoo through a hole in the outer fence. We assumed it was a male from observation and unofficial information from zoo's employees. The second wolf (MOG) inhabited the Ecological Station of Itirapina and its surroundings.

INSERT TABLE 1 AROUND HERE

2.2. *Recordings*

Vocalizations were recorded manually in a single channel by a solid-state digital recorder (Marantz PMD661-MKII) attached to a professional unidirectional microphone (Sennheiser ME67 head with K6 powering module - frequency response: 50–20,000 Hz) suited with a windshield (Rycote classic-softie windshield). Since the maned wolf's extended-barks can reach 20 kHz (as noticed with MOG recorded barks and known by unpublished dissertations), we chose a sample rate of 44.1 kHz. All files were saved in uncompressed 16 bits PCM-WAVE format. We also monitored maned wolves' vocal patterns using remote sound recordings with an automated digital recorder (Wildlife Acoustics, SongMeter 2+), that permitted continuously recording from 17:00 to 07:00, without the experimenter presence. It was installed in the open area of the enclosures. Recording sessions (manual and automatic) were taken in each zoo continuously from 17:00 to 07:00 (UTC-3), from 2013 to 2015, in thirteen 10-days field campaigns. The distance between the emitter and the manual microphone varied from 7 to no more than 15 meters, except for the FR wolf to whom it was impossible to be closer than 40 meters. To get recordings with similar conditions in all of the zoos, we measured the sound pressure level (of vocalizations and environmental noise in dB) using a sound level meter (Instrutemp, ITDEC-4080; range 30dB to 130dB). The amount of data collected was then scanned manually in Song Scope software (Wildlife Acoustics). We also used some of these vocalizations automatically acquired as stimuli for some of the playback tests (see topic 2.4.1 for details).

2.3. Playback soundtracks preparation

Playback tracks were edited in Raven Pro (1.4 version) composed of individual notes (playback stimulus) set in a temporal series similar to a natural extended-bark (Figure 1, top). To avoid sex and oestrous stage influences on vocalizations, we used only notes from males (FA, TU, and OR) as playback stimuli. The notes were randomly selected in R program among those with a good signal-to-noise ratio. The intervals between two stimuli (notes) respected the natural sequence patterns of the maned-wolf extended-bark, of about 5 seconds. We avoided repeating notes in the soundtracks (*i.e.* using different notes to be the stimulus A: A_7, A_8, A_9, B, A_{10}) and we used new test soundtracks for each playback session. That approach increases the consistency of our results by preventing simple pseudo replication (Kroodsma et al., 2001).

INSERT FIGURE 1 AROUND HERE

The quality of the sound played by our equipment was verified. We recorded a playback soundtrack when it was being played during a first playback session, and compared that to the original recording, using Raven Pro. The spectrogram analysis of the recorded playback showed no distortion or anomaly in the notes.

2.4. Experimental Procedure

If the maned wolf is capable of distinguishing its reproductive partner from a possible competitor using only the extended-bark, then we expected this vocalization to have individual variations that provide clues about the sender's identity. In case our hypothesis was valid, the maned wolves would be able to distinguish among different conspecifics' extended-barks. In order to test this prediction, we used the habituation-discrimination paradigm (Friedman, 1972; Cheney and Seyfarth, 1988; Rendall *et al.*, 1996; Hauser, 1998; Reby and McComb, 2003; Mumm et al., 2014; Palacios et al., 2015). Habituation is defined as a gradual decrease in the magnitude and/or frequency of the response due to repeated presentations of a stimulus (Catania, 1999; Rankin et al., 2009). The typical habituation-discrimination paradigm consists on presenting repeatedly a given stimulus (*i.e.* habituation stimulus; *e.g.* extended-bark from individual 'A') at fixed time intervals until the subject habituates to it, followed by the presentation of a different stimulus (*i.e.* test stimulus; *e.g.* extended-bark from individual 'B'). If this subsequent exposure to the test stimulus immediately increases the magnitude and/or frequency of the response, discrimination between stimuli A and B occurred.

Sessions started within the period of activity of the species (Dietz, 1984), in the early morning (6:00), to take advantage of photoperiod for video documentation. After preparing all playback and video recording apparatus, we waited approximately 10 minutes in silence and motionless, before starting each test, to familiarize the subjects to our presence and the apparatus. Acoustic stimuli were played back using a powered speaker (Boxer M-10; output power 100 W; impedance 4-8 Ω; frequency range 0.05 kHz - 20 kHz). The speaker was positioned 30 m away from the subjects and out of their sight. Volume level was adjusted to ensure the stimuli were audible by the subjects and simulated a natural barking bout that we witnessed during the recording phase. To prevent changes in the wave sound caused by ground attenuation (Wiley and Richards, 1978), we placed the powered speakers at a distance of approximately 0.5 m from the ground. During the tests, we documented the maned wolves' behaviours with a Sony Handy cam, except for the couple FA and NY that requested individual cameras.

2.4.1. Standard Playback Protocol

To test whether the maned wolf was able to discriminate among extended-barks of different individuals (A and B) we adopted the following playback protocol (modified from Rendall et al., 1996):

Habituation phase: we used a sequence of 6 extended-barks (A_1, A_2, A_3, A_4, A_5, A_6) from wolf A as the habituation stimulus. The sequence was presented to the subject repeatedly until it was habituated (for details see topic 2.5). We chose to use a sequence as stimulus, rather than a single extended-bark, to expose the subject to six different samples of class A stimulus. Thus, the subject was habituated to the individual content of wolf A's extended-barks, not to the particular acoustic characteristics of a single note, a more reliable approach to the natural situation of emission.

Habituation control phase: subsequent to the subject's habituation, we broadcast new extended-barks from wolf A. According to our prediction, these new stimuli (A_7, A_8, A_9) would retain the acoustic characteristics to which the subject had been habituated. Thus, if the subject had not recovered from habituation, we would assume that it had habituated to the acoustic features virtually present in all stimuli from class A. Otherwise, our postulate would be discarded and we would conclude that the subject had only habituated to the initial habituation series (*i.e.* A_1, A_2, A_3, A_4, A_5, A_6). Again, we choose to use three new stimuli rather than a single one.

Discrimination phase: after the habituation control phase, we broadcast a single stimulus from wolf B (the interval between this discrimination stimulus B and the precedent class A stimulus was of about 10 seconds). Stimulus B was also new. Therefore, if stimulus B elicited a response similar to that obtained at the beginning of the habituation phase (*i.e.* before the wolf was habituated), we would assume that the subject had discriminated from maned wolves A and B extended-barks.

Re-habituation phase: at last we presented a new stimulus from class A (*i.e.* A_{10}). If we had observed a decrease in the magnitude of response, or even no response anywise, we would endorse the statement that the subject was able to discriminate between A and B. Albeit we could re-exposure the subject to a stimulus of type A that had already been used, we preferred to broadcast a new one (A_{10}) as a way to increase experimental control.

Sessions on a single zoo were separated by at least 1 week from one another, except in Sorocaba's zoo, for logistical reasons (1-day interval between sessions). We ran the test more than once in several sessions to test habituation to B. However, we only considered the first trial of each session as valid, since the habituation control phase stimuli (A_7, A_8, A_9) and the re-habituation stimuli (A_7) were no longer new after the first trial (Table 2).

2.4.2. Complementary Playback Protocol for CO wolf

The first playback session of the subject CO was harmed by confounding variables, particular to that zoo: loud background noise, frequent human interference, dog barking and others. Under such adverse conditions, the subject did not show a clear response to the stimuli change in the standard protocol. One of the possible reasons for the absence of response was that the subject did not perceive the B stimulus along the track since there were too many distractions during the test. Another possibility was that the subject did not have enough time to react to B stimulus. Thus, even if it had started to react to B, the following stimulus (*i.e.* A_8) could have interrupted it. We could not elongate the interval between notes since the silence itself could be a confounding factor. So, we ran a new test: (1) we played a series of stimulus from wolf A (A_1, A_2, A_3, A_4, A_5, A_6), and then (2) a series of stimulus from wolf B (B_1, B_2, B_3, B_4, B_5, B_6). The criteria of response (see topic 2.5) and the procedure were the same of topic 2.4.1.

2.4.3. Control playback procedure evaluations

2.4.3.1. Background noise effect evaluation

Since recordings were made in uncontrolled environments, the background noise differed from one recording to another. That difference was especially pronounced between automatic and manual recordings. The recordings obtained by Song Meter are much noisier (*i.e.* more background noise is captured with the signal of interest), probably due to the nature of its microphones, and this difference could not be eliminated without inserting other confounding variables (e.g., filtering natural acoustic characteristics of that environment). To guarantee that the subjects' responses occurred due to the change in the stimuli and not to the difference among the types of background noise of each track, we used two forms of control:

(1) We inserted background noise extracted from track B (background noise B) in the habituation soundtrack and just before stimulus B (Figure 1). This strategy allowed us to know if alteration in the subjects' behaviour was due to the change of stimulus (*i.e.* from A to B) or due to the background noise change. If the subject did not react to the inserted fragments of background noise, we assumed that discrimination between A and B took place. The duration of each background noise fragment was of approximately 5 seconds (the same adopted in extended-bark series).

(2) We also held a playback trial with subjects NY and FA using only noise as stimuli to test their responses to the difference in background noise alone. The protocol we used was simpler than the standard one: we played a track which contained an excerpt of noise extracted from the stimulus A recording (background noise A), followed by an excerpt of noise extracted from the stimulus B recording (background noise B). Our expectation was that the subject would react to none of them or, if there was a reaction, the behaviours presented would differ from the ones that occurred in the presence of the extended-barks. For this test, we used only background excerpts that evidently differed from each other, such as background from Song Meter recordings against the background from manual recordings.

2.4.3.2. Stimulus control test

With one of the subjects (male TU), we had the opportunity to play an audio track comprising 15 minutes of surround sound which had been previously recorded before initiating the extended-barks playback tests. This control-track allowed us to evaluate the animal's reaction to artificially played sounds. If the subject responded in some way to the playback of surround sound it would have been harder to guarantee that its reaction to the other playback tests (all artificially played) was due to the stimuli.

2.5. Behavioural Analysis

During the habituation phase of each playback trial, we studied the responses shown by the subjects in order to judge which could be elected as good estimators of the maned wolves' reaction to the stimuli. We adopted the following criteria: (1) the response would have to follow an habituation pattern (*i.e.* gradually decreased in magnitude and/or frequency after repeated presentations of a stimulus); (2) the occurrence of the response would have to be easily identified by an untrained observer; (3) the duration of the response would have to be brief (less than 10 seconds), since the interval between the discrimination stimulus and the re-habituation stimulus was of about 10 seconds. The response that best matched all requirements was the "Oriented Attention" (Figure 2): maned wolf turns its head towards the speaker and remains with ears raised, eyes wide open and outer ears facing the speaker during at least 1 second. The reaction can occur just after the stimulus or have a latency of maximum 4 seconds. (Adapted from Palacios *et al.*, 2015).

In order to comply with the habituation-discrimination paradigm, we only analysed tests in which the subjects displayed the Oriented Attention response at the beginning of the habituation phase. However, any response to the test stimulus (*i.e.* B) might have been influenced by the fact that the subject had already heard extended-barks. Thus, to consider the subject was habituated during playback procedures, both the frequency of the Oriented Attention response and the magnitude of the subject's overall "disturbance state" (examined by the experimenter under Konrad Lorenz 1962's "Gestalten" concept 1959 *apud* Hutt & Hutt, 1974; the experimenter conclusion was checked later by five untrained observers, as explained below) should decrease. Since we used Oriented Attention as a measure of the response, we only played the test soundtrack (*i.e.* A_7, A_8, A_9, B, A_{10}) when the subject was not facing the speaker (we waited until it happened, that never took more than one minute) and when no other noticeable acoustic stimulus was present.

To score the subject's responses, we watched the playback videos in the Camtasia Studio software (version 8.6.0; TechSmith Corporation). This software displays the video and the audio's waveforms on the same screen, which allowed us to accurately visualize the timing between stimulus (sound) and response (image). To measure the subjects' responses to the stimuli we used a single measure: Oriented Attention presence. Thus, discrimination between A and B happened if the subject displayed Oriented Attention only in the beginning of the habituation phase and during/immediately after B. To prevent "experimenter bias" we elected five untrained observers to judge the validity of our "gestalt" Disturbance. Each judge watched video excerpts from CO's playback tests. Four of the excerpts corresponded to moments in which we considered the subject was "very perturbed" (*i.e.* high magnitude level of Disturbance). The other four excerpts were from moments we considered it "less perturbed" (*i.e.* lower magnitude level of Disturbance). We randomly mixed the video excerpts and, as a result, we obtained four pairs of video containing excerpts we judged as different "gestalten", one of them corresponding to the transition from stimuli A to stimuli B (Table 3). Judges were asked to watch each pair of video excerpts and score if the animal's activity had decreased or increased from the first excerpt to the second. The judges were familiar with our definition of the "gestalt" Disturbance and evaluated the videos on mute mode. To determine inter-observer agreement, we used Fleiss' Kappa statistic calculated with and without author's scores. To determine the strength of agreement, we adopted Landis and Koch (1977) benchmarks.

3. RESULTS

3.1 Recordings

We sampled nine captive maned wolves (6 males and 3 females) and one wild wolf (unknown sex) and gathered 1911 notes distributed in 107 sequences (Table 4). We recorded five duet events between the couples FA-NY (n=3) and FR-TU (n=2) (Figure 3). Male and female were out of each other's sight before and during the events.

During fieldwork in São Carlos' zoo, we recorded two very long sequences with the automatic recorder. The first sequence contained 712 extended-barks emitted in a row and the second one contained 208. Both sequences were emitted on the same day, 20 minutes apart from each other. Unfortunately, all these sequences, those of FA, NY and CO (n = 34) and most of LUs notes (n = 42), could not be used due to recordings or digitalization errors.

3.2. Standard Playback Protocol

We ran 19 playback sessions with 10 different subjects from six different zoos. We discarded nine of the sessions, which did not fulfil minimal experimental conditions. We considered as invalid every trial in that the subject did not displayed Oriented Attention in the beginning of the habituation phase (i.e., did not habituate: 1st sessions of CO, PE, PL, TI, WO, and 2nd or 3rd sessions of FA, LU, NY, PL). All of them, except in WO sessions, were harmed by confounding variables as loud background noise, human interference; subject was not used to unfamiliar humans. In two of the 10 valid trials, the subjects (FA and NY) did not respond as expected.

3.3. Complementary Playback Protocol

The Complementary Playback Protocol to which CO was submitted generated a clear reaction: the magnitude of the "gestalt" Disturbance suddenly increased in the presence of the B stimuli (excerpts from Video-B). The judgment on validity of our "gestalt" Disturbance indicated moderate agreement without the author as judge (Fleiss' kappa statistic: k = 0.56; n judges = 5; P-value 0.001) and substantial agreement with the author as judge (Fleiss' kappa statistic: k = 0.625; n judges = 6; P-value 0.001). The agreement in the increase of Disturbance magnitude from stimuli A to stimuli B was unanimous in both cases (Table 3).

3.4. Control playback procedures evaluations

3.4.1. Background noise effect

The difference of background noise did not produce any response in the subjects. We used the first type of control in four playback soundtracks (two with TU; two with FA and NY) and the subjects did not show any response to the fragment of background noise preceding the test stimulus. In addition, the subjects FA and NY did not react to the second type of control.

3.4.2. Stimulus control test

The subject TU did not show any response to the background noise playback. It remained resting before, during and after the test, which increases the credibility of the other playback tests' results.

4. DISCUSSION

Our study showed that the maned wolves are able to distinguish among different conspecifics' extended-barks. Individual variations in this vocalization have been reported, but the possibility that they could be perceived by the species was not known until now.

The extended-bark functioning as a mechanism to increase the spatial distance among conspecifics is in accordance with the "distance communication hypothesis" (Bouchet et al., 2012), which states that individual acoustic distinctiveness is related to the typical distance between the sender and the target receivers (Bouchet et al., 2012; Marler, 1967). The key idea is that the acoustic channel may be the only efficient modality for individual recognition if the animals are out of each other's visual range (Bouchet et al., 2012). As the vocalizations used for long-distance communication cannot benefit from visual or olfactory clues, they should be easier to recognise than the ones used for short-range communication (Bouchet et al., 2012). In addition, this individual distinctiveness should be especially pronounced in species whose individuals are often separated from each other (Bouchet et al., 2012; *e.g.* penguins: Aubin and Jouventin, 2002) and have nocturnal habits (coyote, *Canis latrans*, Mitchell et al., 2006). Both characteristics are found in the maned wolf, a species with nocturnal habits in which the spatial distance between male and female varies according to the female's reproductive period (Dietz, 1984). The emission rate of the maned wolf's extended-barks increases during the breeding season (Dietz, 1984) and couples' duets were observed only when male and female were out of each other's visual range (Dietz, 1984; personal observations). Thus, the extended-bark may also function in aiding the localization between pair-mates (Dietz, 1984) and it is expected to contain individual acoustic variation (Brady, 1981).

Vocal individuality only has biological relevance if it can be perceived by receivers. There are two ways in which receivers can perceive vocal individuality: (1) discrimination_ when the receiver uses the similarity or dissimilarity of acoustic stimuli to discriminate between senders (Mumm et al., 2014) [note that the term "discrimination" does not follow the behaviourist concept, defined by differences in responding in the presence of different stimuli; Catania, 1999]; and (2) recognition_ when the receiver matches the individual distinctive characteristics of the emitter's signal with specific information about this emitter ('true recognition' for Tibbetts and Dale, 2007). Although these definitions are not always distinct among authors (Tibbetts and Dale, 2007; Mumm et al., 2014), they may have different implications. Recognition implies that the emitter is somehow familiar to the receiver and, in this sense, one may expect that the latter's ability to learn must be quite sophisticated and requires memory capacity. On the other hand, one could state that if the receiver is not able to perceive similarities or dissimilarities in the acoustic stimuli, it would not be able to recognise it either. Thus, discrimination is a previous condition to recognition.

