SHEINA KOFFLER

Avaliação da biologia reprodutiva, genética quantitativa e consequências do manejo a longo prazo em abelhas sem ferrão para subsidiar programas de melhoramento genético

Assessing stingless bee reproductive biology, quantitative genetics, and the consequences of long-term management to support breeding initiatives

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**São Paulo
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Peixoto Kleinert

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Abstract

Meliponiculture (or stingless beekeeping) is a powerful tool for sustainable economic development in the tropics. However, meliponiculture has many challenges as it lacks standardization in management and breeding practices. The aim of this thesis was to investigate stingless bee reproductive biology combined to management and breeding as background to meliponiculture improvement. In chapter 1, we performed a meta-analysis of heritability across the Hymenoptera in order to review current knowledge and discuss the challenges for bee breeding and conservation. In chapter 2, we assessed how sexual selection acts on male traits in the stingless bee *Scaptotrigona* aff. *depilis* and identified which traits may affect male fitness. In chapter 3, we estimated heritability and genetic correlations for the traits studied in the previous chapter, to understand how evolution shapes male traits and to identify important traits for breeding programs. Finally, in chapter 4 we investigated how the environment and long-term management influence colony productivity in *Melipona subnitida* (jandaíra), a commercially managed species in Northeastern Brazil. Our results revealed that morphological traits exhibit higher heritability estimates than fitness related traits, and in general, colony productivity traits showed potential for breeding. However, few studies are available for stingless bees yet. Male aggregations in *S. aff. depilis* selected competitive males with higher quality sperm, indicating the importance of this mechanism in stingless bee mating biology. The studied male traits exhibited high heritability estimates, with exception of sperm length. Since aggregations selected males with shorter sperm, these results suggest selection for long-term sperm storage by queens and higher fertilization potential. The assessment of *M. subnitida* records revealed that honey production was affected by climate and management, and strategies to improve beekeeping practices were discussed. We believe this thesis provides important guidelines to establish successful stingless bee breeding programs.

Resumo

A meliponicultura, criação racional de abelhas sem ferrão, é uma poderosa ferramenta para o desenvolvimento econômico sustentável em regiões tropicais. No entanto, a prática da meliponicultura enfrenta diversos desafios, como a falta de padronização das técnicas de manejo e melhoramento genético. O objetivo desta tese foi investigar a biologia reprodutiva de abelhas sem ferrão aliada ao manejo e ao melhoramento genético, a fim de fornecer subsídios para o aprimoramento da meliponicultura. No capítulo 1, realizamos uma meta-análise sobre herdabilidade em Hymenoptera, a fim de compreender o conhecimento atual e os desafios associados ao melhoramento genético de abelhas e sua conservação. No capítulo 2, avaliamos como a seleção sexual atua em caracteres dos machos da espécie *Scaptotrigona* aff. *depilis* e identificamos quais caracteres podem influenciar o sucesso reprodutivo. No capítulo 3, estimamos a herdabilidade e correlações genéticas para os caracteres avaliados no capítulo anterior, a fim de entender como a evolução atua moldando os caracteres dos machos e quais desses caracteres podem ser utilizados em programas de melhoramento genético. Finalmente, no capítulo 4, investigamos o efeito do ambiente e do manejo na produtividade de colônias em *Melipona subnitida* (jandaíra), uma espécie manejada comercialmente no Nordeste brasileiro. Nossos resultados revelaram que caracteres morfológicos exibem estimativas de herdabilidade mais altas do que caracteres ligados ao sucesso reprodutivo. No entanto, poucos estudos com abelhas sem ferrão foram realizados até o momento. Agregações de machos em *S. aff. depilis* selecionaram machos mais competitivos que apresentaram maior qualidade espermática, indicando a importância desse mecanismo na biologia reprodutiva de abelhas sem ferrão. Os caracteres estudados apresentaram alta herdabilidade, com exceção do comprimento do espermatozoide. Como agregações selecionam machos com espermatozoides mais curtos, esses resultados sugerem seleção direcionada para um maior tempo de armazenamento do esperma pelas rainhas

e maior potencial de fertilização. A avaliação dos registros de *M. subnitida* revelou que a produção de mel foi afetada pelo clima e pelo manejo, e estratégias a fim de melhorar as práticas da meliponicultura foram discutidas. Acreditamos que essa tese fornece importantes resultados para o estabelecimento de programas de melhoramento genético em abelhas sem ferrão.

Introduction

Animal husbandry is an important activity for human well-being, which may be improved by species domestication and breeding. Apiculture, the maintenance of honeybee (*Apis* spp.) hives, dates back to the 9th century (Bloch et al. 2010), mainly for the exploitation of honey and wax (Crane 1990). Since then, many advances have been made regarding management techniques and breeding, which led to a worldwide expansion of apiculture. Beekeeping operations now may rely on selection of high quality colonies to improve production, instrumental insemination of queens, and long-distance hive transportation to reach good foraging patches (Büchler et al. 2013). Besides honey production, honeybees are important providers of pollination services, and the use of hives for crop pollination is essential to food supply (Aizen et al. 2009), consisting also in an important revenue for beekeepers.

The high demand for apiculture products has led to the introduction of honeybees in many non-native areas, which may negatively impact native species, through resource competition and disease spillover (Goulson 2003; Fürst et al. 2014). In addition, recent declines reported for bee populations related to distinct triggers as land use change, pesticide exposure, pathogens, invasion by alien species, and climate change (summarized in Potts et al. 2016) threaten beekeeping operations. This scenario reveals an increasing need to initiate a sustainable management of other social bees, focusing on native species, in order to avoid new species introductions, diversify supply, and improve local development. Bee domestication may be a long and difficult task, requiring the control of different steps of colony development. However, recent achievements in bumblebee domestication show the viability of this operation (Velthuis and van Doorn 2006). In the

tropics, stingless bees are a key candidate for commercial management, exhibiting high diversity and a growing beekeeping industry (Cortopassi-Laurino et al. 2006; Jaffé et al. 2015).

Stingless bees (Meliponini tribe) occur in the tropical and subtropical regions of the globe, comprising 500 described species (Silveira et al. 2002; Ascher and Pickering 2017). Due to its large distribution and high diversity, stingless beekeeping (also known as meliponiculture) may provide local options for beekeeping and honey production, without the necessity of recurring to introduced species. Meliponiculture may also contribute to increasing income in developing regions due to commercialization of hive products as honey, propolis (plant resin collected by bees), or even hives (Cortopassi-Laurino et al. 2006; Jaffé et al. 2015). Indeed, stingless bee honey is much more valuable than honeybee's, being also appreciated for its medicinal properties (Alves 2013; Maia et al. 2015). In addition, crop pollination by stingless bees was reviewed in many studies, revealing a high potential for employing different species in pollination (Heard 1999; Cortopassi-Laurino et al. 2006; Slaa et al. 2006; Giannini et al. 2014). Hence combining beekeeping, selling of hive products, and pollination may become an interesting alternative for economic sustainable use of stingless bees in the future. However, despite the high diversity and management interest, stingless bees are threatened by land use change, which reduce suitable habitat for nesting and foraging (Brown and Albrecht 2001; Villanueva-G et al. 2005). In addition, honey hunting is a common practice in many regions, in which nests are destroyed to achieve honey stores. Meliponiculture may thus act also as a conservation tool, preserving species and populations of stingless bees.