The maned wolves should be able to recognise at least their reproductive partner's extended-barks and discriminate them from unfamiliar ones. If they are not able to do that, the efficiency of this vocalization at long distances would be compromised, as the hearer would not be able to discriminate its reproductive partner from a rival. We have focused our efforts on testing discrimination of senders through extended-barks' vocal individuality and our results present clear evidence of it. In the future, it might be interesting to further investigate the recognition capacity of conspecifics through extended-barks, given its potential relevance in social dynamics of the maned wolf. Since discrimination is a prerequisite to recognition, our results are the first step to further research in that direction.

The "single measure approach" (McGregor et al., 1992) assumes that the response elicited by playback is adequately described by a single measure. The use of this approach can be inadequate if the measure chosen is not sufficient to describe the subject response (McGregor et al., 1992). The criteria of response chosen for the standard playback tests (*i.e.* Oriented Attention) left no doubt regarding the subjects' responses: they have clearly discriminated between stimuli. Many mammals adopt loud vocalizations to signal territory occupancy (*e.g.* Lion *Panthera leo*: McComb et al., 1994; Grinnell & McComb, 2001; other great cats: Darden *et al.*, 2003; gray wolf *Canis lupus*: Harrington & Mech, 1983; chimpanzees *Pan troglodytes*: Mitani & Nishida, 1993). A loud vocalization would be expected to occur in a species with wide territories such as the maned wolf (ranging from 20 to 115 km²: Dietz, 1984, 1985; Carvalho & Vasconcellos, 1995; Bandeira de Melo et al., 2007). The loud extended-bark presents several acoustic characteristics that may favour its perception and source location (*e.g.* high amplitude, abrupt start and broadband frequency: McGregor, 2005), making it the proper vocalization for territorial defence. Other evidences of the extended-bark territorial function were recorded during the recording phase in the present study. The vocalization was usually emitted at dawn and dusk, optimal times for sound transmission in open areas such as the Cerrado's phytophysiognomy in which the species occurs (*i.e.* Wiley & Richards, 1978; Brady, 1981). In addition, we noticed that the female NY always climbed the mound of dirt placed on the centre region of the enclosure (the highest place there) before vocalizing and often turned its head up during the emission of the extended-barks (Figure 2), which may have facilitated the sound spreading.

The extended-bark functioning for territorial boundaries disputes may find support in the very long sequences (712 notes and 208 notes) recorded in São Carlos's Zoo, where the captive wolves, male TU and female FR, lived in interaction with the free-living male HOR. The longest extended-bark sequences recorded in the wild by Dietz (1984), and in captivity by Kleiman (1972), were of only 23 and 30 notes, respectively. We noted many events of aggressive interactions between the captive wolves and HOR; when HOR was around the enclosures, the younger male TU was always submissive and we observed agonistic interactions between HOR and FR. Thus, the caretakers had reported that another male wolf (which shared the enclosure with FR before TU) had been injured in a fight with HOR, through the fences. The long extended-bark sequences may be an honest signal of the wolf's physical conditions and could be used to avoid physical confrontation (if TU had the chance to get away from HOR, maybe he would). A wolf in better physical condition may be able to broadcast a longer sequence of extended-barks, thus communicating its disposition to fight for territory, and identityng itself to familiar competitors.

In addition to the difficulty of gathering data in so many different places, there were numerous technical and logistical problems to overcome. These problems may have limited the validity of some results. As previously reported, our soundtrack stimuli were restricted to the maned wolves FA, TU and OR. The maned wolves FA and TU were manually recorded with Marantz, and OR was automatically recorded with SongMeter 2+. Since we could not play the stimuli produced by one wolf to itself, all playback soundtracks played to the subjects TU and FA (including NY, that shared the enclosure with FA), contained stimuli recorded with different types of equipment. The remarkable background noise difference between the recordings of each equipment was controlled in every trial by a simple procedure (see "Background noise effect evaluation"). By exploiting every relevant variable, we eliminated as many confounding factors as possible, given the quasi-experimental character of our work. Thus, the number of subjects is not crucial to the validity of our experiment. In fact, even if only one subject had corroborated our hypothesis, it would be more plausible to generalize the validity of our results to the whole species than to assume that the one subject tested was the only wolf capable of discriminating between stimuli. Furthermore, the robust and consistent responses of the subjects make it unlikely that the results are odd or deviant.

4. CONCLUSIONS

Individual variations in the extended-bark can be discriminated by maned wolves. If they perceive differences, probably they can state territories limits by voice, and at a distance. That turns the acoustic channel an efficient long-distance signalling system for the species that moves in the dark in a vast home range and whose hunting territory is a valuable resource to be defended.

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Table 1. Maned wolves that participated in this study. They were held in captivity in different zoos of the State of São Paulo, Brazil, either isolated or in pairs. Sexes are presented as M = male, F = female. The contribution of each animal in the different phases of the research is presented in the last column.

CITY/STATE	**SUBJECT**	**SEX**	**PARTICIPATION**
Botucatu/ SP	Wolverine (WO)	M	Playback tests
Botucatu/ SP	Logan (LO)	M	Playback tests
Catanduva/ SP	Mel (ME)	F	Recording
Ribeirão Preto/ SP	Orfeu (OR)	M	Recording
São Carlos/ SP	Frutti (FR)	F	Recording
São Carlos/ SP	Tutti (TU)	M	Recording and Playback tests
São Carlos/SP	Hortelã (HOR)**	M	Recording
São José do Rio Preto/ SP	Mostarda (MO)	M	Recording
Sorocaba/ SP	Fantasma (FA)***	M	Recording and Playback tests
Sorocaba/ SP	Nymeria (NY)***	F	Recording and Playback tests
Sorocaba/ SP	Perônio (PE)*, ***	M	Playback tests
Sorocaba/ SP	Tibio (TÌ)*, ***	M	Playback tests
Americana/SP	Colombo (CO)	M	Recording and Playback tests
Paulinia/SP	Pluto (PL)	M	Playback tests
Piracicaba/SP	Lupin (LU)	M	Recording and Playback tests
Itirapina/SP	Mogli (MOG)	Unknown	Recording (pilot procedures)

*All subjects out of PE and TI, were adults (more than 2 years old according to Rodden et al., 2004). **HOR was a free-living male (unofficial information from Zoo´s employees and personal verification) at reserve bordering the Zoo of São Carlos, that occasionally visited Tutti and Frutti's enclosure. The only wolves that were kept in pairs were: (1) FA and NY; (2) TÌ and PE. TU and FR shared the same enclosure during part of the day, but were kept apart most of the time.

Table 2. List of the valid standard playback protocol trials. Discrimination between stimulus B and A occurred when "Oriented Attention" in the beginning of the habituation phase was recorded, and during/immediately after B.

		DID IT DISPLAY ORIENTED ATTENTION?					
Subject	**Session**	**A_1 - A_6 (BEGINNING)**	**A_1 - A_6 (END)**	**A_7 - A_9**	**B**	**A_{10}**	Discrimination
CO	2nd	Yes	No	No	Yes	No	Yes
FA	1st	Yes	No	No	Yes	No	Yes
FA*	2nd	Yes	No	No	No	No	No
LO	1st	Yes	No	No	Yes	No	Yes
LU	1st	Yes	No	No	Yes	No	Yes
NY	1st	Yes	No	No	Yes	No	Yes
NY*	2nd	Yes	No	No	No	No	No
TU	1st	Yes	No	No	Yes	No	Yes
TU	2nd	Yes	No	No	Yes	No	Yes
TU	3rd	Yes	No	No	Yes	No	Yes

* "Inconclusive", since the subjects failed discrimination in the second trial, but responded as expected in the first trial.

Table 3. Evaluation of the "gestalt" Disturbance based on video excerpts of the subject CO by 5 naive adult judges of both sexes.

	Author	**Judge 1**	**Judge 2**	**Judge 3**	**Judge 4**	**Judge 5**
Video-A	Increases	Increases	Increases	Increases	Decreases	Increases
Video-B*	Increases	Increases	Increases	Increases	Increases	Increases
Video-C	Increases	Increases	Increases	Increases	Decreases	Increases
Video-D	Decreases	Decreases	Decreases	Decreases	Decreases	Decreases

*Video-B corresponds to the transition from stimuli A to stimuli B and the agreement among judgments was unanimous.

Table 4. Recording samples acquired during 13 field campaigns in 7 Zoos. The total number of notes is indicated, followed by number of sequences (parenthesis) and the individual average number of notes per sequence. Recording procedure is indicated: automatic (recorded with SongMeter 2+) or manual (recorded with Marantz).

Subject	**Notes (sequences)**	**Average Notes/ Sequence**	**Recording Procedure**	**Recording Effort**	**Zoo's Location**
OR	337 (8)	42,1	Automatic	37:52 h	R. Preto
ME	0	0	Automatic	111:40 h	Catanduva
MO	0	0	Automatic	181:58 h	S. J. R. Preto
NY	111 (12)	9,2	Manual	221:00 h	Sorocaba
FA	145 (33)	4,4	Manual	221:00 h	Sorocaba
FR	54 (7)	7,7	Manual	120:00 h	São Carlos
TU	32 (3)	10,7	Manual	120:00 h	São Carlos
CO**	34 (5)	6,8	Manual	1:00 h	Americana
LU**	52 (5)	10,4	Manual	1:00 h	Piracicaba
LU	323 (30)	10,8	Automatic	98:00 h	Piracicaba
FA or NY*	215 (31)	6,9	Automatic	50:00 h	Sorocaba
HOR or FR*	931 (3)	310,3	Automatic	5:00 h	São Carlos

* Since there was more than one wolf near the autonomous recorder, it is not possible to be certain of the emitter (FA or NY in Sorocaba's Zoo, and FR or HOR in São Carlos' Zoo). ** All the vocalizations of the CO and most vocalizations of the LU were anomalous and exceeded the typical highest frequency threshold of extended-barks. Thus, the sample rate previously adjusted for the recordings was insufficient leading to aliasing artefacts in all notes emitted by CO (n = 34) and most of the ones emitted by LU (n = 42).

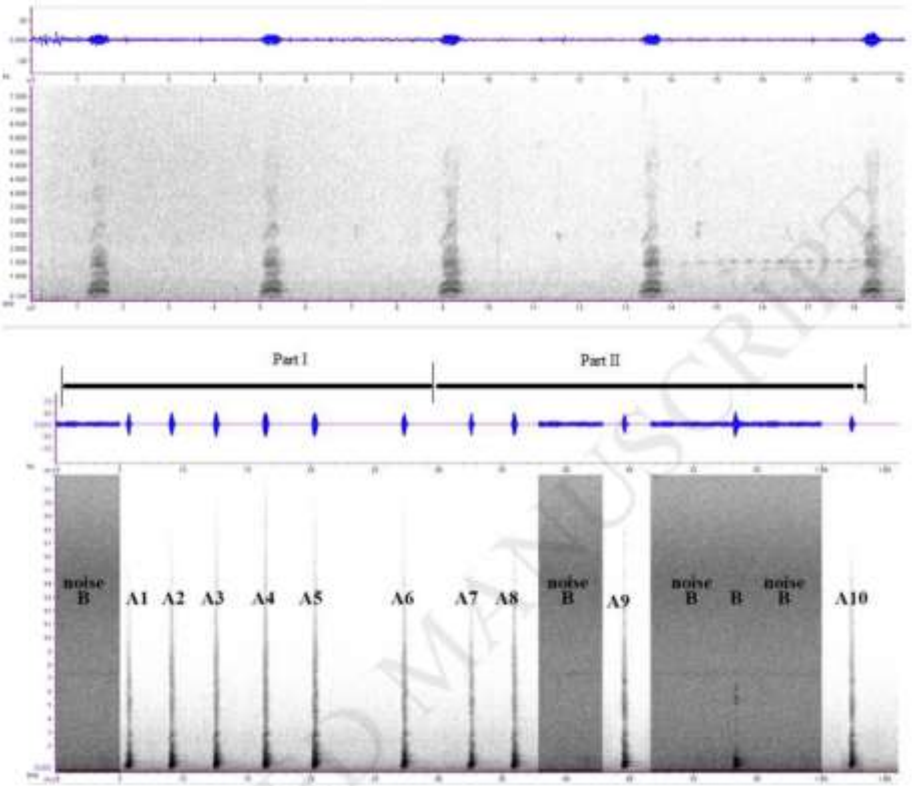

Figure 1. Spectrograms of a natural sequence of extended-barks (top) and a soundtrack used in standard protocol playback test (bottom). In the natural sequence, five notes are present and showed in detail at the 0.2-3kHz band. In the edited sequence below, Part I corresponds to the habituation phase: it was played continuously (*i.e.* in loop mode) until the subject was habituated. Part II corresponds to the test phase (discrimination soundtrack). The dark regions are background noise extracted from track B. The spectrogram was generated in Raven Pro 1.4.

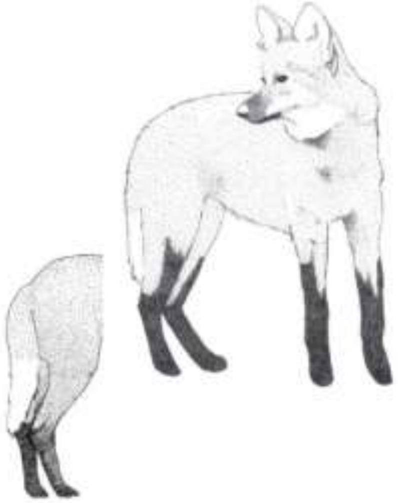

Figure 2: The "Oriented Attention" posture (left) of the maned wolf that characterized stimulus discrimination: the animal turns its head towards the speaker and remains with ears raised, eyes wide open and outer ears facing the speaker during at least 1 second. Barking (right): maned wolf emits a sequence of extended-barks. The wolf is usually standing up with its tail relaxed. Between one note and another, its ears rotate in a back and forth movement as if searching for sounds (Drawings made by Juliana Takata based on video excerpts of our playback tests and author's verbal description, respectively).

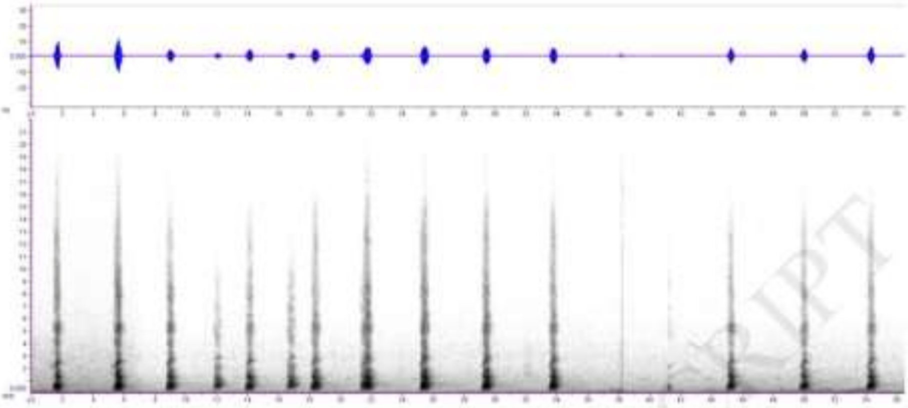

Figure 3. Duetting extended-bark of FR and TU from São Carlos' Zoo personally recorded by Flora Balieiro. The sequence is initiated by the female FR. The TU males' first note occurred after female's third. The female emitted 12 notes and the male 2.

Apêndice C _ *Allosuckling in a captive group of wild cavies* Cavia aperea

DE GRUYTER Mammalia 2017; aop

Short Note

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Allosuckling in a captive group of wild cavies *Cavia aperea*

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Abstract: We took the rare opportunity to observe and quantify spontaneous allosuckling in a captive group of *Cavia aperea* captured in the wild (a male, two females and their offspring). We recorded behavior interactions between all offspring and each of the adults between days 6 and 20 of life. Infants suckled equally from their mother and from the other female, which differs from a previous report in which mothers typically nursed own young. In addition, infants stayed closer to the other female than to mother or to the father. We discuss these results in the light of the common occurrence of allosuckling in Cavioidea and social structure.

Keywords: communal nursing; non-offspring; parental investment; sociality.

Milk provision is a specific feature of maternal care in mammals and involves physiological and behavioral costs for the mother (Roulin 2002). Nonetheless, milk provision to unrelated offspring (allonursing) is phylogenetically widespread in Mammalia, although restricted to a relatively small number of species (68 according to Packer et al. 1992; Tecot and Baden 2015). Evolutionary theories for the origin of allonursing behavior evoke reciprocal altruism (Tecot and Baden 2015), kin selection, misdirection of parental care (Roulin 2002) and offspring-related advantages (e.g. milk theft: Zapata et al. 2009; cross-transfer between litters of passively acquired antibodies: Garnier et al. 2013; resource optimization strategy, Takamatsu et al. 2003). An association between allonursing and both the size of the litter and the group was found to be more frequent in multiparous than uniparous species and in small groups composed of related individuals than in larger groups composed of less related individuals (Packer et al. 1992). These trends are consistent with explanations based on reciprocity and kin-based benefits, but exceptions to this rule urge the improvement of our knowledge about proximal and ultimate causations of this behavior in a wider variety of species.