Many challenges still exist to the implementation of large-scale meliponiculture. Stingless beekeeping lacks standardized management practices and parameters for colony evaluation, which are well established in apiculture (Cortopassi-Laurino et al. 2006; Jaffé et al. 2015). In addition, to a large scale implementation, breeding and artificial selection of traits are needed, aiming to ensure colony health and fecundity, and increase production and

quality of hive products. These practical limitations are derived from knowledge gaps in basic stingless bee biology, which has received growing attention recently (Alves et al. 2011; Wenseleers et al. 2011; Francisco et al. 2014; Jaffé et al. 2014), but still constrain further management development. Studying bee biology and reproductive strategies thus contributes to improve management practices and elaborate stingless bee breeding programs. In this context, the aim of this thesis was to investigate stingless bee reproductive biology and how to implement and optimize stingless bee breeding programs. We decided to focus our reproduction studies on stingless bee males, since most studies have investigated colony life cycle and queen biology. In addition, bee breeding programs also rely on selecting colonies that produce high quality males or selecting males for instrumental insemination. However, which male traits are related to higher quality in stingless bees is not known. The thesis is divided in four chapters, three of which are published articles and one is a manuscript edited for submission (Fig. 1).

Chapter 1. Quantitative genetic studies as background for bee breeding and conservation

[article: Quantitative conservation genetics of wild and managed bees]

We performed a systematic review of quantitative genetic parameters (heritability estimates and genetic correlations, which are usually employed in breeding programs) in Hymenoptera (ants, bees, and wasps). Using a meta-analysis approach, we investigated which factors influenced heritability estimates. Our results allowed assessing the potential for bee breeding and identify available information for stingless bee breeding specifically. Since breeding may affect genetic diversity and evolutionary potential, implications for bee conservation were also discussed.

Chapter 2. Reproductive biology of stingless bees: identifying male quality traits and sexual selection episodes

[article: Competitive male have higher quality sperm in a monogamous social bee]

By performing a behavioral study in male aggregations during reproductive events, we assessed how sexual selection acts on stingless bee male traits, and identified which traits may affect male fitness. We investigated the relationship between pre and post-copulatory sexual selection, since selective pressures for both mating success (high male-male competition) and fertilization success (long-term sperm storage and fertilizations) were identified in this group. We used the stingless bee *Scaptotrigona* aff. *depilis*, which exhibits large and long-lived colonies, shows potential for crop pollination and honey production, and is resistant to manipulation.

Chapter 3. Quantitative genetic parameters of stingless bee male traits important for bee breeding

[article: When size matters: low heritability reveals selection for sperm length in a monogamous stingless bee (*Scaptotrigona* aff. *depilis*)]

We estimated the heritability and genetic correlations for *Scaptotrigona* aff. *depilis* male traits studied in the previous chapter, in order to assess how selection acts on male traits and identify which traits may be used in breeding programs. Even though we have estimated which traits were under sexual selection, the present approach is important to determine if those traits show a heritable basis. We employed a mixed model using Restricted Maximum Likelihood, which is more accurate than previous methods in quantitative genetics. The potential for breeding schemes using high quality males is discussed.

Chapter 4. Optimizing stingless bee breeding practices

[article: Temporal variation in honey production by the stingless bee *Melipona subnitida* (Hymenoptera: Apidae): long term management reveals its potential as a commercial species in Northeastern Brazil]

In order to assess how the environment and management practices affect stingless bee colony performance, we analyzed a long term record of colony productivity for a managed stingless bee in Northeastern Brazil. *Melipona subnitida* (Jandaíra) is an important species for meliponiculture in this region, especially for honey production, and this is the longest dataset available for a commercial stingless bee. Based in our results, we discussed different management strategies that can be adopted by a beekeeper, the future of meliponiculture in this region considering climate change, and potential traits for colony performance that can be used in breeding programs.

Figure 1. Thesis organization: four chapters aiming to investigate stingless bee reproductive biology and how management and breeding practices may improve stingless beekeeping.

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Quantitative conservation genetics of wild and managed bees

Sheina Koffler, Astrid de Matos Peixoto Kleinert & Rodolfo Jaffé

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Chapter 1.

Quantitative genetic studies as background for bee breeding and conservation

Resumo

Caracteres quantitativos informam sobre o potencial evolutivo das populações, sendo que as estimativas de herdabilidade medem a capacidade da população em responder a mudanças globais. Embora abelhas silvestres e manejadas estejam em crescente ameaça devido à degradação dos habitats naturais e mudanças climáticas, ameaçando também a biodiversidade de plantas e a produção agrícola, até o momento nenhum estudo revisou sistematicamente as estimativas de herdabilidade no grupo. Neste trabalho, contribuimos para preencher essa lacuna, reunindo as estimativas de herdabilidade disponíveis para abelhas, formigas e vespas, analisando quais fatores influenciam essas estimativas e avaliando as correlações genéticas entre os caracteres. Por meio da seleção de modelos analisando um conjunto de dados com mais de 800 estimativas, encontramos que a herdabilidade é influenciada pelo tipo do carácter. Caracteres morfológicos apresentaram as estimativas de herdabilidade mais altas, enquanto caracteres relacionados à defesa e metabolismo apresentaram as estimativas mais baixas. Sistema de estudo, grau de socialidade, desenho experimental, tipo de estimativa (sentido amplo ou restrito) e tamanho amostral não afetaram as estimativas de herdabilidade. Os resultados não foram alterados pela inclusão da inércia filogenética ou analisando as abelhas sociais separadamente. As correlações genéticas entre os caracteres de *Apis mellifera* revelaram tanto correlações positivas, geralmente para caracteres dentro da mesma categoria, como coeficientes negativos, sugerindo trade-offs entre os caracteres. Nós discutimos esses resultados e ressaltamos a importância da manutenção da variância genética nos caracteres associados ao sucesso reprodutivo. Nosso estudo revela a importância de se considerar estimativas de herdabilidade e correlações genéticas no planejamento de programas de melhoramento genético e conservação. Esperamos que essa meta-análise auxilie na identificação de práticas de seleção artificial sustentáveis e estratégias de conservação que garantam a manutenção do potencial evolutivo de abelhas silvestres e manejadas.

Abstract

Quantitative genetic traits provide insights into the evolutionary potential of populations, as heritability estimates measure the population's ability to respond to global changes. Although wild and managed bees are increasingly threatened by the degradation of natural habitats and climate change, risking plant biodiversity and agriculture production, no study has yet performed a systematic review of heritability estimates across the group. Here we help fill this knowledge gap, gathering all available heritability estimates for ants, bees, and wasps, evaluating which factors affect these estimates and assessing the reported genetic correlations between traits. Using a model selection approach to analyze a dataset of more than 800 heritability estimates, we found that heritability is influenced by trait type, with morphological traits exhibiting the highest heritability estimates, and defense and metabolism-related traits showing the lowest estimates. Study system, sociality degree, experimental design, estimation type (narrow or broad-sense heritability), and sample size were not found to affect heritability estimates. Results remained unaltered when correcting for phylogenetic inertia, and when analyzing social bees separately. Genetic correlations between honeybee traits revealed both positive coefficients, usually for traits in the same category, and negative coefficients, suggesting trade-offs among other traits. We discuss these findings and highlight the importance of maintaining genetic variance in fitness-related traits. Our study shows the importance of considering heritability estimates and genetic correlations when designing breeding and conservation programs. We hope this meta-analysis helps identify sustainable breeding approaches and conservation strategies that help safeguard the evolutionary potential of wild and managed bees.