Among rodents, there are two infraorders that, among other aspects, differ in relation to the developmental state of offspring on birth: the altricial Sciurognathi and the precocious Hystricognathi (Woods and Kilpatrick 2005). It was suggested that precociality would favor communal nursing, as mothers would have diminished costs in giving milk to offspring that are already able to eat solid food just after birth (Ebensperger et al. 2002). However, despite the demonstration that allosuckling is more common in Hystricognathi than in Sciurognathi (Ebensperger et al. 2002) and that precocious offspring demand less energy from their mother during lactation than altricious offspring, the mothers' total energetic expenditure (i.e. summing up gestation and lactation periods) was found to be similar among the compared precocial and altricial rodent species (Künkele and Trillmich 1997, Künkele 2000). Moreover, milk production in the precocious guinea pig will not respond to environmental variability in the number of offspring or food offer (Laurien-Kehnen and Trillmich 2003), a condition that could further increase the cost of milk production for these mothers. Thus, why do they share milk with alien offspring? In a long lasting study in which semi-free living populations of wild cavy *Cavia aperea* Erxleben 1777, the domestic form *Cavia porcellus* and hybrids of *C. aperea* and *C. porcellus* were observed, allosuckling occurred indiscriminately in domestic and hybrid groups but was noted just once in *C. aperea* groups (Rood 1972). Out of the genus *Cavia*, communal nursing and allonursing were recorded frequently

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in the bigger yellow-toothed cavy *Galea musteloides* Meyen 1832, under laboratory conditions (Künkele and Hoeck 1995), and in another yellow-toothed cavy, the *Galea spixii* Wagler 1831, under seminatural conditions (Lacher 1981). *Microcavia australis* I. Geoffroy and D'Orbigny 1833, the more ancestral species of Caviinae, was described as a colonial and communal nursing species by Rood (1972). Out of Caviinae, allonursing in capybara *Hydrochoerus hydrochaerus* Linnaeus 1766 (Caviidae: Hydrochoerinae, Rowe and Honeycutt 2002) seems similarly frequent in captivity (Nogueira et al. 2000) and in nature (Macdonald 1981). No further information about allonursing behavior is available for other caviidae species.

We describe here the occurrence of allosuckling in *Cavia aperea*, challenging the conclusion of Rood (1972) that wild cavy "typically nurse only own young" (p. 65), by conducting systematic observations of a single captive group. This species matches the prevision of Packer et al. (1992) of the occurrence of allosuckling in multiparous species living in small and related groups (Rood 1972, Asher et al. 2004) that tend to exhibit birth synchrony (a feature of *Cavia porcellus* reported by Künkele and Hoeck 1995, and to whom *C. aperea* is closely related, Spotorno et al. 2004). Under a low-density population of 12.5 individuals/ha, *C. aperea* lives in small social groups of one to four adult animals organized by female distribution, which is possibly related to food distribution (Lacher 1981). Males exhibit a tendency to polygyny through female defense (Asher et al. 2004). The bond between mother and offspring is strong and involves the use of specific sounds for the attraction and maintenance of physical contact (Rood 1972, Monticelli and Ades 2013). Fathers exhibit frequent playing and grooming behaviors towards offspring in contrast to the promiscuous species *Galea musteloides* (Adrian et al. 2005).

The cavies used in this study were born in the wild and captured on a rural property near the municipality of Jaboticabal, State of São Paulo (more details of population and capture are in Monticelli and Ades 2011). The animals, one male (M1) and two females (F1 and F2), were kept in a 6-m^2 enclosure in an outdoor pen. After 3 months living together, both females gave birth to two offspring each, within a 2-day interval, F1 to f1a (female) and f1b (male), and F2 to f2a (female) and f2b (male). Animals were individually identified by selective shaving (offspring) or dyeing the fur with hydrogen peroxide (adults). Specimens of this wild cavy population were confirmed to be *Cavia aperea* at the Museu de Zoologia de São Paulo (Monticelli and Ades 2011).

We used scan sampling to record the behavior of adults and offspring once a week, for 3 weeks. Scans were performed every 2 min during a 30-min session (i.e. 15 scans/session). Sessions were separated by, at least, 30-min intervals and occurred during day light (from 8 to 18 PM). Observations started when the youngest litter was 6 days old and the oldest litter was 8 days old (day 1) and ended when offspring reached 20 days of age (day 3). There were two sampling sessions on day 1, 11 on day 2 and 10 on day 3. We completed 345 scans corresponding to 11.5 h of observation over the 3 days of observation. The distance between offspring and each adult was registered according to the following categories: (1) physical contact, (2) one to two offspring-body length distance, (3) three offspring-body length distance and (4) distant (more than three offspring-body length distance). The behavior of the offspring and its direction (to the mother, to the other female and to the male) were recorded as follows: suckling, suckling attempt, approaching and following.

In order to control for the lack of independence of multiple observations of the same behavior or distance within scans, we used the means of data obtained in each 30-min session as an independent data point. To control for the possibility of non-independence of behavior among different offspring, we used the pooled data from the four offspring obtained in each 30-min session as an independent data point. In this way, we obtained data points that represented sums of the distance or the frequency of each behavior directed for each adult by the four offspring in each scan, which allowed for comparisons between each pair of adults. To determine whether there were differences in the distance and frequencies of behaviors directed to each adult by the offspring, we used Friedman ANOVA tests, followed by paired comparisons (Wilcoxon tests) and the Holm-Bonferroni correction for multiple comparisons (Holm 1979). In this case, the corrected probability value (p′) is given.

Offspring did not differ significantly in the frequency of suckling from mother or the other female (Wilcoxon $Z=-0.82$, $p'=0.41$) or in the frequency of approaching the three adults (Friedman $\Sigma^2=4.9$ $p=0.086$, Table 1). However, they differed in the frequencies of attempts to suckle ($\Sigma^2=21.38$ $p<0.001$) and follow ($\Sigma^2=19.39$ $p<0.001$), directing significantly lower frequencies of these behaviors to the male (attempt to suckle: male vs. mother $Z=-3.44$ $p'<0.001$, male vs. other female $Z=2.97$ $p'=0.005$; follow: male vs. mother $Z=-3.44$ $p'<0.001$, male vs. other female $Z=2.97$ $p'=0.003$), but attempted to suckle ($Z=-1.38$ $p'=0.17$) and followed ($Z=-1.13$ $p'=0.26$) both females similarly. Offspring significantly differed in the distance they kept from the adults inside the enclosure ($\Sigma^2=21.48$ $p<0.001$) (Table 2). They stayed closer to the other female than to the mother ($Z=-2.39$ $p'=0.03$), but more to F1 than to the more aggressive and supposedly dominant female F2, or the male ($Z=-2.39$, $p'=0.02$) and

Table 1: Mean (SE) of the occurrences of behaviour categories exhibited by offspring directed to their mother, the other female and male, per 30-min sampling session.

Offspring	Suckling		Attempting to suckle			Following			Approaching		
	Mother	Non-mother	Mother	Non-mother	Male	Mother	Non-mother	Male	Mother	Non-mother	Male
f1a	1.2 (0.2)	1.3 (0.2)	1.2 (0.2)	1.2 (0.2)	1 (0)	1.2 (0.1)	1.2 (0.1)	1 (0)	1 (0)	1.2 (0.2)	1 (0)
f1b	1.2 (0.1)	1.7 (0.4)	1.7 (0.2)	1 (0)	0	1.2 (0.2)	1.3 (0.1)	1 (0)	1.1 (.1)	1 (0)	1 (0)
f2a	1 (0)	1.3 (0.2)	1.2 (0.1)	2 (1)	0	1.2 (0.4)	1.2 (0.2)	1 (0)	1 (0)	1.2 (0.2)	1 (0)
f2b	1.5 (0.2)	1.7 (0.3)	1 (0)	2 (1)	0	1.4 (0.2)	1.3 (0.2)	1 (0)	1.2 (.2)	1.2 (0.2)	1.2 (0.2)

Table 2: Mean (SE) distance (in number of offspring's bodies) between offspring and their mother, the other female and male, per 30-min sampling session.

Offspring			Distance
	Mother	Non-mother	Male
f1a	3.1 (0.1)	3.2 (0.1)	3.4 (0.1)
f1b	3.1 (0.1)	3.1 (0.1)	3.3 (0.1)
f2a	2.8 (0.1)	3.1 (0.1)	3.6 (0.1)
f2b	3.0 (0.2)	2.1 (0.1)	3.3 (0.1)

closer to the mother than to the male ($Z = -2.87$, $p' < 0.01$, Table 1). The male was seen approaching and lying down in contact to the offspring twice and was tolerant to most of their interactions with him.

Our results suggest that offspring do not prefer to direct their parental solicitations to their own mother, as predicted from Rood (1972). Also, they clearly directed less behavior to their father, the only male present in the enclosure. Even considering suckling and attempt to suckle, two social interactions typically involving the mother and her genetic offspring, there was no prevalence of directing these behaviors to their own mother. The systematic observation of allosuckling in this group is in disagreement with the rarity of occurrence of this behavior in the long-term study of Rood (1972). This author performed naturalistic observations of wild cavies in the field and under seminatural conditions concluding that *Cavia aperea* "typically nurse only own young" (p. 65). Despite this conclusion, the *only* observation of allonursing registered in Rood's study occurred in conditions that were very similar to ours: females recently captured from nature that were kept together and gave birth within just 5 days apart. Rood's observations of allosuckling in *C. aperea* also departed from reports of allosuckling in other species of the family Caviidae as *C. porcellus*, *Galea musteloides*, *Galea spixii*, *Microcavia australis* and *Hydrochoerus hydrochaerus* (Rood 1972, Lacher 1981, Künkele and Hoeck 1995, Nogueira et al. 2000, Takamatsu et al. 2003).

The pervasive occurrence of allosuckling in Caviidae contrasts with the lack of evidence of its adaptive function (Künkele and Hoeck 1995, Takamatsu et al. 2003). A possible explanation to this apparent paradox may be linked to the social structure of these species. Although the great diversity of the social systems within Caviidae, allosuckling seems more frequent in those species that form larger and cohesive groups, as *Microcavia australis* and *Hydrochoerus hydrochoerus*, with females overlapping home ranges. On the other hand, although allosuckling was not observed in free-living *Cavia aperea* and *Galea musteloides*, their social organization could also promote its occurence. Social organization of *G. musteloides* involves non-cohesive multi-female-multimale groups (Adrian and Sachser 2011), whose individuals frequently interact during social foraging. *C. aperea* form hierarchically structured harems (Künzl and Sachser 1999, Adrian and Sachser 2011) in which females live in superimposed home ranges inside the male's home range area (Ashser et al. 2004, 2008). The frequent interactions among females and the resulting hierarchy may have led to increased tolerance and decreased aggression seen among them (Sachser 1986, Künzl and Sachser 1999). This organization, associated with environmental food availability and synchronized births, could promote the occurrence of allosuckling. This hypothesis should be tested through observation of groups of these species under different ecological conditions. In environments with high food availability and high levels of interaction among females, we should expect higher frequencies of allosuckling compared with environments with low food availability and high females' dispersal.

Allosuckling in these species does not seem to result in direct benefit to the young or to the mothers. This might be related to the precociality of the offspring, which begin eating solid food around the third day after birth (Künkele and Trillmich 1997), and to the female's lack of ability to regulate milk production (Laurien-Kehnen and Trillmich 2003). The lack of immediate benefits to allonursing parallels previous findings in other, more distantly related caviomorphs. For instance, in degus (*Octodon degus*) neither the number and body weight of offspring nor the physical condition of the mothers are enhanced in social groups breeding communally compared with solitary breeding females (Ebensperger et al, 2007)

The hypotheses that allosuckling may be a by-product of the basic social organization of Caviomorph rodents, that includes the formation of harems or multimale-multifemale groups gathered around food patches, does not exclude the possibility that it may have some adaptive value in those more cohesive and complex social groups, such as *Hydrochoerus hydrochaerus* and *Microcavia australis*, two species in which with female philopatry might be a powerful promoter of cooperation among related females. In conclusion, we propose that the widespread presence of allosuckling in the super family Cavioidea can be a by-product of their basic social organization, but as social complexity evolved in certain groups, allosuckling may have been selected for its benefits. Future efforts will help to understand the selective pressures leading to the expansion and diversification of allomaternal care throughout the Hystricognathi infraorder, thus expanding research to investigate the causes and consequences of less derived forms of allomaternal care. Finally, we are aware that this is a small study and that further studies are needed to better understand allonursing in *Cavia aperea*.

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Apêndice D _ O repertório acústico de uma espécie com baixo nível de socialidade: a lontra neotropical

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Ao contrário de seu parente mais próximo, a ariranha (*Pteronura brasiliensis*), que vive em bando e se comunica por uma variedade de sinais químicos e acústicos, na natureza, a lontra (*Lontra longicaudis*), só foi vista na companhia de coespecíficos na estação reprodutiva e até o desmame dos filhotes. Em cativeiro, a lontra é capaz de passar a vida toda com um coespecífico, o que sugere algum nível de socialidade. Há na literatura estudos sobre o uso de sinais químicos (muco e fezes) para marcação de território, e apenas descrições subjetivas de 4 ou 5 vocalizações. Esta será a primeira descrição sonográfica do seu repertório e uma contribuição ao conhecimento desta espécie brasileira pouco estuda e já ameaçada pela perda de habitat. Acompanhamos 14 exemplares cativos em 6 instituições diferentes no Brasil, totalizando mais de 11 meses de registros. Em uma análise preliminar identificamos 10 sons que ainda precisam ser categorizados em tipos e variantes. Essas vocalizações foram obtidas em contextos variados, como aprendizado aquático em profundidade, aproximação de casais, aproximação de fêmeas e encontros agonísticos. Notamos que um filhote criado em cativeiro sem a mãe dirigia um chamado emitido em situação de risco para a pessoa que o criou; e uma fêmea adulta emitiu chamado de contato na presença da pesquisadora AMP com quem já estava habituada. A próxima fase do trabalho inclui a descrição sonográfica desses sinais e uma discussão comparativa do repertório de L. longicaudis e outros mustelídeos com níveis variados de socialidade. Palavras-chaves: lontra, comportamento, vocalização, Carnívora, Mustelidae.

Acesso via: http://www.etologiabrasil.org.br/media/upload/eae/anais-2010.pdf, p. 223

Apêndice E _ O repertório acústico de um especialista dos rochedos da caatinga brasileira, o mocó, Kerodon rupestris (Rodentia: Caviidae)

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Embora seja um parente próximo do preá (gênero Cavia, também da subfamília Caviinae), o mocó é um especialista de habitat que sofreu adaptações morfológicas, ecológicas e comportamentais. Trocou o hábito pastador de Cavia, Galea e Microcavia pela capacidade de escalar arbustos para se alimentar de folhas; desenvolveu almofadas palmares, alongou a duração da gestação e envolveu o pai no cuidado com os filhotes. Muitas dessas modificações são compartilhadas por outras espécies de ambiente rochoso ("rock-dwelling") distribuídas ao redor do mundo. Interessados em saber de que forma a comunicação acústica teria se ajustado a sobrevivência da espécie no ambiente isolado dos rochedos da caatinga, gravamos e começamos a analisar o repertório de mocós da cidade de Patos, PB. Foram 90 dias de trabalho de campo e coleta de dados nas dependências da Fazenda Tamanduá. Capturamos 13 animais (6 machos e 7 fêmeas) e fizemos registros em áudio e vídeo em 3 situações: (1) animais em ambiente natural, antes da captura e após a soltura, (2) animais em grupo em um cercado, e (3) pareamento de animais em caixa-teste (dimensões 80x80x80cm), nas condições macho-macho, macho-fêmea e fêmea-fêmea. A análise parcial dos registros apontou 8 vocalizações mais o entrechoque-de-incisivos comumente exibido por outros cavídeos em interações agonísticas. Os resultados, embora preliminares, sugerem semelhanças com outras espécies de Caviinae, com um repertório rico como o do preá, e ao menos uma convergência com espécies de ambientes rochosos: o uso de um assobio de alarme emitido por um animal estrategicamente posicionado, como será discutido. Palavras chaves: Bioacústica, comunicação animal, Caviomorpha, especialista de habitat, ambiente rochoso. Suporte financeiro: CNPq, Instituto Fazenda Tamanduá.

Acesso via: http://www.etologiabrasil.org.br/media/upload/eae/anais-2010.pdf, p. 222

Capítulo 3.

Aplicações da bioacústica enquanto ferramenta tecnológica

> *Depois de passarmos de uma região para outra, surpreendiam-nos os gritos de viventes que nos eram desconhecidos, ao passo que desapareciam outros que já se nos tinham tornado familiares ou, se continuavam a fazer ouvir, era com modificação sensível no órgão vocal . . . . Quando subíamos o manso curso do Paraguai, ouvíamos por vezes uma espécie de canto gutural de alguns bugios que se reuniam em uma das árvores da floresta. De repente, cessava a singular harmonia; um deles recomeçava e os outros, cada um por sua vez, entravam novamente no concertante. Um grito rouco e fortemente repetido duas ou três vezes anunciava-nos a presença de outro animal, o jacaré; ou então urros, quais gigantescos miados, avisavam-nos da aproximação da onça; vozes totalmente diversas, indicadoras do gênio dos seres que as produziam e tão diferentes do quase relincho da pacífica anta que, lembrando o do cavalo, dele contudo tanto se diferencia no modo de assoviar . . . . Quando se considera a quantidade de vozes dos animais e sua infinita variedade, tende-se a pensar que é quase impossível transcrevê-los sem o uso de um número infinito de sinais. O método que apresento é apenas uma primeira tentativa.* Um trecho da *Zoofonia* de Hercule Florence, selecionado por Miguel Flori.[3].

Curiosos da vida animal com tecnologia para registrar o momento: essa foi a combinação inicial da Bioacústica. Como colocado por Jacques Vielliard, a década de 1960 armou os ornitólogos (leigos ou profissionais) para a observação, gravação, armazenamento e posterior análise dos sons produzidos pelos animais (Vielliard & Silva, 2010). Nesse sentido, é bem interessante que a primeira tentativa de registrar som animal tenha sido feita por quem é considerado o inventar da fotografia, Hercules Florence (Kossoy, 2006).

Quando eu comecei a estudar a biofísica acústica para descrever e medir os assobios da cobaia, no laboratório de Psicoetologia do Instituto de Psicologia da USP, o programa utilizado é o Signal RTS, em linguagem DOS, e esse era um avanço tecnológico para a época. Hoje, estamos em um ponto em que as gravações e as análises podem ser automatizadas, abrindo, dentre tantas possibilidades, a chance de se gravar espécies exclusivamente

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[3] Visite o instituto criado e mantido pelos filhos de H. Florence, em http://www.ihf19.org.br/pt-br/. Endereço físico: Instituto Hercule Florence. Alameda Santos, 787, 8º andar, cj. 82 • Cerqueira César • CEP 01419-001. São Paulo • SP • Brasil

noturnas, ou em áreas difíceis de chegar; algumas sempre estiveram nas nossas trilhas no interior das matas, mas escondiam-se ou paralisavam ao menor sinal da aproximação humana. O estado da arte e uma pequena história da mudança tecnológica e das possibilidades da bioacústica estão apresentadas em artigos já publicados (Faria, Gasco & Monticelli 2015; Monticelli, Paula, Balieiro & Faria, 2016; Apêndices A e B deste capítulo).

De lá para cá, o uso da bioacústica para estudo e monitoramento de espécies acústicas através do Monitoramento Bioacústico Passivo (i.e., automatizado, em inglês, passive acoustic monitoring PAM), é ainda mais concreto. O projeto de monitoramento de elefantes (*The Listening Elephant Project*, da Cornell Lab of Ornitology, Ithaca, EUA) é um exemplo. Uma das possibilidades mais novas trazidas por eles, foi a da transmissão de dados em tempo real (Bjorck, Rappazzo, Chen, Wrege & Gomes, 2019). Isso já podia ser feito via internet, mas dependia da possibilidade e da qualidade do sistema de internet, por causa do volume e tamanho dos arquivos que tinham que ser *wav* (ou equivalente). A solução dos autores é uma fórmula de compreensão do som sem o viés da faixa audível humana (Bjorck *et al.*, 2019). Arquivos mp3, por exemplo, são uma solução para a música, mas não preservam toda a faixa de frequência de vocalizações de mamíferos (Faria, Gasco & Monticelli, 2015). A outra novidade tecnológica de Bjorck e colaboradores está relacionada à automação da detecção de sons de interesse, algo imprescindível à análise de milhares de horas de gravação das janelas acústicas obtidas com o PAM (Faria, Gasco & Monticelli, 2015). O método comum usado há cinco anos atrás era o de criação de um algoritmo de reconhecimento de som usando modelos ocultos de Markov (e. g., Aide, Corrada-Bravo, Campos-Cerqueira, Milan, Vega, & Alvarez, 2013). A proposta de Bjorck *et al.* (2019), baseia-se em redes neurais e usa modelos *end-to-end* de reconhecimento de fala (*State-of-the-art speech recognition*), com perspectiva de uso em aplicativos de *smartphones*. A questão é: pode ser extrapolado para os sons audíveis de outros mamíferos terrestres, que usam toda a faixa de frequência e se sobrepõe com uma variedade de ruídos?

Apesar de avançar rapidamente em termos de inovações tecnológicas, o uso de programas para PAM é considerado incipiente para espécies acústicas além de morcegos (Sugai, Silva, Ribeiro Jr. & Llusia, 2018; há uma grande rede de colaboração entre estudiosos de morcegos, que alimenta uma base de dados que mantém avanços no sentido da identificação em tempo real de espécie pelo som, e que pode ser feita a partir de um aplicativo em celular: Paulo Brodowiec, comunicação pessoal). A recente revisão sistemática do estado da arte do uso de programas de PAM, feita por Sugai *et al.* (2018; em Thomson Reuters Web of Science, no período de 1900–2018, a partir de uma amostra final de 460 artigos), aponta: a prevalência de estudos com morcegos (50%; e 6% para mamíferos não-voadores), em regiões temperadas setentrionais (65%), que geraram dados sobre padrão de atividades (25%) e de uso de habitat (16%), mas quase nada sobre outros padrões comportamentais; a maioria dos trabalhos usou gravadores não-programáveis (61%), talvez uma decisão financeira, e realizou análises manuais ao invés de automatizadas (58%).

Ao meu ver, ainda estamos muito aquém do que precisamos para alavancar a enorme contribuição que a Bioacústica pode dar ao estudo, levantamento, monitoramento de populações naturais, e ser usada em práticas de resolução de conflito (como no projeto dos elefantes) e de conservação das espécies. Esbarramos essencialmente, para mamíferos neotropicais, na análise das gravações, que tem que ser feita de forma não manual; tem que ser automatizada para ser factível para muitas dessas aplicações (Monticelli *et at.*, 2016). Sendo automatizada a detecção de sons de interesse, é possível o recebimento em tempo real de dados (identificados), para tomadas imediatas de ações.

Para a Etologia da conservação, a identificação deveria chegar a nível individual, porque o foco de análise do comportamento é esse. Fizemos algumas contribuições nesse sentido, usando essencialmente o aulido do lobo-guará como modelo. Em Balieiro (2016; Apêndice C), Balieiro e Monticelli (2018; Apêndice B do Capítulo 2), Ferreira, Faria e Monticelli (2018; Apêndice 3.D) e Ferreira, Monticelli e Faria (2017; Apêndice E), investigamos aspectos que melhor descreveriam os aulidos a nível individual (a automação do reconhecimento de sons depende de uma amostra de partida que sirva como modelo do sinal, durante um procedimento de treino Ferreira, Monticelli & Paula, 2016, Apêndice F). No projeto de iniciação científica de Bruna Lima Ferreira (Apêndice F), modelamos o aulido do lobo no programa Songscope (Wildlife Acoustics@), usando como amostra de treino gravações do acervo FOCA (obtidas por Flora Balieiro manualmente e usando gravadores automáticos SM2 Wildlife Acoustics). O nosso melhor modelo encontrou 87,93% de aulidos em uma janela conhecida (Ferreira *et al.*, 2016), e esse já é um desempenho melhor do que o obtido por Rocha *et al* (2015), nos programas XBAT (84,86%) e Raven (32,43%). Para que a detecção chegue a nível individual, a análise LPC revelou melhores parâmetros de discriminação de animais com base em seus aulidos, do que a Cepstral (Ferreira *et al.*, 2017, Apêndice E). Mas ainda não pudemos testar nossa hipótese de que a análise de wavelets do aulido daria um nível ainda melhor de distinção dos aulidos entre indivíduos.

Nossa outra contribuição é com a manutenção de um acervo de paisagens acústicas de domínio público: da Estação Ecológica de Itirapina (2016-2017, obtidas por Bruna Campos Paula; Paula, 2017) e do Parque Ecológico de Vassununga (2018; antes da instalação de um clube de tiros, há poucos metros da borda do parque). Essas paisagens constituem dados científicos e um registro conservado de um momento espaço-temporal para comparação com paisagens futuras, em busca de impactos de alterações nas paisagens. Usamos essas paisagens e gravações automatizadas do zoológico da cidade, em busca de efeitos que pudessem indicar alteração em padrões comportamentais por alteração no ambiente (ruído de festas, implantação de um clube de tiros efeitos de poluição sonora de origem antrópica

(Paula, 2017).

Deichmann, Hernández-Serna, Campos-Cerqueira e Aide (2017) usaram o estudo da paisagem acústica de uma floresta no Peru para investigar efeitos decorrentes da exploração de gás natural, nas fases de construção e perfuração. Puderam dizer como e quando (em metros de distância e ao nascer e pôr do sol) o ruído da plataforma de exploração afetava a riqueza de espécies de anuros e aves e a atividade vocal, a partir da analise visual de espectogramas das paisagens. A riqueza espectral (variabilidade de sons) e a detecção de espécies de aves aumentaram com a distância da plataforma, enquanto que a de anuros diminuíram, tanto em número de detecções como em riqueza de espécies. Estudos como esse mostram como programas PAM podem beneficiar discussões no âmbito de uma ecologia acústica, a partir de paisagens ao invés de detecção de sons de interesse. Mais uma ferramenta que pode ser usada para se avaliar o impacto da atividade humana sobre comunidades de animais acústicos, e que podem ser implementadas para orientar estratégias de mitigação de impactos (por exemplo, ajustam as atividades mais barulhentas aos horários de menor atividade vocal de espécies que não podem mover-se para longe das fontes de ruído).

Vocalizações como itens de enriquecimento ambiental

A bioacústica também tem servido de instrumento para o estudo de emoções e bem-estar animal (e.g., Grandim 2001; Briefer, Tettamanti & McElligott, 2015). Nós testamos algo novo, em ambiente de cativeiro: a possibilidade de uso de uma vocalização coespecífica como elemento de estresse agudo, que funcionasse como item de enriquecimento ambiental. O rugido de bugios é um comportamento típico da espécie, está relacionado ao hábito territorialista, e evoca a mesma resposta em grupos concorrentes (grupos sociais independentes que compartilham uma área cujos limites dos territórios se tocam e precisam ser defendidos diariamente). Em cativeiro, bugios estão privados desse comportamento. A menos que haja um grupo vizinho solto ou preso, os estímulos evocadores do sinal não estarão presentes. Oferecer aos animais cativos a oportunidade de exibir os comportamentos típicos, especialmente aos que apresentam comportamentos estereotipados, é uma prática indicada em programas de enriquecimento ambiental (Martins, 2015).

Testamos a eficácia dos rugidos na melhoria do bem-estar dos macacos bugios em cativeiro, medindo as respostas comportamentais e fisiológicas de treze Alouatta caraya (6 fêmeas e 7 machos; Martins, 2015). Adotamos o delineamento experimental ABABA, no qual as fases de controle A são intercaladas com fases de tratamento B (reprodução do rugido), primeiramente executadas ao mesmo tempo (previsivelmente) e em uma programação aleatória (imprevisível). A duração relativa do rugido e de outros comportamentos esperados (alerta, atenção orientada, interações sociais positivas) aumentou nas fases B em relação às fases A e os comportamentos estereotipados (que são interpretados como indicativos de estresse ao cativeiro) diminuíram (Martins, 2015). A redução do estresse nas fases B foi confirmada por análise dos metabólitos fecais, pelo colaborador Rupert Palme. Concluímos que tanto as rotinas previsíveis quanto as imprevisíveis melhoraram o bem-estar e propusemos a prática (o uso da reprodução de chamados específicos das espécies) como uma nova categoria de enriquecimento ambiental. O artigo ainda está em preparação.

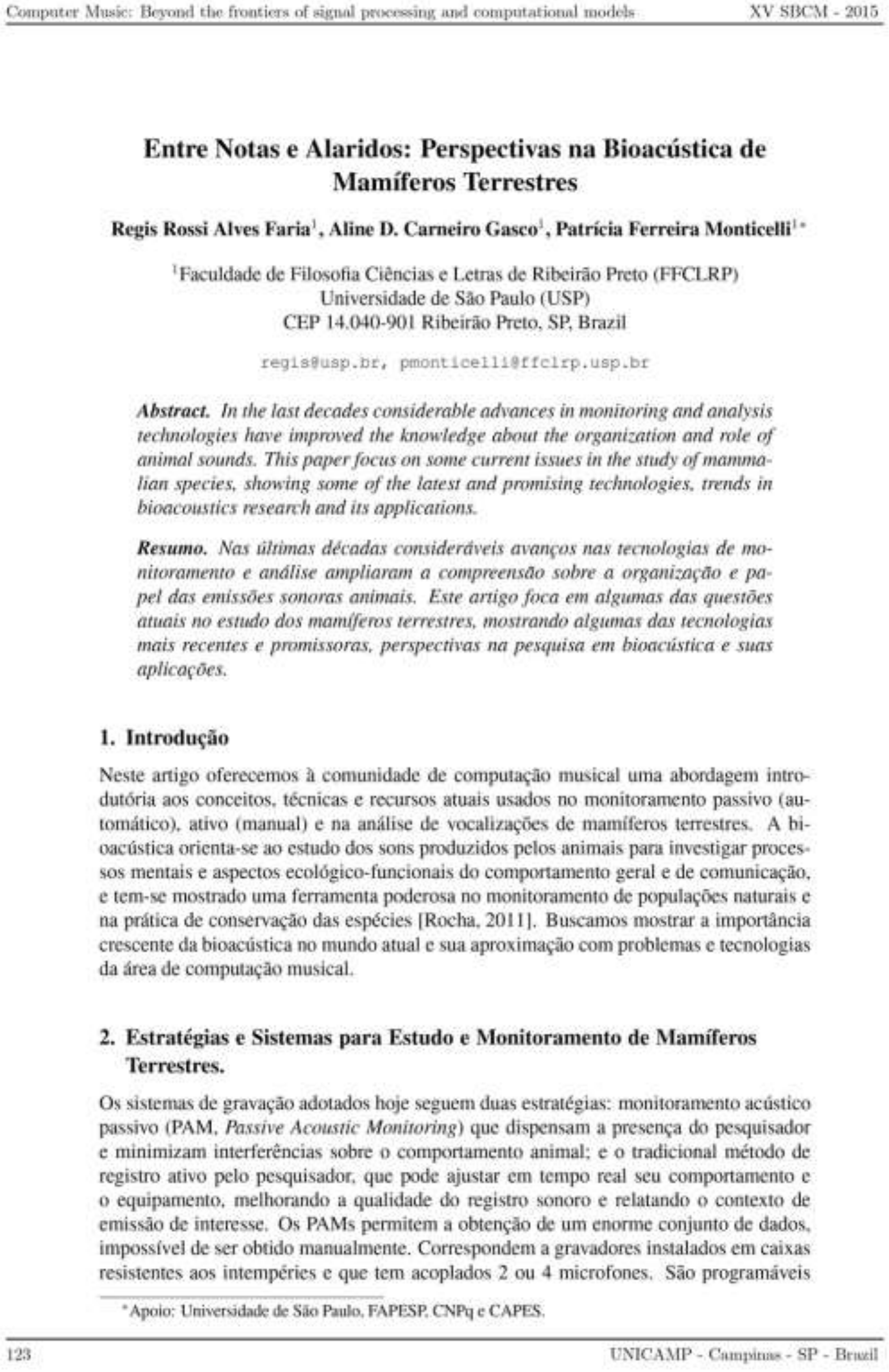
Arquivos Sonoros: conservação, pesquisa e ação social

Há dez anos, no *Internacional Bioacoustics Conference*-2009, foi apresentada uma proposta de criação de uma enorme base de dados "globalizada" que poderia ser usada por toda a comunidade científica. Seria uma biblioteca dos sons produzidos pelas espécies animais, a partir da qual seria possível identificar *em campo* qualquer espécie que produzisse um som já identificado. Isso já é uma realidade para morcegos, não com o fim de se conhecer o repertório das espécies, mas como ferramenta de identificação de espécies (Russo & Voigt, 2016).

O catálogo global ainda não se concretizou, e talvez precise de uma nova geração de pesquisadores e agentes de apoio à pesquisa, que vejam mais valor no coletivo do que em ter domínio sobre um acervo. Cada grupo de pesquisa, com interesse em um conjunto determinado de espécies, vem criando seu próprio arquivo sonoro. E nós criamos o nosso, um arquivo sonoro voltado ao grupo menos representado até aqui, os mamíferos não-voadores, a Fonoteca César Ades (FOCA). Ainda não consegui abrir o link para acesso à comunidade, mas já existe a estrutura: há um endereço *http* com uma ferramenta de busca por som, grupo taxonômico ou comportamento, ferramentos de contato, download, e tocadores de vídeo e áudio. Um acervo inicial de áudio (.wav) e vídeo gravado pelo nosso grupo de pesquisa (basicamente caviomorfos e alguns carnívoros, e aves anuros das paisagens de Itirapina; e as paisagens de Itirapina, Vassununga e Ribeirão Preto) vem sendo transferido de HDs para uma nuvem destinada para isso. Seguimos o padrão da Fonoteca Neotropical Jacques Vielliard (https://www2.ib.unicamp.br/fnjv/), que mantem todo o acervo criado por Vielliard, digitalizado a partir de fitas de rolo, e uma coleção mais recente de herpetofauna. A Macaulay Library of Natural Sounds (http://macaulaylibrary.org/search.do), a maior e mais antiga, tem um rico acervo de aves e alguma coisa de mamíferos, mas faltam informações sobre o comportamento associado às emissões. E há bibliotecas particulares, que vendem amostras de som, como o www.sonidosdelanaturaleza.com.