Introduction

Wild and managed bees are increasingly threatened by the modification of natural habitats and climate change (Biesmeijer and Roberts 2006; Brown and Paxton 2009; Potts et al. 2010; Kennedy et al. 2013), which poses risks to agricultural production (Gallai et al. 2009) and the maintenance of plant biodiversity (Ollerton et al. 2011). The human-mediated modification of natural habitats can affect the long-term viability of bee populations, fragmenting them, reducing gene flow, and increasing Allee effects (Stephens and Sutherland 1999; Allendorf et al. 2012). For instance, urbanization and agricultural land use have been shown to restrict gene flow in some bee species (Davis et al. 2010; Jha and Kremen 2013; Jha 2015). Likewise, management practices can have a profound influence over genetic differentiation and the maintenance of valuable adaptations to local environmental conditions (De la Rúa et al. 2009; Byatt et al. 2015a). While most studies assessing the conservation status of bee populations have focused on quantifying bee abundance, species richness or genetic diversity, they have largely neglected quantitative traits. However, quantitative genetic traits provide a direct assessment of the evolutionary potential of populations, and thus their ability to respond to global change (Frankham et al. 2002; Visscher et al. 2008; Zayed 2009).

Quantitative traits are polygenic (determined by a group of genes) and exhibit complex inheritance patterns (Frankham et al. 2002). To measure trait inheritance, heritability estimates are calculated as the ratio of genetic variance to total phenotypic variance (Falconer and Mackay 1996). Heritability estimates thus represent a proportion, and range between 0 (indicating no genetic variance, or traits which are solely affected by

the environment) and 1 (for traits regulated exclusively by genetic variance, which are unaffected by the environment). While narrow sense heritability indicates the proportion of the phenotypic variance attributable to additive genetic variance, broad sense heritability is estimated as the ratio between genotypic variance (additive, dominance and epistasis effects) and total phenotypic variance. Traits with high heritability estimates provide a higher ability to respond to selection, as a large range of phenotypes will be available for selection to act upon. In contrast, traits showing low heritabilities have a more restricted response to selection.

Fitness-related traits generally exhibit low heritability estimates, because the fixation of advantageous traits reduces additive genetic variance (Frankham et al. 2002; Visscher et al. 2008). For example, life-history traits in 75 species from wild and outbred populations were found to show much lower heritabilities than morphological traits (Mousseau and Roff 1987). Such fitness-related traits are of primary conservation interest, because they indicate the ability of populations to respond to selection (Frankham et al. 2002). Additionally, fitness-related traits show higher dominance genetic variance, which increases the susceptibility of inbreeding (Frankham et al. 2002). Populations that face severe declines are under strong genetic drift, which reduces genetic diversity, increases inbreeding, and may eventually erode heritability of fitness-related traits. However, heritability estimates may not be directly related to genetic diversity, as traits can show a low (or high) genetic variance regardless of population-wide genetic diversity (Reed and Frankham 2001). Integrating a population's response to selection and traits heritability is thus essential to understand adaptive response in changing environments (Hendry et al. 2003).

A potential application of quantitative genetic studies for the conservation of wild bee populations is the estimation of heritability for disease resistance, as different species are currently threatened by the spillover of pathogens from managed populations (Sachman-Ruiz et al. 2015). Quantitative genetic variability is also the basis of breeding initiatives, which select and enhance traits of interest. In honeybees, for instance, quantitative genetic

studies can help breed for traits like resistance to *Varroa jacobsoni* (Harbo and Harris 1999), honey production, colony defensive behavior (Bienefeld et al. 2007). Using heritability estimates, Harbo and Harris (1999) identified four potential traits for breeding to increase mite resistance. Also relying on quantitative genetic approaches, Bienefeld et al. (2007) increased the selection response to honey production from 0.04% to 0.54% per year, and to colony defense behavior from 0.03 to 0.62%. On the other hand, body size of *Osmia lignaria* (a solitary bee used in commercial pollination) exhibited a low heritability, indicating that breeding to modify body size is unlikely to yield the desired results in this species (Tepedino et al. 1984). These examples illustrate how conservation and breeding efforts can benefit from quantitative genetic studies.

In the Hymenoptera (ants, bees, and wasps), quantitative genetic studies pose important challenges, due to the haplodiploid sex determination system and complex genetic architecture (Oxley and Oldroyd 2010). Early initiatives adapted existing methods and protocols available for diploid species (Rinderer 1977; Oldroyd and Moran 1983; Moritz 1985). More recent developments include protocols for narrow sense heritability estimations (Liu and Smith 2000) and the use of BLUP models (Best Linear Unbiased Predictors) for estimating breeding values (Bienefeld et al. 2007; Gupta et al. 2013). Although a body of studies have used quantitative genetic approaches for breeding purposes (Harbo 1992; Conte et al. 1994; Brascamp et al. 2016), no previous efforts have been made to systematically review heritability estimates across the group and assess their implication for bee conservation. In addition to heritability estimates, genetic correlations between traits are important to gain a more complete understanding of inheritance, as multiple traits respond simultaneously to selection.

Here we perform the first systematic review of heritability estimates across the Hymenoptera. Our main aim was to assess how natural and artificial selection operate across multiple traits, and how bee breeding practices could cause a reduction in evolutionary potential. We first evaluate how different factors influence heritability

estimates, and then compare estimates and genetic correlations among different traits across social bees. We predicted that heritability estimates would be affected by trait type, study system, sociality degree, experimental design, estimation type (narrow or broad-sense heritability), and sample size; and that similar traits would show positive correlations.

Materials and Methods

We performed a systematic review on available studies reporting heritability estimates across the Hymenoptera. Three online databases were accessed (Google Scholar, Scopus, and Web of Science), using different combinations of key words (heritability, quantitative genetics, social insects, bees, ants, wasps). Each study was checked for heritability estimates and a library of selected studies was prepared. Articles cited in the selected studies were also included in our dataset. Overall, we collected information from 90 studies (Supp. Table 1). For each study we retrieved: (a) study system (ant, bee or wasp); (b) species; (c) sociality degree (social or solitary); (d) experimental design (diallel cross, ratio between genetic and total phenotypic variance, breeder's equation, sibling analysis, and parent-offspring regression); (e) estimate type (narrow or broad-sense heritability); (f) number of families employed (number of colonies for social species or number of maternal lineages for solitary species); (g) number of individuals analyzed; (h) trait category (behavior, communication, defense, fertility, metabolism, morphology, and colony productivity); and (i) analyzed trait. Heritability estimates were classified as narrow-sense when the study design allowed additive genetic variance estimation, following the methods discussed in Liu and Smith (2000). In addition, we also considered as narrow-sense the estimates obtained by regression methods among generations, corrected by the relatedness coefficients; as well as realized heritability estimates, which are an indirect method of measuring additive genetic variance. The ratio between genetic and total phenotypic variance (henceforth G/T estimation) was always classified as broad-sense heritability since it does not allow differentiating additive variance from dominance or epistasis effects. When sample sizes

(number of families or individuals) were given in intervals, we conservatively considered the lower number. In order to reduce the number of trait types in our dataset, we assigned them to seven categories: Behavior, Communication, Defense, Fertility, Metabolism, Morphology, and Colony productivity (Table 1).