A FOCA tem interesse em fomentar a pesquisa, oferecendo dados científicos (arquivos em wav e com um metadados objetivamente descritos e detalhados do contexto das emissões), e servir à comunidade. Para isso foi pensada e inclui em seu termo de concessão de uso a doação de amostras de sons com cláusulas que garantem o reconhecimento da fonte e autoria (FOCA e o recordista), sem privar do acesso e do uso de seu acervo como dado ou ferramenta para a pesquisa, pesquisadores, educadores e interessados no comportamento animal. Eu gostaria que a expansão dos programas de monitoramento bioacústico (gravação e detecção automatizada) e das bibliotecas sonoras, acontece no sentido de gerar material para uso público, entendendo-se sua aplicação em causas globais que são atuais e urgentes (e.g., conservação da biodiversidade, mitigação de conflito humano-fauna, avaliação de riscos da exposição à poluição sonora) e não como nicho de atuação do mercado capitalista. Trabalhamos tanto para chegar até aqui e o caminho à frente ainda é longo (veja como os desafios que ainda têm a quiropterologia que é quem está mais à frente, Russo & Voigt, 2016). Transformar o desenvolvimento de tecnologia em bioacústica em mercado de lucro é, ao meu ver, andar na contramão em relação ao objetivo primordial dos pesquisadores que iniciaram essa linha de pesquisa: os benefícios da ciência e tecnologia devem ser públicos, de acordo com seu fim de preservação do patrimônio mundial da Biodiversidade. Assim espero!

Apêndice A – *Entre notas e alaridos: perspectivas na Bioacústica de mamíferos terrestres*



Entre Notas e Alaridos: Perspectivas na Bioacústica de Mamíferos Terrestres

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Abstract. *In the last decades considerable advances in monitoring and analysis technologies have improved the knowledge about the organization and role of animal sounds. This paper focus on some current issues in the study of mammalian species, showing some of the latest and promising technologies, trends in bioacoustics research and its applications.*

Resumo. *Nas últimas décadas consideráveis avanços nas tecnologias de monitoramento e análise ampliaram a compreensão sobre a organização e papel das emissões sonoras animais. Este artigo foca em algumas das questões atuais no estudo dos mamíferos terrestres, mostrando algumas das tecnologias mais recentes e promissoras, perspectivas na pesquisa em bioacústica e suas aplicações.*

1. Introdução

Neste artigo oferecemos à comunidade de computação musical uma abordagem introdutória aos conceitos, técnicas e recursos atuais usados no monitoramento passivo (automático), ativo (manual) e na análise de vocalizações de mamíferos terrestres. A bioacústica orienta-se ao estudo dos sons produzidos pelos animais para investigar processos mentais e aspectos ecológico-funcionais do comportamento geral e de comunicação, e tem-se mostrado uma ferramenta poderosa no monitoramento de populações naturais e na prática de conservação das espécies [Rocha, 2011]. Buscamos mostrar a importância crescente da bioacústica no mundo atual e sua aproximação com problemas e tecnologias da área de computação musical.

2. Estratégias e Sistemas para Estudo e Monitoramento de Mamíferos Terrestres.

Os sistemas de gravação adotados hoje seguem duas estratégias: monitoramento acústico passivo (PAM, *Passive Acoustic Monitoring*) que dispensam a presença do pesquisador e minimizam interferências sobre o comportamento animal; e o tradicional método de registro ativo pelo pesquisador, que pode ajustar em tempo real seu comportamento e o equipamento, melhorando a qualidade do registro sonoro e relatando o contexto de emissão de interesse. Os PAMs permitem a obtenção de um enorme conjunto de dados, impossível de ser obtido manualmente. Correspondem a gravadores instalados em caixas resistentes aos intempéries e que tem acoplados 2 ou 4 microfones. São programáveis

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*Apoio: Universidade de São Paulo, FAPESP, CNPq e CAPES.

para gravar a intervalos predefinidos, têm alta capacidade de armazenamento de dados e alimentação de longa duração. Com isso, pode-se coletar dados sem voltar ao campo, de forma contínua ou espaçadas no tempo (sazonalmente ou em janelas de interesse) e no espaço (por uma área de interesse amostral).

Sistemas mais sofisticados podem incluir ainda meios de comunicação remota (transmissão por radiofrequência acionados/monitorados à distância) e dispositivos GPS para rastreamento de posição. O monitoramento de espécies de interesse é feito através do rastreamento de suas vocalizações previamente conhecidas nas gravações, que acusam a sua presença no local e fornecem estimativas populacionais e outras informações sobre a dinâmica intra/entre espécies. A empresa Wildlife Acoustics produz gravadores autônomos (com microfones inclusive à prova d'água) para captação e análise sonora.

O monitoramento passivo não permite ainda, contudo, a gravação seletiva e contextualizada de sons de uma espécie, com a identificação da situação de emissão que levará a hipóteses sobre sua função comunicativa. Também ainda não se alcançou um nível de qualidade de registro que permita seu uso na descrição física e sirva como testemunho da espécie, muito menos para uso em estudos comparativos entre indivíduos ou espécies que levariam a inferências sobre o nível de socialidade e de complexidade de seus processos mentais, ou de relações filogenéticas, respectivamente. Esse tipo de registro ainda é obtido pessoalmente, com um gravador portátil profissional acoplado a um microfone direcional e um fone de ouvido.

Diversos modelos de gravadores profissionais portáteis são usados atualmente, como Marantz PMD660/661/671, Tascam DR100, Zoom H4N, Sony PCM-D100, Roland R26 ou Sound Device 722/788-T, acoplados a microfones supercardióides condensadores (*shotguns*). O levantamento e a descrição contextualizada do repertório de vocalizações de mamíferos terrestres da nossa fauna tem sido feito no Laboratório de Etologia e Bioacústica (EBAC) desde 2011. Requer a obtenção de vocalizações em uma variedade de contextos e a posterior categorização dos sinais segundo sua morfologia. Em um segundo nível de análise, os "tipos" de sinais são categorizados quanto ao "contexto de emissão", como a defesa de território (o aulido do lobo-guará [Rocha et al., 2015]), o alerta e intimidação de predadores (o latido de quatis [Gasco, 2013]), o cortejo (o *purr* do macho preá [Monticelli and Ades, 2011]) e o reencontro entre membros do grupo, incluindo entre mães e filhotes (assobios de capivaras [Suzuki, 2015]). Os avanços na nossa compreensão sobre os processos biológicos e estados internos dos animais nos permitem hoje identificar parâmetros vocais que codificam inclusive suas emoções [Briefer et al., 2015].

Um problema clássico em bioacústica é detectar e isolar os sons produzidos simultaneamente pelas diversas espécies dentro da "paisagem acústica" registrada. A segregação e o isolamento das interferências do meio podem contar com técnicas de separação de fontes, matrizes de microfones e *beamforming* (separação baseada na localização). Ao estudo de mamíferos terrestres impõe-se uma série de requisitos à escuta, ao registro, à reprodução fidedigna para fins de testes experimentais de função comunicativa (*playback*), e à descrição e análise para fins científicos. Por iniciarem-se em uma faixa de frequências abaixo de 100Hz e terem enfatizadas frequências dominantes ou *peak frequency* abaixo de 1kHz, esses sons sobrepõem-se ao ruído de fundo ambiental.

Uma vez registradas as amostras, a próxima tarefa é detectar as vocalizações e extraí-las para análise. Esse processo de identificação e segmentação é guiado observando-se níveis de sinal/ruído e as características temporais e espectrais dos sinais. Uma correta calibração de parâmetros como intensidade, tamanho, tipo e percentual de *overlap* das janelas de análise, profundidade de bits e resolução de tempo e frequência será determinante para o sucesso. Cada tipo de ambiente e espécie implicam em um conjunto de parâmetros mais adequado para o processamento e análise dos sinais, sendo esta busca, no caso da detecção e análise manual, um dos processos mais demorados.

O pesquisador atualmente dispõe de pacotes e recursos computacionais projetados para detecção automática e análise de sons animais, como os *softwares* Raven, do Laboratório de Ornitologia da Cornell University, o Avisoft SASLab, e a plataforma Arbimon. Rocha e outros [Rocha et al., 2015] descreveram o uso do pacote de análise sonora XBAT (*Extensible Bioacoustics Tool*) para a plataforma MATLAB na detecção automática de vocalizações de lobos em gravações de longo prazo, através da busca por sons que se autocorrelacionem com modelos preestabelecidos e o rastreio de eventos para os quais o valor de correlação exceda um determinado limiar. Preconizando uma arquitetura extensível, que permite que componentes compartilhem uma mesma infraestrutura, os usuários deste *software* podem acessar, visualizar, buscar, anotar e medir eventos de interesse nos sons.

Os formatos de codificação perceptuais voltados para voz humana, como o AMR (*Adaptive Multi-Rate*) e o 3GPP disponíveis em *smartphones*, não são adequados para um registro fidedigno de vocalização animal, para o que é usado o formato não comprimido WAV (PCM), com amostragem de 16-24bit@44-48kHz.

3. Monitoramento Mediado por Auralização.

Uma estratégia relativamente recente de estudo está relacionada à captura de emissões vocais dos animais induzidas artificialmente, por meio da auralização no ambiente que os estimulariam a dar uma resposta. Tal estratégia é usada desde a 2ª Guerra por ornitólogos e herpetólogos (estudiosos de anuros) para verificar a ocorrência (induzindo a aproximação) e um aumento na emissão de uma resposta, vocal ou não, que permita caracterizar comportamentos e inferir funções comunicativas. Mas, até hoje foi pouco usado com mamíferos. O realismo da auralização parece ser um requisito crítico de sucesso. A capacidade de cobertura espacial da projeção sonora dependerá de um sistema de auralização com alto nível de imersão e com resolução e qualidade suficientes para garantir uma comunicação efetiva.

4. Desafios e Perspectivas na Bioacústica de Mamíferos Terrestres.

Muitos são os aspectos importantes a considerar em um sistema de monitoramento bioacústico, como a diversidade de espécies, o uso de detectores de atividade para disparar eventos de gravação, variações climáticas, interferências acústicas, ruídos e separação de sons. Do ponto de vista funcional, os objetivos da análise irão determinar as ferramentas de processamento a serem utilizadas. Usualmente, o vocabulário de emissões vocálicas de mamíferos e outros animais são analisáveis a partir de uma segmentação em unidades sonoras, que podem se assemelhar às sílabas. Uma série de ferramentas utilizadas em *Musical Information Retrieval*, como analisadores espectrais, descritores sonoros, transformadas e segmentadores são utilizados na análise desses sons. Todavia há diferenças substanciais entre estes e os sinais musicais, particularmente na estrutura temporal e em parâmetros espectrais [Stowell and Plumbley, 2011]. Enquanto na música encontramos timbre e uma estrutura temporal bem definidos, em emissões animais encontramos sílabas de diferentes conteúdos soando com durações próximas a ${}^{1}/_{2}$ segundo, e compondo estruturas ou frases que se repetem. A identificação do tipo de emissão e seu significado só será possível a partir da segmentação de toda a sequência (frase).

Na FFCLRP-USP, o EBAC em colaboração com o Laboratório de Acústica e Tecnologia Musical (LATM) tem buscado levantar requisitos e técnicas de processamento de sinais para melhor isolar as interferências ambientais, rumo a uma análise diferenciada para sons de mamíferos terrestres. O grupo vem definindo metas em direção a um sistema para monitoramento, lançando mão de técnicas para auralização espacial em campo e para captura sonora espacial. O monitoramento acústico com múltiplos microfones é útil para localizar animais e rastrear seus movimentos em uma escala espacial maior [Blumstein et al., 2011]. Considerando a migração dos aplicativos para as plataformas móveis e a oferta de acessórios para este segmento, atualmente é praticável a captação de sons em boa resolução em tabletes (ex: iPads). Ainda, os sistemas que utilizam áudio *wireless* expandem as possibilidades, principalmente se aliados a um dispositivo de recarregamento autossustentável de baterias.

Finalmente, um estudo interessante prospectivo é a avaliação da emoção e expressividade no registro sonoro de animais silvestres [Moura et al., 2008]. Assim como no caso do canto humano, correlações entre duração, tonalidade e intensidade de diversas vocalizações forneceriam subsídios para uma análise aprofundada do conteúdo da *cantoria*, identificando contornos melódicos e harmônicos em frases longas e caracterizando modulações. Avaliações de longo prazo do registro sonoro assim, extrapolariam aspectos puramente ecológicos do monitoramento tradicional, avançando sobre análises semânticas como hoje temos na área de música.

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Apêndice B – *Um relato atual da prática da bioacústica de mamíferos terrestres no Brasil*

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Um relato atual da prática da bioacústica de mamíferos terrestres no Brasil

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RESUMO

Apresentamos um relato de uso de equipamentos para registro de vocalizações de animais em campo e ferramentas utilizadas para a análise destes sinais. Nosso objetivo é contextualizar a prática da pesquisa em bioacústica de mamíferos terrestres atualmente em curso no Brasil com os recursos tecnológicos disponíveis, discutindo suas virtudes e limitações, e contrapondo características existentes e recursos desejáveis para os sistemas de captura, gravação e análise sonora que ainda não atendem plenamente às necessidades da área.

0. INTRODUÇÃO

A prática da pesquisa em bioacústica fundamenta-se no estudo do comportamento dos animais por meio de suas vocalizações e relações sonoras com o meio ambiente. O acesso ao registro sonoro é viabilizado pelo uso de equipamentos eletrônicos como sistemas de captura e gravação, e além disso a análise dos sinais também requer o uso extensivo de ferramentas de processamento de áudio.

Neste relato pretendemos apresentar os diferenciais na prática da bioacústica realizada no Brasil, em suas motivações, interesses, processos adotados e animais abordados. No Laboratório de Etologia e Bioacústica (EBAC) do Departamento de Psicologia da Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (FFCLRP-USP) estudam-se os mamíferos neotropicais sob uma abordagem etológica, particularmente focalizando o comportamento de comunicação acústica de alguns ícones da fauna sul-americana, como o lobo-guará, a capivara e o quati. Além do levantamento das vocalizações usadas para a comunicação intra e interespecíficas, no EBAC analisamos a associação entre tamanhos de repertórios e o nível de sociabilidade das espécies, a influência do ruído antrópico na comunicação à longa distância (por exemplo, com o lobo-guará), a seleção sexual através de sinais acústicos, e a presença e significado de efeitos não-lineares nas vocalizações de mamíferos. Além disso, descrevemos paisagens acústicas para fins de conservação e avaliação do impacto de atividades humanas sobre a fauna, o que vem despertando um interesse adicional em conhecer e mapear a ecologia sonora-musical destes habitats.

Em colaboração com o Laboratório de Acústica e Tecnologia Musical (LATM) do Departamento de Música da mesma faculdade, temos atuado no sentido de identificar requisitos e selecionar métodos de aquisição e processamento de sinais sonoros para obter uma melhor qualidade de captura sonora do ambiente e isolamento das interferências de múltiplas fontes, em direção a um sistema integrado para monitoramento ativo com cobertura espacial capaz, por exemplo, de localizar animais e rastrear seus movimentos em uma escala espacial maior, conforme Blumstein et al (2011) sustentam ser viável com o uso das novas tecnologias de áudio.

Considerando também a migração dos aplicativos para as plataformas móveis e a oferta de acessórios para este segmento, atualmente é praticável a captação de sons em boa resolução em dispositivos portáteis como *tablets* e *smartphones*. Os sistemas que utilizam áudio *wireless* expandem mais ainda as possibilidades, principalmente se aliados a um dispositivo de recarregamento auto-sustentável de baterias.

Neste relato buscamos identificar os materiais e tecnologias utilizados, mostrar alguns dos métodos de monitoramento que utilizamos em campo para o registro sonoro manual e alguns dos equipamentos utilizados, como o sistema "Song Meter"™ (da *Wildlife Acoustics, Bioacoustics Monitoring Systems*) e Arbimon™ (*Automated Remote Biodiversity Monitoring Network*) da *Sieve Analytics Inc.* (Aide et al 2013). Nosso foco em particular endereça o uso de equipamentos de gravação e monitoramento bioacústico em florestas tropicais no Sudeste brasileiro.

Os blocos funcionais e os componentes fundamentais que integram estes equipamentos incluem os microfones, o sistema de alimentação, as interfaces de áudio e sistemas de gravação. Abordamos os meios e formatos de registro sonoro mais comuns, bem como características de equipamentos e acessórios de controle que se tornam cada vez mais comuns, como *tablets* e *smartphones*.

No que concerne à análise dos sinais sonoros, identificamos algumas das ferramentas mais utilizadas e as dificuldades clássicas em se caracterizar as vocalizações de mamíferos que frequentemente estão combinadas a um fundo ruidoso.

Colocamos em pauta discussões sobre a qualidade dos registros obtidos e *reports* de uso dos sistemas em campo, destacando a falta de um sistema especializado para estudo de mamíferos terrestres, apontando limitações nos sistemas atuais e perspectivas para inovações tecnológicas desejadas para o futuro.

1. PRIMÓRDIOS DA BIOACÚSTICA NO BRASIL

Para o fundador da bioacústica no Brasil, o Francês Jacques Marie Edme Vielliard[1], a história desta ciência começa com a *Zoophonia* de Hercule Florence (Vielliard & Silva 2006), o inventor da fotografia. Foi ele o autor do relato ilustrado e detalhado da Expedição Langsdorff pela Amazônia e entorno entre os anos de 1825 e 1829. Para preservar os cantos que ouviu, Florence usou notações musicais.

A bioacústica se firmou como ciência no final da década de 1960 com o surgimento dos gravadores "portáteis" de fitas de rolo (Au 1997), ainda muito pesados, mas que serviam bem principalmente aos ornitólogos e os *bird watchers* e, logo depois, atraiu a atenção de interessados em cantos de anuros e insetos. A análise de gravações era inicialmente feita com o auxílio de sonógrafos, e depois, em programas de computador. A partir da década de 1980 os gravadores tornaram-se de fato portáteis, primeiro utilizando fitas no formato digital DAT (*Digital Audio Tape*) e hoje chegam à facilidade de se gravar e transportar uma grande quantidade de sons em algo tão pequeno e leve como um cartão de memória de estado sólido (*flash*).

É muito mais recente o interesse de pesquisadores trabalhando com mamíferos pela bioacústica. Ainda hoje se encontram trabalhos que fazem descrições qualitativas de sons de mamíferos não-humanos, vide as publicações da *Mammalian Species*, tão detalhadas na descrição da anatomia e fisiologia das espécies, mas extremamente vagas na descrição do comportamento vocal. Os estudos que oferecem descrições quantitativas, por meio de análise sonográfica, são, em sua grande maioria, de primatas, morcegos ou cetáceos. Entre as explicações para isso está a dificuldade de se localizar e observar as espécies terrestres. São animais noturnos ou muito ariscos, que evitam a presença humana e que também não vivem restritos a uma pequena área. Tudo isso torna quase impossível a gravação de um mamífero terrestre em campo por vias manuais.

A seguir apontaremos algumas das principais limitações tecnológicas dos equipamentos de gravação acústica e diretrizes para possíveis soluções, com foco no estudo de mamíferos terrestres.

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[1] Vide *website* da Fonoteca Neotropical Jacques Vielliard em www.ib.unicamp.br/museu_zoologia/colecao_sonora

2. TECNOLOGIAS USADAS NA PESQUISA BIOACÚSTICA

O cenário funcional clássico de trabalho no campo envolve (a) um sistema para captura e registro, e (b) um sistema para síntese e auralização, controlados por meio de um dispositivo eletrônico/digital micro-processado com memória. O sistema de captura e registro deve dispor de um ou mais microfones e eventualmente poderá conter embutido um sub-sistema de análise acústica e semântica dos sons. O sistema de síntese e auralização, quando usado, deve dispor de alto-falantes para produzir sons no ambiente. Nos dispositivos micro-processados os dados são armazenados em inventórios digitais, que recentemente podem inclusive contar com espaços de armazenamento à distância (ex: *data clouds*) acessíveis por meio de redes de telecomunicações sem fio (*wireless*). Fora do campo, conta-se ainda com plataformas computacionais adicionais para análise e processamento do material coletado.

Em campo a operação clássica manual é ainda considerada a de maior efetividade, mas é a modalidade de operação automática que encontra o maior campo de interesse hoje e, melhorando-se a relação benefício/custo, tende a crescer. Nesta, o monitoramente é de longo termo e pode contar com vários graus de autonomia. Incluem-se nesta modalidade os sistemas conhecidos popularmente como ARS (*Automatic Recording System*) e PAM (*Passive Acoustic Monitoring*). A temporização em sistemas assim pode por exemplo realizar coletas de 1 minuto de áudio a cada 10 minutos (ex: sistema Arbimon™ com 144 1-min. gravações por dia) ou ser programável para disparo por evento acústico detectado.

Na escolha dos microfones é importante considerar o tipo de transdutor que se deseja e os materiais construtivos. Isto determinará quais serão os requisitos para alimentação da polarização (ex: de 3 a 48V tipicamente), para resposta em frequência e sensibilidade, para capacidade de embutir os dispositivos e oferecer entradas adicionais e circuitos de pré-amplificação que condicionem os sinais. Os sistemas de microfonação podem ainda considerar a modelagem do padrão de captura (característica direcional), a focalização (que poderá ter repercussão na capacidade de determinação de posição territorial de espécimes em função da resolução métrica conseguida) e a possibilidade de separação de sinais pelo arranjo espacial (ex: com uso de sistemas multi-microfonados; Blumstein et al 2011). Considere ainda que os microfones podem vir embutidos ou montáveis, usando-se conectores e cabos acessórios, caso que oferece maior flexibilidade e opções ao usuário.

Dispositivos de campo podem ter várias interfaces, portas e conexões para interligar a outros equipamentos e dispositivos. Conectores de 2 canais como miniplugs estéreo (P2) e USB são os mais comuns e de maior penetração, embora interfaces seriais (SPI) e rede IP (CAT5 ou superior) sejam possíveis de uso. Os dispositivos processadores mais populares incluem microcontroladores e microprocessadores (ex: ARM™ Cortex™ 32/64 bits), integrados DSPs, FPGA's e *codecs* dedicados, capazes de suportar vários formatos de mídia digital.

Os formatos mais comuns de registro de áudio incluem o PCM ou ADPCM (não comprimido) e os formatos de compressão perceptual (com perdas) como MP3 (MPEG 1&2 *Audio Layer* III, I & II) e MPEG 4 AAC-LC ou HE-AAC (*level* 3, SBR +PS). Formatos comprimidos sem perdas (ex: FLAC) não são ainda popularmente adotados em plataformas para bioacústica, possivelmente devido ao ainda baixo fator benefício/custo para sua adoção.

Minimamente as plataformas de monitoramento de campo devem contar com microfones, alimentação própria, um dispositivo de gravação, interfaces de controle (de usuário) e invólucros adequados à exposição ambiental. Como exemplo citamos a plataforma "Song Meter"™, basicamente um gravador de campo com recursos de microfonação e autonomia para até 300 horas.

Poucos sistemas que realizam aquisição, processamento e gerenciamento integrados estão atualmente disponíveis para o pesquisador. Entre eles citamos o Arbimon™ apresentado em Aide et al (2013). Dispositivos disponíveis em suas estações de monitoramento podem incluir painéis solares, *iPod's*™, baterias de 12V e reguladores de tensão, controladores de potência, antena (para rádio 900 MHz) e microfones de faixa plena (20-20kHz) e à prova d'água. Nas estações base de análise, encontram-se antenas de recepção, *switches* de rede e acesso à internet, computadores (servidores) para processamento e armazenamento (bases de dados ou repositórios digitais), além de programas para visualização.

Com relação à análise do material sonoro, podemos citar algumas plataformas e recursos mais comumente utilizados por equipes de pesquisadores que buscam principalmente identificar padrões de vocalização, identificar espécies para objetivos de taxonomia e estudos estatísticos sobre populações animais, e ainda identificar mensagens e funções comunicativas para compreender os modelos sociais aplicados às espécies. Este estudo a longo prazo tem impacto na geração de novo conhecimento e na definição de políticas para preservação ambiental.

São utilizados programas (*software*) em plataformas computacionais (inclusive em *grids* computacionais, como o *Biophony Grid Portal*) que perfazem a análise no domínio do tempo e da frequência. Entre eles citamos o XBAT (*Extensible*

Bioacoustic Tool), um pacote de funções para MATLAB™ para análise e anotação de sons, que opera sobre longas gravações usando técnicas de reconhecimento de padrões[2], o Raven *Interactive Sound Analysis*, *software* desenvolvido no *Cornell Lab of Ornithology*, junto ao seu programa de pesquisa em bioacústica[3], e o AviSoft SAS-Lab Pro, programa para investigação da comunicação acústica de animais da empresa *Avisoft Bioacoustics*[4] (Alemanha), com recursos para análise, edição, classificação e síntese sonora.

A título de informação sobre funções de análise, o *software* Raven, por exemplo, possui uma série de recursos como a detecção, seleção de eventos; determinação de níveis (limiares, picos e valores médios); seleção adaptativa de níveis em pontos diferentes de espectrogramas; medidores e exibidores de contornos de frequências; calibração de níveis e escalas do sinal; detectores de silêncio, indicadores de duração e posição temporal absoluta e relativa; envelopes de *fade-in* e *fade-out*; modos de audição/playback com filtros, etc.

3. LIMITAÇÕES TECNOLÓGICAS ATUAIS

Embora os equipamentos remotos automatizados sejam bons, relata-se ainda grande dificuldade com a qualidade e clareza da captura com os microfones e técnicas de captura existentes nestes sistemas. Os sons frequentemente são prejudicados por ruídos difíceis de se isolar em comparação ao que se consegue com sistemas de captura/operação manuais, em que temos mais ajustes a serem feitos presencialmente.

A título de ilustração de um tipo de vocalização de mamífero terrestre brasileiro e suas características, a figura 1 mostra dois registros bem caracterizados de uma vocalização de longa distância do lobo-guará obtidos de animais de cativeiro, manualmente por Flora Balieiro (à esquerda), e através de um sistema de gravação automático (à direita, com muito ruído ao fundo). O aulido da espécie é uma nota que soa grave (maior energia nas frequências inferiores) e ruidosa em uma ampla faixa de frequências. A figura mostra a forma de onda temporal do som acima, e seu espectrograma abaixo.

As vocalizações emitidas por mamíferos vão desde ultrassons (e.g. morcegos) até infrassons (e.g. elefantes) e para cada faixa de frequência de interesse é necessário que o pesquisador use um equipamento específico (Fletcher 2004, McQuay 2008). Microfones, como instrumentos transdutores que convertem energia mecânica (ondas de pressão sonora) em energia elétrica, funcionam também como "filtros" registrando determinadas faixas de frequência e intensidade, e descartando ou atenuando outras (Burbidge et al. 2001).

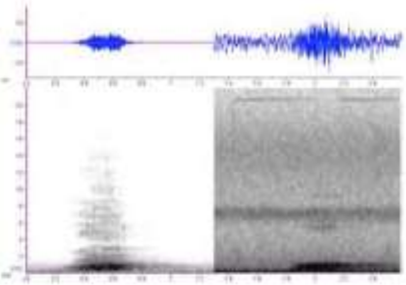

Figura 1 – Duas notas de aulido de lobos-guará em cativeiro, obtidos manualmente com gravador Marantz PMD660 acoplado a Sennheiser ME67 (esq.) e por gravador automático "Song Meter"™ (dir.). Repare na diferença no nível de ruído entre as duas gravações, tomadas em situações similares.

O desenvolvimento da maioria dos microfones portáteis disponíveis no mercado foram impulsionados pela indústria da música e, portanto, criados para captar sons audíveis para humanos (i.e. 20-20kHz) e para operar em ambientes controlados (i.e. longe de condições adversas comuns durante trabalhos em campo com mamíferos terrestres, tais como alta temperatura e umidade). Embora a disponibilidade de microfones capazes de captar a diversidade de vocalizações de mamíferos esteja crescendo, ela ainda é restrita em certa medida.

Os gravadores portáteis digitais foram um importante avanço tecnológico para o estabelecimento da bioacústica como ciência (Au 1997). No entanto, a duração da carga de suas baterias ainda é um fator limitante para trabalhos de campo, pois gravações contínuas sem fonte de alimentação externa normalmente duram poucas horas. Tipicamente encontramos células alcalinas, de NiMH ou Li-ion com duração de até 300 horas. A autonomia entretanto depende de diversos fatores, inclusive da possibilidade de alimentação externa (como no uso de painéis solares) e da boa regulação de tensão e gerenciamento de potência.

Outro parâmetro importante a ser considerado por pesquisadores é a escolha do formato de gravação dos arquivos de áudio e a capacidade de armazenamento do gravador. Gravações em formatos compactados como (ex: MPEG-1/2 *Audio Layer* III, I ou II) ocupam menos espaço, o que aumenta a quantidade de dados que podem ser armazenados. Por outro lado, a baixa fidelidade deste tipo de arquivo de áudio impede que alguns tipos de análises

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[2] https://code.google.com/archive/p/xbat-devel/

[3] www.birds.cornell.edu/brp/raven/RavenVersions.html

[4] www.avisoft.com

acústicas sejam realizadas (Fischer et al. 2013). Gravações fidedignas à vocalização original são gravadas em formatos de arquivo não compactados (ex: WAV/AIFF PCM – *Pulse Code Modulation*) e requerem mais espaço de armazenamento, o que pode aumentar muito o custo de certos tipos de pesquisa.

Dentre os principais problemas enfrentados com gravações de mamíferos terrestres em campo destacamos:

(1) microfones com resposta de frequência não plana dentro da faixa de frequências da vocalização de interesse;

(2) baixa relação sinal/ruído das gravações, especialmente quando lidamos com microfones onidirecionais em ambientes ruidosos;

(3) sensibilidade do microfone que pode ser permanentemente afetada quando exposto a temperaturas acima da faixa de tolerância de seus parâmetros operacionais;

(4) baixa resiliência em condições de alta umidade (especialmente quando lidamos com microfones condensadores);

(5) alto consumo de energia mesmo nos gravadores digitais, o que restringe o tempo de gravação em campo;

(6) capacidade restrita de armazenamento de dados acústicos para monitoramento de longo prazo por alguns gravadores digitais para determinados tipos de pesquisa;

Com relação aos métodos de análise de sinais e detecção de vocalizações, os problemas mais comuns incluem a ocorrência e as dificuldades de tratamento de casos de falsos positivos e falsos negativos. Exemplos de causas de falsos positivos incluem ruídos meteorológicos (ex: vento), mecânicos e vocalizações de espécies parecidas. Como exemplo de causas para falsos negativos citamos a microfonação deficiente (em que vocalizações distantes, não sendo marcadas, não serão achadas) e parametrização excessivamente restritiva para evitar falsos positivos (que acaba por elevar o número de falsos negativos, como relata Aide et al 2013).

4. PERSPECTIVAS PARA INOVAÇÕES TECNOLÓGICAS

Nesta sessão desejamos sugerir algumas diretrizes a serem observadas no desenvolvimento de soluções para as limitações atuais. Soluções tecnológicas focadas em gravações acústicas de mamíferos terrestres em ambientes naturais devem levar em conta aspectos diretamente relacionados às espécies de interesse e ao ambiente em que vivem. Elencamos abaixo alguns aspectos fundamentais:

(1) conhecer aspectos do repertório vocal das espécies de interesse (ex: qual é a faixa de frequências que se quer amostrar; qual é a amplitude média das vocalizações alvo);

(2) caracterização da biofonia do ambiente (i.e. leque de sons da paisagem acústica que tem origem biológica). Esta etapa servirá, por exemplo, para verificar previamente se existem outras vocalizações que podem se sobrepor às de interesse e que poderão ser eliminadas pelo equipamento;

(3) caracterização da geofonia (i.e. sons provenientes de fenômenos naturais como chuva e vento; Krause 1993) e antropofonia (i.e. sons oriundos de atividades humanas; Krause 1993) do ambiente, que podem interferir no registro de sons de interesse;

(4) características climáticas, fitofisionômicas e de relevo do ambiente que podem influenciar na propagação do som e durabilidade do equipamento de gravação;

(5) tipos de pesquisa que se beneficiarão com a tecnologia a ser empregada. Por exemplo, pesquisas com levantamento de fauna podem se beneficiar de gravadores autônomos (i.e. que fazem gravações programadas sem a necessidade da presença do pesquisador em campo) que tenham microfones capazes de amostrar amplas faixas de frequências, enquanto pesquisas que buscam gravar uma única espécie ou vocalização podem se beneficiar de microfones desenhados especialmente para este fim.

5. CONSIDERAÇÕES FINAIS

O avanço da tecnologia eletrônica e o aperfeiçoamento de equipamentos de gravação e análise acústica tem ampliado as fronteiras de pesquisas na bioacústica. Todavia existem ainda barreiras tecnológicas que limitam a transposição no uso destes para o estudo de mamíferos terrestres. Não se trata de melhorar o sucesso no registro de sons por eles produzidos somente ajustando a qualidade dos instrumentos de gravação. É necessário se investir em automação no processo. Assim, existe hoje uma demanda crescente de equipamentos pensados para registro de vocalizações em campo na ausência do pesquisador, e com uma cobertura maior de alcance.

Equipamentos para monitoramento de campo na forma de plataformas autônomas parece ser uma tendência, em que a autonomia, além da questão da durabilidade de baterias ou do tempo máximo de operação sem intervenção humana, acrescenta funções de decisão e análise, de auto-gestão, ou de maior controlabilidade à distância. Não obstante à aparente robotização dos dispositivos que isto possa sugerir, caber notar que em casos onde a intervenção do aparelho no meio ambiente for maior, também será maior a necessidade de disfarçá-lo.