Negative heritability estimates and estimates above 1 were excluded from the analyses, since they reflect negative genetic variances or limitations related to inbreeding. In order to identify which factors influenced heritability estimates across the Hymenoptera, we ran linear mixed effects models with the arcsine-transformed heritability as response variable and species as a random factor. All factors which could potentially affect heritability estimates (study system, sociality degree, experimental design, estimate type, number of families, number of individuals, and trait category) were included as predictors. Because some of these factors contained missing data and small sample sizes in certain categories, we could not fit a full model containing all predictors. Instead, we ran simple models using complete cases datasets, containing a single predictor. Models with individual predictors were contrasted to null models (without the predictor), using likelihood ratios tests (LRT) with $\alpha = 0.05$. We then built a full model containing the predictors showing a significant influence on heritability estimates. This full model was compared to reduced models without each predictor (also employing LRT), until we reached the best model, where the exclusion of any predictor significantly decreased the model's log-likelihood. We also tested whether including a variance structure improved the model's likelihood or not, using a constant variance structure to allow different variances among categories. Models with a variance structure were compared to homoscedastic models using LRT. To account for phylogenetic dependence among species, we ran phylogenetic controlled regressions, using a tree constructed with the species in our dataset (Quicke and van Achterberg 1900; Downton and Austin 1994; Cameron et al. 2007; Hughes et al. 2008; Munro et al. 2011; Danforth et al. 2013) (Fig. S1, see also the supplementary R file with the tree). Phylogenetic relationships among corbiculate bees were retrieved from Danforth et al. (2013), multiple observations from the same species were included as polytomies, and branch lengths

Table 1 Classification of the analyzed traits.

Trait category	Trait type	Traits
behavior	learning (PER)	learning (proboscis extension reflex), resistance of the conditioned response to extinction
	number of matings	estimated and effective number of matings
communication	colony defense	colony defense, honey robbing
	reaction to alarm pheromone (time)	time to react to alarm pheromone (laboratory essays, field essays), time to first sting, time for clustering
	reaction to alarm pheromone (number of bees)	number of bees reacting to alarm pheromone (laboratory essays, field essays), number of stings in target
	reaction to alarm pheromone (oxygen consumption)	
	alarm pheromone production	production of isopentyl acetate, 1-hexanol, 2-heptyl acetate, octyl acetate, 2-nonyl acetate, 2-heptatone
	worker dominance	trophallactic dominance, 9-ODA in mandibular gland
	kin recognition	
defense	hygienic behavior	hygienic behavior after 24h, 48h, 72h, towards Varroa-infested brood cells
	mite population	final mite population, mites on adult bees, mites on brood cells
	mite reproduction	total mite reproduction rate, effective mite reproduction, suppression of mite reproduction
	grooming behavior	grooming behavior, physical damage to mites (total, dents in body, broken legs or bodies)
	immune response	immune gene expression, innate immune response

fertility	number of ovarioles	
	ovary development	
	oviposition	number of eggs laid, days of oviposition
metabolism	worker development	total development, duration of uncapped stage, duration of postcapping stage
	queen development	duration of queen postcapping stage
	drone maturation rate	age at the onset of flight, initial locomotor activity (light and dark)
	worker longevity	
	cold resistance	
	oxygen consumption	
morphology	worker pupal weight	
	male size	
	color pattern	worker, drone and queen color patterns
	proboscis length	
	forewing size	forewing length and width, wing venation (L1, L2, cubital A, cubital B)
	forewing angles	forewing angles (29, 30, 31, 32, 33, 34, 35, 36, 38, 39)
	cubital index	
	hindwing size	hindwing length, hindwing width, hindwing area
	number of hamuli	
	wax mirror morphology	wax mirror length, wax mirror width, distance between wax mirrors
	third sternum length	
	hind leg morphology	leg area and length, tibia length, femur area and length, basitarsis length and width, metatarsus length and width, length of corbicula hairs

Trait category	Trait type	Traits
...morphology	sting morphology	sting length, stylet length, bulb length, maximum width of bulb, lancet length, height of the valve of right lancet, number of tooth barb of sting
	sperm length	individual sperm length, mean sperm length
colony productivity	colony development	colony weight, weight gain, spring development (score)
	colony population	brood production, brood viability, number of bees covering combs
	honey production	honey production (annual or seasonal), number and dimensions of honey pots
	wax production	
	propolis production	
	royal jelly production	royal jelly production (per colony, per cup), larvae acceptance
	foraging success	hoarding behavior, syrup collection rate, pollen hoarding, number and dimensions of pollen pots, sunflower pollen collection, avocado foraging, allometry coefficient between glossa and wing lengths
	colony aggressiveness	aggressiveness or calmness during colony inspection (score)
	swarming	

were considered equal (length = 1). We then constructed generalized least squares models controlling for phylogenetic effects (Pagel 1999), using the arcsine-transformed heritability as response variable and the predictors contained in the best linear mixed models. Both linear mixed models and phylonegenetic controlled regressions were implemented using the full dataset and a data subset including social bees only. All analyses were performed in R, using the *lme4* (Bates et al. 2014) and *nlme* (Pinheiro et al. 2016) packages. The R packages *ape* (Paradis et al. 2004), *geiger* (Harmon et al. 2008), and *phytools* (Revell 2012) were used to construct and manipulate the phylogenetic tree. Plots were produced with the package *ggplot2* (Wickham 2009).

Genetic correlations between traits were also assessed and correlation coefficients for *Apis mellifera* traits were compiled and grouped in categories (Table 1), since this species exhibited most of the available information on genetic correlations (Supp. Table 2). As most studies did not present significance tests for genetic correlation coefficients, we included all coefficients in our dataset. Correlation coefficients below -1 and above 1 were discarded from the analysis, because they do not fit in the parameter space of coefficients of correlation (Wielewski et al. 2014) and may be related to problems in the covariance estimation.

Results

We gathered a total of 806 heritability estimates for 32 Hymenoptera species (Supp. Table 1). Ants were the worst represented group, with 7% of the estimates, followed by wasps (15%) and bees (78%). Solitary species were well represented in wasps (61% of the estimates); while in bees few estimates were found for solitary species ($n = 8$, *Osmia lignaria* and *Megachile rotundata*). Social bees included *Apis mellifera*, four bumblebee species (*Bombus huntii*, *B. occidentalis*, *B. rufocinctus*, and *B. terrestris*), and two stingless bees (*Melipona quadrifasciata* and *M. scutellaris*). Broad-sense estimates were far more common (78%) than narrow-sense estimates.

Fig. 1 Heritability estimates for seven trait categories for all Hymenoptera species. While median values are represented by the lines inside boxes, which span the first and third quartiles, sample sizes are provided in brackets.

Trait category was the best predictor of heritability (LRT: $\chi^2 = 100.22$, $p < 0.001$, Table 2), with morphological traits exhibiting the highest estimates and defense and metabolism-related traits showing the lowest estimates (Table 3, Fig. 1). None of the other tested predictors (study system, sociality, experimental design, estimate type, number of families, and number of individuals), nor a variance structure in the best model were found to improve the model's likelihood (LRT: 3.37, $p = 0.76$). Variance was similar for broad and narrow-sense estimates (the inclusion of a variance structure did not improve the model's log-likelihood of a model containing trait category and estimate type as predictors; LRT: 1.75, $p = 0.17$, Fig. S2). Results remained unaltered when controlling for phylogenetic effects (LRT: $\chi^2 = 86.12$, $p < 0.001$, Tables 2 and 3). Additionally, the same results were found when analyzing social bees only (not accounting for phylogenetic effects LRT: $\chi^2 = 99.41$, $p < 0.001$, accounting for phylogenetic effects LRT: $\chi^2 = 152.60$, $p < 0.001$, Tables 2 and 3, Fig. 2).

Table 2 Best models describing heritability. N is the number of observations of each model, while the p-values and degrees of freedom (d.f.) refer to the likelihood ratio tests (using a χ^2 test statistic), in which the full model was compared to a reduced model without trait category.

Response (Dataset)	N	Model	Predictor	χ^2	d.f.	P-Value
Heritability (Hymenoptera species)	620	LMM	trait category	100.22	6	< 0.001
Heritability (Hymenoptera species)	635	GLS controlling for phylogenetic effects ^a	trait category	86.12	6	< 0.001
Heritability (social bee species)	444	LMM	trait category	99.41	4	< 0.001
Heritability (social bee species)	476	GLS controlling for phylogenetic effects ^b	trait category	152.60	6	< 0.001

^a $\lambda = 0.34$; ^b $\lambda = -0.05$.

Trait type

Fig. 2 Heritability estimates for social bee traits. While median values are represented by the lines inside boxes, which span the first and third quartiles, trait categories are represented by different colors.

Genetic correlations between honeybee traits are shown in Figure 3. Traits related to the same trait category usually exhibited positive genetic correlations, with the exception of communication traits. In general, morphological traits were positively correlated. Although sting pheromones were positively correlated, there was a negative association between sting pheromones and mandibular gland pheromones. Honey production and hoarding behavior were negatively correlated to hygienic behavior and mite presence. In addition, honey production also exhibited negative genetic correlations with colony aggressiveness. Positive genetic correlations were found between colony aggressiveness and swarming, hygienic behavior and propolis production, mite presence and propolis production, honey production and propolis production, defense and comb cell size, and defense and longevity.

Table 3 Parameter estimates for the best models describing heritability. Estimates for each trait type are given with morphological traits as comparison base (the negative estimates values indicate that morphological traits exhibited the highest estimates). Trait types with low sample sizes ($n < 8$) were excluded from the analyses. SE is the standard error, d.f. the degrees of freedom, and P-values were computed using the Satterthwaite approximation (*lmerTest* package).

Response (Dataset)	Model	Predictor (Trait category)	Estimate	SE	d.f.	P-Value
Heritability (Hymenoptera species)	LMM	behavior	-0.079	0.054	601.9	0.14
		communication	-0.008	0.031	611.2	0.79
		defense	-0.195	0.028	612.1	< 0.001
		fertility	-0.110	0.036	522	0.002
		metabolism	-0.223	0.037	599.4	< 0.001
		productivity	-0.152	0.019	609.7	< 0.001
Heritability (Hymenoptera species)	GLS controlling for phy- logenetic effects	behavior	-0.093	0.053	-	0.08
		communication	-0.012	0.030	-	0.70
		defense	-0.165	0.027	-	< 0.001
		fertility	-0.093	0.035	-	0.008
		metabolism	-0.217	0.037	-	< 0.001
		productivity	-0.136	0.019	-	< 0.001
Heritability (social bee species)	LMM	communication	-0.010	0.034	439	0.76
		defense	-0.219	0.029	439	< 0.001
		metabolism	-0.223	0.045	439	< 0.001
		productivity	-0.154	0.020	439	< 0.001
Heritability (social bee species)	GLS controlling for phy- logenetic effects	behavior	-0.141	0.063	-	0.03
		communication	-0.009	0.035	-	0.80
		defense	-0.172	0.034	-	< 0.001
		fertility	-0.150	0.043	-	< 0.001
		metabolism	-0.202	0.039	-	< 0.001
		productivity	-0.124	0.027	-	< 0.001

Fig. 3 Coefficients of genetic correlations between *Apis mellifera* traits. The dashed line shows the null coefficient, so negative correlations are exhibited left, and the positive correlations right. Median values are represented by the lines inside the boxes, which span the first and third quartiles.

Discussion

Heritability was best explained by trait category, with morphological traits showing the highest heritability estimates and defense and metabolism-related traits showing the lowest. Our collection of available heritability estimates for Hymenoptera species revealed that social bees, and particularly honeybees, are the best represented group. Additionally, genetic correlations between honeybee traits revealed both positive coefficients, usually for similar traits, and negative coefficients.

Even though the main Hymenoptera groups (ants, bees and wasps) were represented in our dataset, there was a strong bias towards honeybee studies. This was expected since honeybees are key managed pollinators and an important model system for genetic studies (Oxley and Oldroyd 2010; Evans et al. 2013). However, we highlight the underrepresentation of other social and solitary bees, which are also important providers of pollinator services (Nunes-Silva et al. 2013; Giannini et al. 2014) and may be experiencing population declines (Brown and Albrecht 2001; Kennedy et al. 2013). In addition, the commercial use of stingless bees has been highlighted as a key tool to achieve sustainable rural development, although management practices are still less developed than in apiculture (Jaffé et al. 2015; Koffler et al. 2015). The lack of quantitative genetic studies in bees other than honeybees thus hinders breeding and conservation efforts.

Estimate type did not improve our model's likelihood, indicating that studies calculating narrow-sense heritability obtained similar estimates to those computing broad-sense estimates. Narrow-sense heritability estimates were less represented in our dataset, perhaps because their estimation requires a more complex experimental design. Narrow-sense estimates usually involve more than one generation (realized heritability in breeder's equation, parent-offspring regression and diallel crosses), thus requiring longer studies. Since broad-sense estimates are based on total genetic variance, they are expected to be more variable than narrow-sense estimates, which only include additive genetic variance

(Falconer and Mackay 1996; Bennett and Hoffmann 1998; Hoffman et al. 2008; Kovacs et al. 2009; Stürup et al. 2011). However, our results show that variance was similar for broad and narrow-sense estimates.

Our dataset shows there are numerous methods employed to estimate heritability, still a subject of debate (Fjerdingstad 2005). Although experimental design was not detected as a factor influencing heritability estimates, a separate analysis of the best represented trait in our dataset (honey production in honeybees) revealed that GT estimates were higher than those obtained through parent-offspring regression. These results thus indicate that estimation method can influence heritability, even though other confounding factors and low sample sizes might have masked its effects when analyzing our full dataset. Our study thus highlights the need to standardize methodological approaches to estimate quantitative genetic parameters in bees (Liu and Smith 2000; Bienefeld et al. 2007). A recent review of heritability estimates in wild vertebrates showed that estimates obtained using the animal model were lower and more precise than other methods (sib-analyses, parent-offspring and grandparent-offspring regressions), suggesting that future studies should embrace this new approach, which integrates different relatedness degrees and common environment dependence (Postma 2014).

We found a large variation in heritability estimates within traits and trait categories (Figs. 1 and 2), although variance did not vary among categories. Such variation could be related to temporal or environmental changes, since heritability values estimated in the same population at different times may vary (Fjerdingstad 2005; Kovacs et al. 2009). Higher heritability estimates are usually found under more favorable conditions (Charmantier and Garant 2005). In addition, because heritability is a population measure (Visscher et al. 2008), the investigation of different populations of the same species may provide different heritability estimates. Population-level variation in heritability suggests that each population comprises a different genetic repository worthwhile preserving (Collins et al. 1984; Oldroyd et al. 1991).

Trait category was the best predictor for heritability across the Hymenoptera as well as within social bees. Many social bee traits exhibited medium to high heritability estimates, indicating large potential for breeding and genetic improvement. Morphological traits showed the highest heritability estimates, a result expected for traits not directly related to fitness (Falconer and Mackay 1996; Frankham et al. 2002; Visscher et al. 2008; Postma 2014). Colony productivity indicators (such as honey production, colony population, and foraging success) showed a high heritability, and thus a great potential for genetic improvement. Defense and metabolism traits showed the lowest heritability estimates, which suggest their close relation with fitness. Our work thus suggests that breeding programs focused on traits directly related to disease resistance (as hygienic behavior) may only lead to a modest increase in trait expression.