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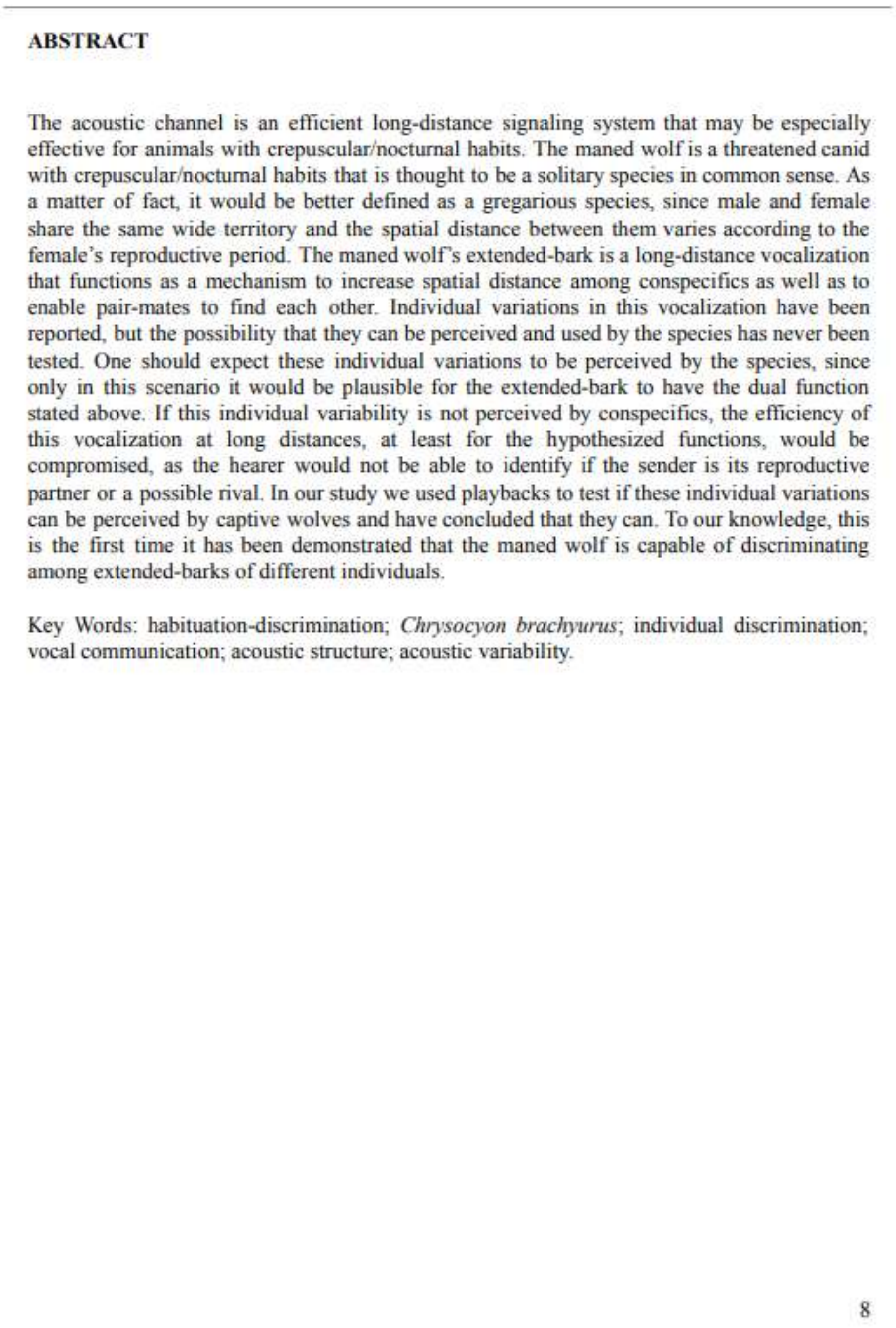
Apêndice C – *The voice of the not so lonely maned wolf: evidence of individual discrimination via playback of the long-distance extended-bark.*



ABSTRACT

The acoustic channel is an efficient long-distance signaling system that may be especially effective for animals with crepuscular/nocturnal habits. The maned wolf is a threatened canid with crepuscular/nocturnal habits that is thought to be a solitary species in common sense. As a matter of fact, it would be better defined as a gregarious species, since male and female share the same wide territory and the spatial distance between them varies according to the female's reproductive period. The maned wolf's extended-bark is a long-distance vocalization that functions as a mechanism to increase spatial distance among conspecifics as well as to enable pair-mates to find each other. Individual variations in this vocalization have been reported, but the possibility that they can be perceived and used by the species has never been tested. One should expect these individual variations to be perceived by the species, since only in this scenario it would be plausible for the extended-bark to have the dual function stated above. If this individual variability is not perceived by conspecifics, the efficiency of this vocalization at long distances, at least for the hypothesized functions, would be compromised, as the hearer would not be able to identify if the sender is its reproductive partner or a possible rival. In our study we used playbacks to test if these individual variations can be perceived by captive wolves and have concluded that they can. To our knowledge, this is the first time it has been demonstrated that the maned wolf is capable of discriminating among extended-barks of different individuals.

Key Words: habituation-discrimination; *Chrysocyon brachyurus*; individual discrimination; vocal communication; acoustic structure; acoustic variability.

Resumo da Dissertação de Mestrado de Flora Balieiro, apresentada ao Depto de Psicologia da FFCLRP, USP em 2016.

Apêndice D _ *Pré-processamento de sinais acústicos para análise e*

XXVIII Encontro da Sociedade Brasileira de Acústica
3 a 5 de outubro de 2018
Porto Alegre - RS

PRÉ-PROCESSAMENTO DE SINAIS ACÚSTICOS PARA ANÁLISE E CARACTERIZAÇÃO DE VOCALIZAÇÃO DE LOBOS-GUARÁ

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RESUMO

A possibilidade de identificação automática de espécies por meio da detecção de seus sinais acústicos de comunicação abre novas possibilidades para o estudo do comportamento e a preservação da biodiversidade. Apesar de disponíveis comercialmente, ferramentas de análise automáticas ainda deixam a desejar em termos de acerto e acurácia. O sucesso de um método automatizado requer a validação de uma estratégia de sequenciamento de processos visando à determinação de parâmetros acústicos vocais em meio a uma complexa paisagem sonora. Um desafio neste sentido recai na extração do ruído existente nas gravações em campo que, por vezes, torna difícil a interpretação correta de parâmetros acústicos. Neste trabalho exploramos uma combinação de etapas de pré-processamentos de sinal para extrair o ruído de gravações de aulidos de indivíduos de lobo-guará (*Chrysocyon brachyurus*) em gravações feitas por um gravador digital amostradas a 44,1 kHz com 16 bits de resolução, usando-se ferramentas de processamento do programa Audiosculpt 3.2.7 do IRCAM. O procedimento envolveu uma sequência de etapas de: seleção dos melhores áudios e segmentação dos arquivos; normalização volumétrica; identificação e remoção das zonas de ruído. Os resultados incluem o processamento de mais de 10 horas de gravação de campo com a produção de um corpo de mais de 131 vocalizações, em condições adequadas para a análise de caracterização de indivíduos que será feita a seguir.

Palavras-chave: bioacústica, detecção automática de indivíduo, processamento de sinais de áudio, conservação de biodiversidade, Audiosculpt.

ABSTRACT

The possibility of automatic species identification through their communication acoustic signals opens new possibilities for the study of behavior and the preservation of biodiversity. Although commercially available, automated analysis tools still lack success in terms of outcome and accuracy. The success of an automated method requires the validation of a process sequencing strategy aiming at the determination of vocal acoustic parameters in the middle of a complex soundscape. A challenge in this regard lies in the extraction of noise from field recordings, which sometimes makes difficult the correct interpretation of acoustic parameters. In this study, we explore a combination of signal pre-processing steps to extract the noise of recordings of roar bark vocalizations of maned wolves (*Chrysocyon brachyurus*) in recordings made by a digital recorder sampled at 44.1 kHz with 16-bit resolution, using processing tools of the software Audiosculpt 3.2.7 by IRCAM. The procedure involved a sequence of steps: selection of the best audios and file segmentation; loudness normalization; identification and removal of noise zones. The results include processing over 10 hours of field recording with the production of a body of more than 131 vocalizations, under conditions suitable for the individual characterization analysis that will be done next.

Keywords: bioacoustics, automatic individual detection, sound signal processing, biodiversity conservation, Audiosculpt.

1. INTRODUÇÃO

A possibilidade de identificação de espécies por meio da detecção de seus sinais acústicos abre uma gama de possibilidades na ciência e em práticas de preservação de biodiversidade. O levantamento e o monitoramento de fauna, por exemplo, que são pontos de partida para ações de manejo e de preservação ambiental [1], são tradicionalmente feitos por meio da identificação visual de animais por avistamento direto, ou de pistas de sua presença (i.e., pegadas, fotografias obtidas por câmeras autônomas, pela identificação de DNA em material contido em fezes). Alternativamente, a utilização de ferramentas bioacústicas, como a captura de som por meio de gravadores automáticos, permite a coleta de informação sem a presença humana, e com vantagens em relação à identificação visual [2]. Os sinais acústicos usados por animais para orientação e comunicação podem ser percebidos a grandes distâncias e não são facilmente obstruídos como os sinais visuais [2]. Além disso, gravadores automáticos à disposição no mercado hoje, podem gravar por longos períodos de tempo, aumentando muito a variedade de animais amostrados e favorecendo o reconhecimento de padrões de atividade mesmo de espécies raramente encontradas [3]. Como alguns sinais de comunicação são individualmente distintos, há um potencial de identificação por sensoriamento automático remoto ao nível do indivíduo, um interesse da Etologia da Conservação [4].

Um desafio à gravação automatizada de longas paisagens acústicas é o processamento das informações contidas na grande quantidade de arquivos gerados. A forma manual de análise consiste no pesquisador ativamente procurar pelas vocalizações de interesse ao longo das gravações (visualmente, nos espectrogramas, ou ouvindo-as). Este processo pode ser longo e trabalhoso e depende da experiência do analisador [5]. A análise automática, disponível hoje em alguns programas comerciais [6-7], reduz o tempo de processamento desses dados [8]. Contudo, ainda se está longe de ter alcançado um algoritmo que seja flexível o suficiente para detectar toda a variedade de vocalizações usadas pelos animais, e acurado o suficiente para destacar as vocalizações-alvo, separando umas das outras [9]. Faz-se necessário investir no desenvolvimento de tecnologia para a detecção e caracterização automatizada de sons animais que atinja o nível individual de identificação. O primeiro passo desse processo é a extração do ruído ambiente, que é inerente às gravações feitas em locais abertos. Esse ruído se sobrepõe à faixa sonora audível na qual se encontram a maioria dos sinais de comunicação de mamíferos terrestres da nossa fauna. A presença e sobreposição desse ruído às vocalizações dificultam a interpretação correta de parâmetros acústicos caracterizadores de sinais de comunicação individual. O que se apresenta aqui é um esboço de protocolo para o pré-processamento de gravações de paisagens acústicas, um passo importante na direção de um processo bem-sucedido de automação do reconhecimento de vocalizações em longas gravações. O processo completo prevê uma ferramenta não-invasiva, acurada e relativamente barata de monitoramento acústico de animais, a partir do qual será possível estimar o tamanho e a composição de grupos sociais naturais e descrever padrões de uso do habitat e de interação interindividual [10].

O reconhecimento de vocalizações no nível individual, no entanto, só é possível em sinais acústicos que possuem um padrão individual vocal, uma "assinatura vocal" [11-12]. Sinais acústicos individualmente distintos já foram registrados em diversos mamíferos [13-16], incluindo o lobo-guará [17-18].

O lobo guará é um canídeo endêmico do Cerrado brasileiro, de interesse para a conservação, e que tem comportamento arredio e hábitos preferencialmente noturnos, o que dificulta seu avistamento em campo [18-19]. A espécie ocupa extensas áreas territoriais que controla através de uma vocalização de longo alcance, o aulido [17-20]. Trata-se de uma vocalização de alta amplitude em ampla faixa de frequências com ênfase em frequências baixas (F0 média: 300 Hz) [18], o que faz com que possa ser facilmente reconhecido e localizado por ouvintes [21]. Por ser conspícuo no repertório do lobo-guará, o aulido é um bom alvo de detecção para os programas de reconhecimento automático. É uma vocalização de curta duração (mediana = 0,54 segundos) emitida em sequências de 3 a 20 notas [17-18].

Neste trabalho foi explorada uma combinação de operações de pré-processamento de sinal para extrair o ruído de gravações acústicas de campo contendo aulidos de indivíduos de lobo guará (*Chrysocyon brachyurus*). Visou-se criar um conjunto de arquivos sonoros com proporção adequada de relação sinal/ruído que permitisse maior exatidão na tomada de medidas acústicas e análises de caracterização desta vocalização.

2. DESENVOLVIMENTO

A seguir, apresentamos as operações de pré-processamento de gravações de aulidos de indivíduos de lobo guará, e de criação de um conjunto de arquivos sonoros com proporção adequada na taxa sinal/ruído.

2.1 Amostras de aulidos

Foram usadas gravações de vocalizações de quatro lobos-guará, dois machos e duas fêmeas, tomados por Flora Balieiro durante seu mestrado no Laboratório de Etologia e Bioacústica (EBAC) [22]. Essas amostras fazem parte do acervo da Fonoteca César Ades (FOCA) mantido pelo EBAC. As gravações foram obtidas com um gravador *Marantz* PMD-661 acoplado a um microfone *Sennheiser* ME67 com frequência de amostragem de 44,1 kHz e 16 bits de resolução. Durante a gravação, os lobos estavam em condição de cativeiro em dois diferentes zoológicos de São Paulo (Sorocaba e São Carlos). Foram gravados 32 aulidos do macho Tutti (TU) e 54 da fêmea Frutti (FR) do Zoológico de São Carlos (ZSC) e 146 aulidos do macho Fantasma (FA) e 111 da fêmea Nymeria (NY) do Zoológico de Sorocaba (ZSO), totalizando 343 notas. As distâncias em que as gravações foram feitas (entre 7 a 70 metros do emissor) variaram conforme o tamanho e a estrutura dos recintos de cada zoológico, e o comportamento dos lobos. Foram feitas entre 5PM-7AM, nos anos de 2015 e 2016.

2.2 Pré-processamento

A primeira etapa para o pré-processamento dos aulidos consistiu em uma seleção de notas atendendo a critérios de qualidade a partir da amostra inicial de 343 notas. Os arquivos foram abertos no programa *Audacity* versão 2.1.3 e foram selecionados segundo a qualidade de sua gravação por audição e por inspeção visual em espectrograma. Em seguida, foram recortados e gravados como novos arquivos (etapa de segmentação dos aulidos). A fim de preservar informações potencialmente importantes acerca do perfil de ruído e da reverberação do som, o recorte das notas de aulidos foi feito mantendo-se cerca de 1s antes e 1s depois da nota (Figura 1).

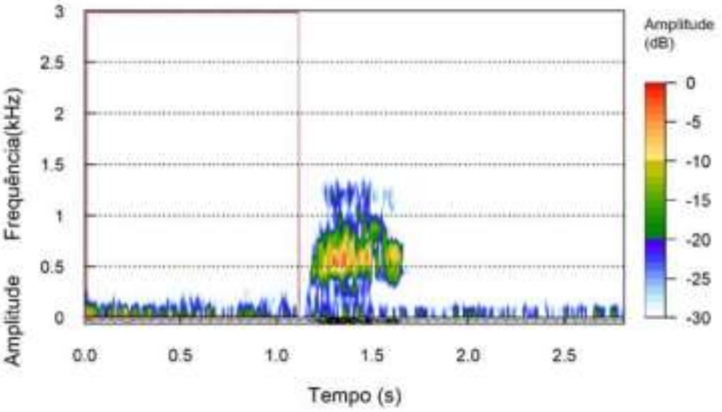

Figura 1. Espectrograma de um aulido do macho do zoológico de São Carlos antes do pré-processamento. A área vermelha demarca 1s preservado antes da nota durante a segmentação do arquivo de som.

Após selecionados e recortados os melhores aulidos, deu-se início ao processo de normalização e limpeza dos arquivos segmentados. Esta etapa era importante pois, no processo automatizado de reconhecimento, é necessária a determinação de parâmetros acústicos das vocalizações em meio a uma complexa paisagem sonora. Para essa remoção de ruídos foram utilizadas ferramentas de processamento do programa *AudioSculpt* do IRCAM (http://anasynth.ircam.fr/home/english/software/audiosculpt) disponível no Laboratório de Acústica e Tecnologia Musical (LATM), que tem a licença de uso. As seguintes etapas foram adotadas para todos os arquivos segmentados:

1) Normalização do som a 3dB, utilizando a função "*normalize*", seguida de inspeção quanto à presença de artefatos da gravação ou gerados após a normalização;

2) Identificação de ruído indesejado no segundo anterior à nota. Zonas de ruído foram demarcadas manualmente para aquisição de seu perfil, na parte inicial do arquivo, antes da nota de aulido, auxiliado por espectrogramas FFT utilizando uma janela de *Hanning* com largura de 4096 amostras;

3) Remoção de ruído identificado. Na função "*noise removal*", usou-se o modo "*energy subtraction*" para retirar o ruído até uma profundidade de 45 dB. Após a remoção do ruído, fez-se nova inspeção da nota, a partir de um espectrograma utilizando os mesmos parâmetros do item 2), em busca de alterações indesejadas na nota de aulido;

4) Identificação de ruído indesejado no segundo posterior à nota. Após a etapa 3, uma nova verificação de presença de ruído indesejado no arquivo foi feita. Como o ruído da parte inicial do arquivo, antecedente à nota, podia não estar totalmente presente no segundo

posterior à nota, foi necessário, em muitos casos, remover o ruído restante do arquivo, com perfil diferente do ruído anterior à nota. Fez-se isso repetindo-se as etapas 2 e 3, agora com as demarcações e aquisições de perfis de ruído feitas após a nota;

5) Verificação final do arquivo e da preservação da nota. Ao final da remoção do ruído, verificava-se novamente a qualidade do som. Foram descartados os aulidos que tivessem perdido componentes importantes de frequência. Esta perda de componentes foi avaliada auditivamente (com uso de monitores de estúdio profissionais) e a partir da comparação manual supervisionada do espectrograma gerado nesta etapa (arquivo limpo).

3. RESULTADOS E DISCUSSÃO

A partir da amostra inicial de 343 aulidos de 4 lobos, obtidos da FOCA, foram eliminados 45% dos aulidos seguindo os critérios de seleção descritos na seção de metodologia. Foram selecionadas para o pré-processamento 188 vocalizações (Figura 2). Destas, 130 foram processadas com sucesso e consideradas satisfatórias, com uma taxa de aproveitamento de 70%. Ao final do processo, 63 vocalizações foram selecionadas para as análises (de caracterização) posteriores.