Breeding initiatives in social bees should also consider genetic correlations between traits, since they can enhance or impair breeding outcomes (Oxley and Oldroyd 2010). In honeybees, similar traits usually showed positive genetic correlations, suggesting the same mechanism of control. Communication traits such as response to alarm pheromones, however, presented negative correlations, indicating trade-offs. The positive genetic correlations observed reveal that breeding may simultaneously decrease colony aggressiveness and swarming behavior, increase hygienic behavior and propolis production, increase honey production and propolis production, and increase communication and longevity. Breeding attempts focused on these traits are thus expected to generate healthier and more productive colonies, although they could quickly deplete genetic variation of low heritability traits (see discussion below). On the other hand, negative genetic correlations may indicate trade-offs and selection acting on opposite directions, so that breeding efforts may not lead to colonies simultaneously expressing all desirable traits. For example, breeding for increase hygienic behavior would reduce honey production. In addition, negative genetic correlations were found between worker and queen effects in traits such wax and honey production (Bienefeld and Pirchner 1990); hygienic behavior (Costa-Maia et al. 2011); and honey yield and colony behavior (Brascamp et al. 2016). By targeting such

negatively-correlated traits, breeding efforts could also help maintain genetic variance in the population, as breeding optima are likely to differ for each trait. Considering both heritability and genetic correlations may thus provide valuable information for breeding programs, regarding the effectiveness of genetic improvement efforts, and the conservation of genetic variance in the traits of interest. In addition, the identification of quantitative trait loci (QTLs) and recent genomic approaches may confirm genetic linkages between traits, measure the strength of the association, and identify epistasis effects among genes, providing a more accurate pattern of multiple trait inheritance (Jensen et al. 2014).

Even though breeding has been highlighted as a long-term sustainable solution for the recent bee conservation challenges (Niño and Cameron Jasper 2015), its possible negative effects must also be considered, which include the loss of genetic variance, an increase in the level of inbreeding, outbreeding depression, introgression, and the loss of local adaptations to environmental conditions (Meixner et al. 2010; Oxley and Oldroyd 2010; Allendorf et al. 2012; Büchler et al. 2014; Niño and Cameron Jasper 2015). It is thus important to promote best practices for breeding programs (Byatt et al. 2015b; Niño and Cameron Jasper 2015) if additive genetic variance is to be maintained in both wild and managed populations. Our work highlights the need to monitor additive genetic variance in fitness-related traits, a practice that is yet uncommon and not sufficiently emphasized by current breeding initiatives (Meixner et al. 2010; Oxley and Oldroyd 2010; Niño and Cameron Jasper 2015). Future conservation programs could also incorporate quantitative genetic information, for example when choosing traits of special interest for reintroduction in areas where genetic variance has already been depleted. The protection of wild populations containing a large genetic variance for specific traits of interest may also help safeguard genetic stocks for future breeding or conservation efforts (Frankham et al. 2002; Zayed 2009).

In places with large wild or feral honeybee populations (like Africa, South America and Australia), breeding efforts are unlikely to deplete genetic variation in wild populations.

However, in places like Europe, where wild honeybee populations have nearly gone extinct (Jaffé et al. 2010), breeding could have a significant influence over the long-term maintenance of genetic variation for desirable traits. Likewise, long-term breeding efforts relying on closed-population systems, instrumental queen insemination, and few sources of queen lineages, which favors inbreeding (Chapman et al. 2008; Meixner et al. 2010; Niño and Cameron Jasper 2015), need to be especially careful to avoid the depletion of additive genetic variance of important fitness-related traits. We posit that some of the reported bee declines (Goulson et al. 2015) could be related to low genetic variance for fitness-related traits, and thus highlight the need for future studies specifically addressing this issue.

Our study summarizes all available heritability estimates for Hymenoptera species, providing a baseline for breeding and conservation studies, and identifying knowledge gaps and research needs. Quantitative genetic studies may contribute significantly towards the conservation of wild and managed bees, as our study shows the importance of considering heritability estimates and genetic correlations when designing breeding and conservation programs. Finally we emphasize the importance of extending quantitative genetic studies beyond honeybees, to address other ecologically and economically important but yet understudied bees. We hope this meta-analysis helps identify sustainable breeding approaches and conservation strategies that help safeguard the evolutionary potential of wild and managed bees.

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Supplementary Material – Chapter 1

Table S1. Dataset of heritability estimates across Hymenoptera, with information on the studied system, species, sociality, experimental design, number of families, number of individuals, trait category, trait, heritability estimate and reference. We only include heritability estimates ranging between 0 and 1 (see footnote^a for the list of excluded studies reporting negative estimates or estimates above 1, as well as the full csv dataset).

Study system	Species ^b	Sociality	Experimental design ^c	Estimate type ^d	Families	Individuals	Trait category	Trait	h^2	Reference
bee	Am	social	-	-	-	-	productivity	colony weight	0.57	[1]
bee	Am	social	-	-	-	-	productivity	honey production spring	0.60	[1]
bee	Am	social	-	-	-	-	productivity	honey production	0.58	[1]
bee	Am	social	-	-	-	-	productivity	brood cells	0.76	[1]
bee	Am	social	-	-	-	-	productivity	brood cells before citrus bloom	0.33	[1]
bee	Am	social	PO	NS	562	-	productivity	honey production	0.54	[2]
bee	Am	social	PO	NS	499	-	productivity	brood cells	0.10	[2]
bee	Am	social	-	-	-	-	communication	time to react to alarm pheromone	0.68	[3]
bee	Am	social	SI	BS	39	764	morphology	number of hamuli	0.68	[4]
bee	Am	social	SI	BS	19	-	communication	time to react to alarm pheromone	0.30	[5]
bee	Am	social	SI	BS	19	-	metabolism	worker longevity	0.32	[5]
bee	Am	social	SI	BS	46	-	productivity	hoarding behavior	0.92	[6]
bee	Am	social	SI	BS	46	-	communication	lab reaction to alarm pheromone (nbees)	0.05	[6]
bee	Am	social	SI	BS	39	-	communication	reaction to alarm pheromone (nbees after 30s)	0.47	[6]
bee	Am	social	SI	BS	39	-	communication	reaction to alarm pheromone (nbees after 60s)	0.73	[6]
bee	Am	social	SI	BS	39	-	communication	reaction to alarm pheromone (nbees after 90s)	0.93	[6]
bee	Am	social	SI	BS	39	-	communication	time to react to alarm pheromone	0.31	[6]
bee	Am	social	SI	BS	39	-	communication	number of stings in target	0.66	[6]
bee	Am	social	SI	BS	50	-	productivity	hoarding behavior	0.66	[6]
bee	Am	social	SI	BS	50	-	communication	lab responsiveness to alarm pheromone (nbees)	0.12	[6]

bee	Am	social	SI	BS	50	-	communication	lab responsiveness to alarm pheromone (time)	0.31	[6]
bee	Am	social	SI	BS	24	-	communication	reaction to alarm pheromone (nbees before)	0.56	[6]
bee	Am	social	SI	BS	24	-	communication	reaction to alarm pheromone (nbees after 30s)	0.71	[6]
bee	Am	social	SI	BS	24	-	communication	reaction to alarm pheromone (nbees after 90s)	0.17	[6]
bee	Am	social	SI	BS	24	-	communication	time to react to alarm pheromone	0.59	[6]
bee	Am	social	SI	-	231	-	morphology	worker pupal weight	0.64	[7]
bee	Ol	solitary	PO	NS	12	-	morphology	body size	0.15	[8]
bee	Ol	solitary	SI	NS	17	-	morphology	male size	0.34	[8]
bee	Am	social	-	-	-	-	productivity	pollen hoarding	0.49	[9]
bee	Am	social	SI	-	26	-	metabolism	worker longevity	0.20	[10]
bee	Am	social	SI	BS	100	-	productivity	hoarding behavior	0.18	[11]
bee	Am	social	SI	BS	28	2776	metabolism	duration of postcapping stage	0.68	[12]
bee	Am	social	PO	BS	28	2776	metabolism	duration of postcapping stage	0.85	[12]
bee	Am	social	SI	BS	11	104	communication	trophallactic dominance	0.32	[13]
bee	Am	social	SI	BS	11	116	communication	9-ODA in mandibular gland	0.89	[13]
bee	Am	social	SI	BS	11	116	fertility	ovary development	0.27	[13]
bee	Am	social	SI	BS	11	116	fertility	number of eggs	0.29	[13]
bee	Am	social	SI	BS	11	116	fertility	days of oviposition	0.57	[13]
bee	Am	social	SI	NS	20	600	morphology	wing length L1	0.59	[14]
bee	Am	social	SI	NS	20	600	morphology	wing angle 32	0.26	[14]
bee	Am	social	SI	NS	20	600	morphology	wing angle 35	0.16	[14]
bee	Am	social	SI	NS	20	600	morphology	wing angle 38	0.40	[14]
bee	Am	social	SI	NS	20	600	morphology	wing angle 32	0.93	[14]
bee	Am	social	SI	NS	20	600	morphology	wing angle 33	0.98	[14]
bee	Am	social	SI	NS	20	600	morphology	wing angle 35	0.29	[14]
bee	Am	social	SI	NS	20	600	morphology	wing angle 38	0.62	[14]
bee	Am	social	PO	NS	20	-	morphology	forewing width	0.93	[14]
bee	Am	social	PO	NS	20	-	morphology	wing length L1	0.56	[14]
bee	Am	social	PO	NS	20	-	morphology	wing length L2	0.91	[14]
bee	Am	social	PO	NS	20	-	morphology	wing angle 31	0.85	[14]
bee	Am	social	PO	NS	20	-	morphology	wing angle 32	0.54	[14]
bee	Am	social	PO	NS	20	-	morphology	wing angle 33	0.75	[14]
bee	Am	social	PO	NS	20	-	morphology	wing angle 35	0.20	[14]
bee	Am	social	PO	NS	20	-	morphology	wing angle 38	0.47	[14]

bee	Am	social	SI	-	-	-	metabolism	cold resistance	0.37	[15]
bee	Am	social	PO	-	-	-	metabolism	cold resistance	0.13	[15]
bee	Am	social	RE	-	-	-	metabolism	cold resistance	0.13	[15]
bee	Am	social	SI	-	-	-	metabolism	oxygen consumption	0.41	[15]
bee	Am	social	PO	-	-	-	metabolism	oxygen consumption	0.15	[15]
bee	Am	social	RE	-	-	-	metabolism	oxygen consumption	0.2	[15]
bee	Am	social	SI	BS	5	-	communication	reaction to alarm pheromone (oxygen consumption)	0.98	[16]
bee	Am	social	SI	BS	10	-	communication	reaction to alarm pheromone (oxygen consumption)	0.96	[16]
bee	Am	social	SI	BS	108	1080	communication	alarm pheromone (isopentyl acetate)	0.50	[17]
bee	Am	social	SI	BS	108	1080	communication	alarm pheromone (1-hexanol)	0.89	[17]
bee	Am	social	SI	BS	108	1080	communication	alarm pheromone (2-heptyl acetate)	0.67	[17]
bee	Am	social	SI	BS	108	1080	communication	alarm pheromone (2-heptanol)	0.54	[17]
bee	Am	social	SI	BS	108	1080	communication	alarm pheromone (octyl acetate)	0.48	[17]
bee	Am	social	SI	BS	108	1080	communication	alarm pheromone (2-nonyl acetate)	0.96	[17]
bee	Am	social	SI	BS	108	1080	communication	alarm pheromone (2-heptanone)	0.59	[17]
bee	Am	social	PO	NS	42	-	communication	number of stings in target	0.38	[18]
bee	Am	social	PO	NS	42	-	communication	number of stings in target	0.32	[18]
bee	Am	social	SI	BS	42	-	communication	number of stings in target	0.81	[18]
bee	Am	social	DC	NS	36	-	productivity	honey production	0.42	[19]
bee	Am	social	DC	BS	36	-	productivity	honey production	0.63	[19]
bee	Am	social	PO	BS	25	1016	behavior	learning behavior (PER)	0.45	[20]
bee	Am	social	SI	BS	28	1129	behavior	learning behavior (PER)	0.54	[20]
bee	Am	social	RE	NS	14	-	behavior	learning behavior (PER)	0.39	[20]
bee	Am	social	PO	NS	16	582	behavior	learning behavior (PER)	0.43	[20]
bee	Br	social	PO	-	43	787	morphology	forewing length	0.20	[21]
bee	Br	social	PO	-	38	680	morphology	forewing length	0.47	[21]
bee	Am	social	PO	BS	5881	-	productivity	honey production	0.26	[22]
bee	Am	social	PO	BS	5881	-	productivity	wax production	0.39	[22]
bee	Am	social	PO	BS	5881	-	productivity	aggressiveness	0.41	[22]
bee	Am	social	PO	BS	5881	-	productivity	calmness during examination	0.91	[22]
bee	Am	social	PO	BS	5881	-	productivity	spring development	0.76	[22]
bee	Am	social	PO	BS	5881	-	productivity	honey production	0.15	[22]
bee	Am	social	PO	BS	5881	-	productivity	wax production	0.45	[22]
bee	Am	social	PO	BS	5881	-	productivity	aggressiveness	0.40	[22]

bee	Am	social	PO	BS	5881	-	productivity	calmness during examination	0.58	[22]
bee	Am	social	PO	BS	5881	-	productivity	spring development	0.46	[22]
bee	Am	social	-	-	-	-	metabolism	duration of postcapping stage	0.23	[23]
bee	Am	social	SI	NS	8	-	communication	kin recognition	0.39	[24]
wasp	Mr	solitary	PO	NS	-	-	fertility	percent of hosts parasitized gregariously	0.41	[25]
wasp	Mr	solitary	PO	NS	-	-	fertility	number of eggs	0.38	[25]
wasp	Mr	solitary	GT	BS	-	-	fertility	percent of hosts parasitized gregariously	0.68	[25]
wasp	Mr	solitary	GT	BS	-	-	fertility	number of eggs	0.66	[25]
bee	Am	social	SI	BS	47	470	morphology	number of hamuli	0.33	[26]
bee	Am	social	SI	BS	47	470	morphology	wing angle 29	0.50	[26]
bee	Am	social	SI	BS	47	470	morphology	wing angle 30	0.42	[26]
bee	Am	social	SI	BS	47	470	morphology	wing angle 31	0.45	[26]
bee	Am	social	SI	BS	47	470	morphology	wing angle 32	0.83	[26]
bee	Am	social	SI	BS	47	470	morphology	wing angle 33	0.63	[26]
bee	Am	social	SI	BS	47	470	morphology	wing angle 34	0.56	[26]
bee	Am	social	SI	BS	47	470	morphology	wing angle 35	0.46	[26]
bee	Am	social	SI	BS	47	470	morphology	wing angle 36	0.67	[26]
bee	Am	social	SI	BS	47	470	morphology	wing angle 38	0.73	[26]
bee	Am	social	SI	BS	47	470	morphology	wing angle 39	0.67	[26]
bee	Am	social	SI	BS	47	470	morphology	wing cubital vein B	0.78	[26]
bee	Am	social	SI	BS	47	470	morphology	wing cubital vein A	0.58	[26]
bee	Am	social	SI	BS	47	470	morphology	distance between wax mirrors	0.76	[26]
bee	Am	social	SI	BS	16	160	morphology	hindwing width	0.83	[26]
bee	Am	social	SI	BS	16	160	morphology	number of hamuli	0.29	[26]
bee	Am	social	SI	BS	16	160	morphology	wing angle 29	0.74	[26]
bee	Am	social	SI	BS	16	160	morphology	wing angle 30	0.91	[26]
bee	Am	social	SI	BS	16	160	morphology	wing angle 31	0.58	[26]
bee	Am	social	SI	BS	16	160	morphology	wing angle 32	0.76	[26]
bee	Am	social	SI	BS	16	160	morphology	wing angle 33	0.52	[26]
bee	Am	social	SI	BS	16	160	morphology	wing angle 34	0.31	[26]
bee	Am	social	SI	BS	16	160	morphology	wing angle 35	0.29	[26]
bee	Am	social	SI	BS	16	160	morphology	wing angle 36	0.67	[26]
bee	Am	social	SI	BS	16	160	morphology	wing angle 38	0.65	[26]
bee	Am	social	SI	BS	16	160	morphology	wing angle 39	0.45	[26]