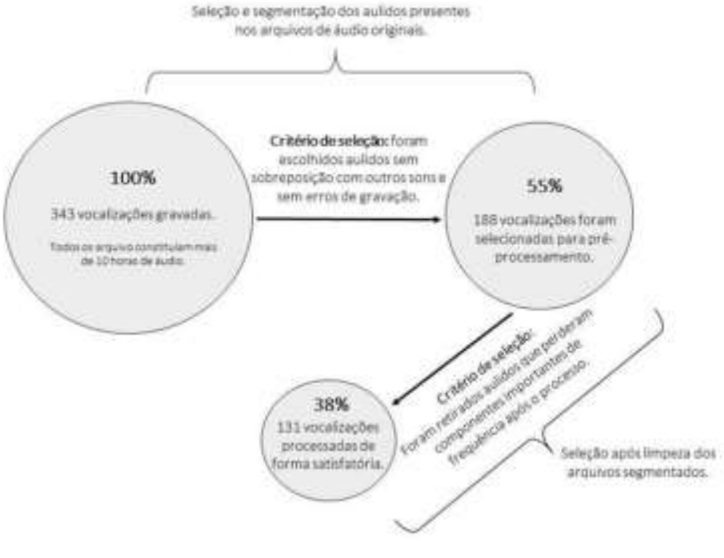

Figura 2. Esquema da quantidade de aulidos selecionados a cada etapa do processo de limpeza dos áudios.

Na Tabela 1 estão listadas as amostras iniciais (obtidas do acervo FOCA) e restantes (após o pré-processamento para análise posterior) de notas por indivíduo. Cerca de 50% das notas de três dos indivíduos (de 47 a 56%) resistiram ao pré-processamento, isto é, permaneceram preservadas após as etapas de normalização e filtragem. As notas do indivíduo Fantasma foram pouco aproveitadas, devido a fatores como ruído externo mais energético nos arquivos. Da sua amostra inicial de 146 notas, a maior dentre os 4 animais, apenas 35 (24%) foram selecionadas como notas com qualidade suficiente, após o pré-processamento, para constituir a seleção final de amostras para a etapa subsequente de análise e caracterização individual (meta deste projeto de pesquisa voltado à modelagem de aulidos para detecção automática).

Indivíduo	Zoológico	Gravados	Selecionados para pré-processamento	Pré-processados com sucesso	Selecionados para análises
Fêmea (Tutti)	São Carlos	54 (100%)	35 (65%)	26 (48%)	12 (22%)
Macho (Frutti)	São Carlos	32 (100%)	22 (69%)	18 (56%)	8 (25%)
Fêmea (Nymeria)	Sorocaba	111 (100%)	88 (79%)	52 (47%)	28 (25%)
Macho (Fantasma)	Sorocaba	146 (100%)	43 (29%)	35 (24%)	15 (10%)
Total		343 (100%)	188 (55%)	131 (38%)	63 (18%)

Tabela 1: Quantidade de aulidos originais gravados, quantidade efetivamente processada e selecionada para análises posteriores, para cada um dos indivíduos de lobo-guará.

Avaliando-se os sinais processados em comparação aos sinais originais, percebe-se sensivelmente uma diferença na relação sinal/ruído e na preservação do conteúdo espectral das notas (Figura 3). Verificou-se que a retirada dos componentes que não eram os sons de interesse na paisagem sonora (i.e., os ruídos e artefatos) favoreceu leituras mais claras e precisas de parâmetros acústicos das vocalizações, sem perdas ou distorções significativas. Considera-se que esta etapa de pré-processamento seja fundamental para análises posteriores usando-se técnicas como *wavelets* e cepstrais, já que a presença de ruídos pode influenciar negativamente os resultados.

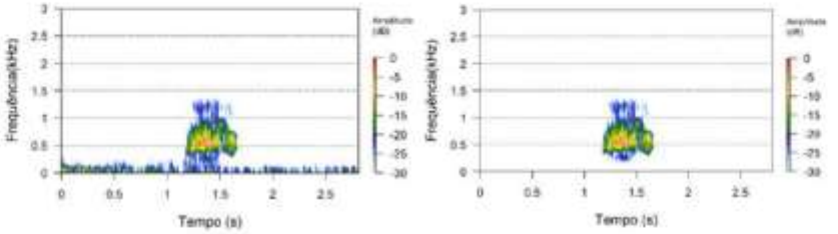

Figura 3. Espectrograma de um aulido do macho do zoológico de São Carlos antes do pré-processamento (à esquerda) e depois (à direita).

4. CONSIDERAÇÕES FINAIS

Neste trabalho foi explorada e verificada a eficácia de uma combinação de operações de pré-processamento de sinal para extrair o ruído de gravações acústicas de aulidos de indivíduos de lobo-guará, e foi produzido um conjunto de amostras sonoras de qualidade para as análises posteriores de caracterização da vocalização. A próxima etapa, nesse processo de criação de um algoritmo para detecção automatizada de aulidos em paisagens naturais, é a caracterização das assinaturas individuais usando diferentes ferramentas analíticas como FFT, *cepstrum* e *wavelets*.

5. AGRADECIMENTOS

Os autores agradecem à Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Processo no. 2017/12147-7) pela bolsa concedida de Iniciação Científica e à autora das gravações, MSc. Flora Balieiro, do acervo da Fonoteca César Ades (FOCA).

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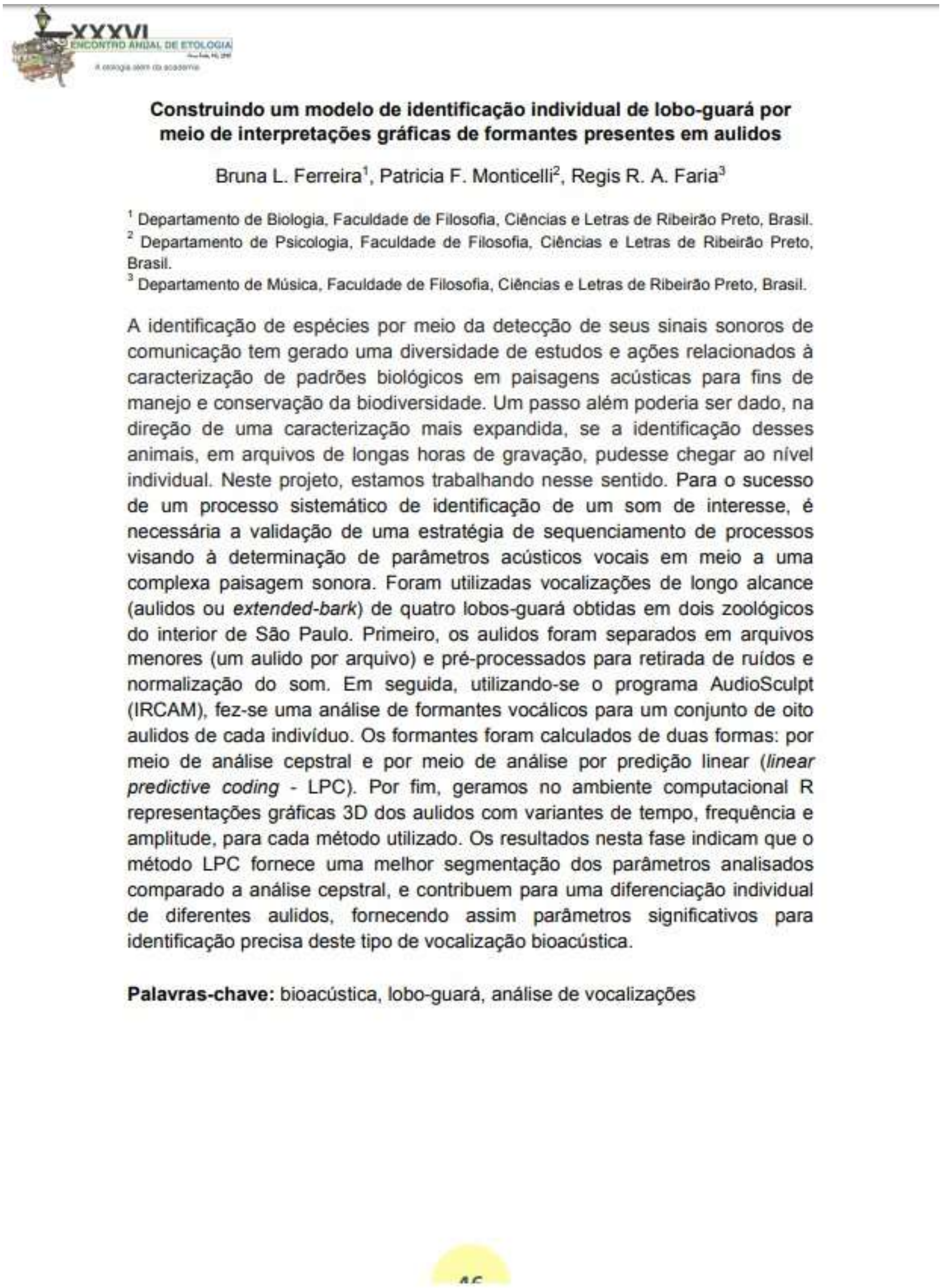
Apêndice E _. Construindo *um modelo de identificação individual de lobo-guará*



XXXVI
ENCONTRO ANUAL DE ETOLOGIA
A etologia além da academia

Construindo um modelo de identificação individual de lobo-guará por meio de interpretações gráficas de formantes presentes em aulidos

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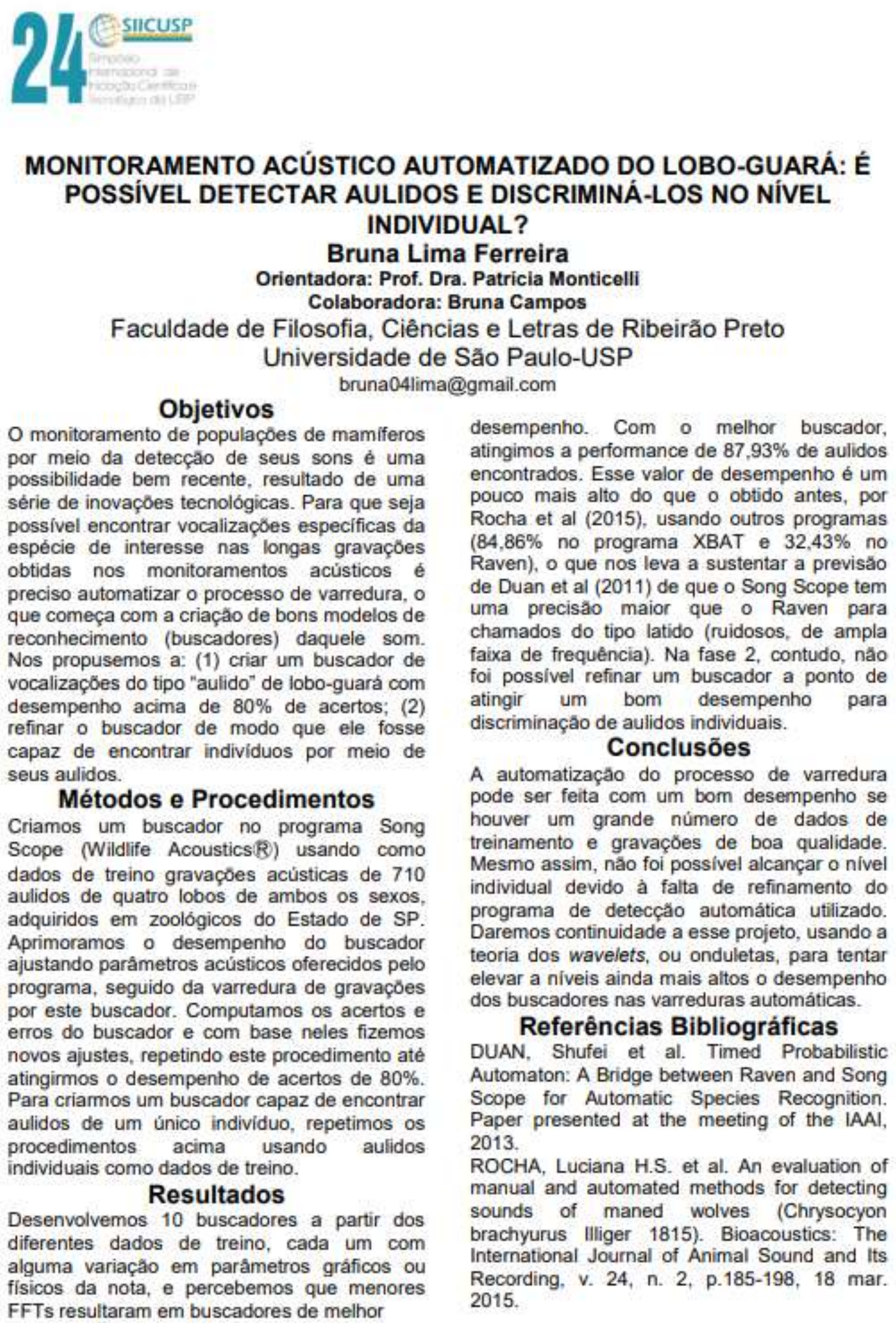
A identificação de espécies por meio da detecção de seus sinais sonoros de comunicação tem gerado uma diversidade de estudos e ações relacionados à caracterização de padrões biológicos em paisagens acústicas para fins de manejo e conservação da biodiversidade. Um passo além poderia ser dado, na direção de uma caracterização mais expandida, se a identificação desses animais, em arquivos de longas horas de gravação, pudesse chegar ao nível individual. Neste projeto, estamos trabalhando nesse sentido. Para o sucesso de um processo sistemático de identificação de um som de interesse, é necessária a validação de uma estratégia de sequenciamento de processos visando à determinação de parâmetros acústicos vocais em meio a uma complexa paisagem sonora. Foram utilizadas vocalizações de longo alcance (aulidos ou *extended-bark*) de quatro lobos-guará obtidas em dois zoológicos do interior de São Paulo. Primeiro, os aulidos foram separados em arquivos menores (um aulido por arquivo) e pré-processados para retirada de ruídos e normalização do som. Em seguida, utilizando-se o programa AudioSculpt (IRCAM), fez-se uma análise de formantes vocálicos para um conjunto de oito aulidos de cada indivíduo. Os formantes foram calculados de duas formas: por meio de análise cepstral e por meio de análise por predição linear (*linear predictive coding* - LPC). Por fim, geramos no ambiente computacional R representações gráficas 3D dos aulidos com variantes de tempo, frequência e amplitude, para cada método utilizado. Os resultados nesta fase indicam que o método LPC fornece uma melhor segmentação dos parâmetros analisados comparado a análise cepstral, e contribuem para uma diferenciação individual de diferentes aulidos, fornecendo assim parâmetros significativos para identificação precisa deste tipo de vocalização bioacústica.

Palavras-chave: bioacústica, lobo-guará, análise de vocalizações

Apêndice F _ *Monitoramento acústico automatizado do lobo-guará: é possível detectar aulidos e discriminá-los no nível individual?*



24 SIICUSP

MONITORAMENTO ACÚSTICO AUTOMATIZADO DO LOBO-GUARÁ: É POSSÍVEL DETECTAR AULIDOS E DISCRIMINÁ-LOS NO NÍVEL INDIVIDUAL?

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Objetivos

O monitoramento de populações de mamíferos por meio da detecção de seus sons é uma possibilidade bem recente, resultado de uma série de inovações tecnológicas. Para que seja possível encontrar vocalizações específicas da espécie de interesse nas longas gravações obtidas nos monitoramentos acústicos é preciso automatizar o processo de varredura, o que começa com a criação de bons modelos de reconhecimento (buscadores) daquele som. Nos propusemos a: (1) criar um buscador de vocalizações do tipo "aulido" de lobo-guará com desempenho acima de 80% de acertos; (2) refinar o buscador de modo que ele fosse capaz de encontrar indivíduos por meio de seus aulidos.

Métodos e Procedimentos

Criamos um buscador no programa Song Scope (Wildlife Acoustics®) usando como dados de treino gravações acústicas de 710 aulidos de quatro lobos de ambos os sexos, adquiridos em zoológicos do Estado de SP. Aprimoramos o desempenho do buscador ajustando parâmetros acústicos oferecidos pelo programa, seguido da varredura de gravações por este buscador. Computamos os acertos e erros do buscador e com base neles fizemos novos ajustes, repetindo este procedimento até atingirmos o desempenho de acertos de 80%. Para criarmos um buscador capaz de encontrar aulidos de um único indivíduo, repetimos os procedimentos acima usando aulidos individuais como dados de treino.

Resultados

Desenvolvemos 10 buscadores a partir dos diferentes dados de treino, cada um com alguma variação em parâmetros gráficos ou físicos da nota, e percebemos que menores FFTs resultaram em buscadores de melhor desempenho. Com o melhor buscador, atingimos a performance de 87,93% de aulidos encontrados. Esse valor de desempenho é um pouco mais alto do que o obtido antes, por Rocha et al (2015), usando outros programas (84,86% no programa XBAT e 32,43% no Raven), o que nos leva a sustentar a previsão de Duan et al (2011) de que o Song Scope tem uma precisão maior que o Raven para chamados do tipo latido (ruidosos, de ampla faixa de frequência). Na fase 2, contudo, não foi possível refinar um buscador a ponto de atingir um bom desempenho para discriminação de aulidos individuais.

Conclusões

A automatização do processo de varredura pode ser feita com um bom desempenho se houver um grande número de dados de treinamento e gravações de boa qualidade. Mesmo assim, não foi possível alcançar o nível individual devido à falta de refinamento do programa de detecção automática utilizado. Daremos continuidade a esse projeto, usando a teoria dos *wavelets*, ou onduletas, para tentar elevar a níveis ainda mais altos o desempenho dos buscadores nas varreduras automáticas.

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