bee	Am	social	SI	BS	16	160	morphology	wing cubital vein B	0.39	[26]
bee	Am	social	SI	BS	16	160	morphology	wing cubital vein A	0.50	[26]
bee	Am	social	SI	BS	16	160	morphology	tibia length	0.46	[26]
bee	Am	social	SI	BS	16	160	morphology	femur length	0.56	[26]
bee	Am	social	SI	BS	16	160	morphology	basitarsis length	0.37	[26]
bee	Am	social	SI	BS	16	160	morphology	third sternum length	0.69	[26]
bee	Am	social	SI	BS	16	160	morphology	wax mirror length	0.48	[26]
bee	Am	social	SI	BS	16	160	morphology	wax mirror width	0.22	[26]
bee	Am	social	SI	BS	16	160	morphology	distance between wax mirrors	0.62	[26]
bee	Am	social	SI	BS	25	250	morphology	number of hamuli	0.58	[26]
bee	Am	social	SI	BS	25	250	morphology	wing angle 29	0.93	[26]
bee	Am	social	SI	BS	25	250	morphology	wing angle 30	0.79	[26]
bee	Am	social	SI	BS	25	250	morphology	wing angle 31	0.68	[26]
bee	Am	social	SI	BS	25	250	morphology	wing angle 32	0.94	[26]
bee	Am	social	SI	BS	25	250	morphology	wing angle 33	0.68	[26]
bee	Am	social	SI	BS	25	250	morphology	wing angle 34	0.96	[26]
bee	Am	social	SI	BS	25	250	morphology	wing angle 35	0.60	[26]
bee	Am	social	SI	BS	25	250	morphology	wing angle 36	0.56	[26]
bee	Am	social	SI	BS	25	250	morphology	wing angle 38	0.66	[26]
bee	Am	social	SI	BS	25	250	morphology	wing angle 39	0.87	[26]
bee	Am	social	SI	BS	25	250	morphology	wing cubital vein B	0.81	[26]
bee	Am	social	SI	BS	25	250	morphology	wing cubital vein A	0.86	[26]
bee	Am	social	SI	BS	25	250	morphology	distance between wax mirrors	0.49	[26]
bee	Am	social	SI	BS	5	50	morphology	hindwing length	0.37	[26]
bee	Am	social	SI	BS	5	50	morphology	number of hamuli	0.63	[26]
bee	Am	social	SI	BS	5	50	morphology	wing angle 31	0.86	[26]
bee	Am	social	SI	BS	5	50	morphology	wing angle 32	0.92	[26]
bee	Am	social	SI	BS	5	50	morphology	wing angle 33	0.86	[26]
bee	Am	social	SI	BS	5	50	morphology	wing angle 34	0.86	[26]
bee	Am	social	SI	BS	5	50	morphology	wing angle 35	0.04	[26]
bee	Am	social	SI	BS	5	50	morphology	wing angle 36	0.66	[26]
bee	Am	social	SI	BS	5	50	morphology	wing angle 38	0.72	[26]
bee	Am	social	SI	BS	5	50	morphology	wing cubital vein B	0.71	[26]
bee	Am	social	SI	BS	5	50	morphology	wing cubital vein A	0.38	[26]

bee	Am	social	SI	BS	5	50	morphology	wax mirror width	0.50	[26]
bee	Am	social	SI	BS	27	270	morphology	hindwing width	0.89	[26]
bee	Am	social	SI	BS	27	270	morphology	number of hamuli	0.6	[26]
bee	Am	social	SI	BS	27	270	morphology	wing angle 29	0.69	[26]
bee	Am	social	SI	BS	27	270	morphology	wing angle 30	0.83	[26]
bee	Am	social	SI	BS	27	270	morphology	wing angle 33	0.46	[26]
bee	Am	social	SI	BS	27	270	morphology	wing angle 34	0.66	[26]
bee	Am	social	SI	BS	27	270	morphology	wing angle 35	0.55	[26]
bee	Am	social	SI	BS	27	270	morphology	wing angle 36	0.40	[26]
bee	Am	social	SI	BS	27	270	morphology	wing angle 38	0.75	[26]
bee	Am	social	SI	BS	27	270	morphology	wing angle 39	0.46	[26]
bee	Am	social	SI	BS	27	270	morphology	wing cubital vein B	0.12	[26]
bee	Am	social	SI	BS	27	270	morphology	wing cubital vein A	0.74	[26]
bee	Am	social	SI	BS	27	270	morphology	basitarsis width	0.88	[26]
bee	Am	social	SI	BS	27	270	morphology	distance between wax mirrors	0.66	[26]
bee	Am	social	SI	BS	80	800	morphology	number of hamuli	0.34	[26]
bee	Am	social	SI	BS	80	800	morphology	wing angle 29	0.51	[26]
bee	Am	social	SI	BS	80	800	morphology	wing angle 30	0.53	[26]
bee	Am	social	SI	BS	80	800	morphology	wing angle 31	0.64	[26]
bee	Am	social	SI	BS	80	800	morphology	wing angle 32	0.61	[26]
bee	Am	social	SI	BS	80	800	morphology	wing angle 33	0.76	[26]
bee	Am	social	SI	BS	80	800	morphology	wing angle 34	0.77	[26]
bee	Am	social	SI	BS	80	800	morphology	wing angle 35	0.95	[26]
bee	Am	social	SI	BS	80	800	morphology	wing angle 36	0.86	[26]
bee	Am	social	SI	BS	80	800	morphology	wing angle 38	0.52	[26]
bee	Am	social	SI	BS	80	800	morphology	wing angle 39	0.67	[26]
bee	Am	social	SI	BS	80	800	morphology	wing cubital vein B	0.96	[26]
bee	Am	social	SI	BS	80	800	morphology	wing cubital vein A	0.69	[26]
bee	Am	social	SI	BS	80	800	morphology	wax mirror length	0.85	[26]
bee	Am	social	SI	BS	80	800	morphology	distance between wax mirrors	0.89	[26]
bee	Am	social	SI	BS	43	430	morphology	number of hamuli	0.58	[26]
bee	Am	social	SI	BS	43	430	morphology	wing angle 29	0.53	[26]
bee	Am	social	SI	BS	43	430	morphology	wing angle 30	0.63	[26]
bee	Am	social	SI	BS	43	430	morphology	wing angle 31	0.38	[26]

bee	Am	social	SI	BS	43	430	morphology	wing angle 33	0.68	[26]
bee	Am	social	SI	BS	43	430	morphology	wing angle 34	0.62	[26]
bee	Am	social	SI	BS	43	430	morphology	wing angle 35	0.16	[26]
bee	Am	social	SI	BS	43	430	morphology	wing angle 36	0.63	[26]
bee	Am	social	SI	BS	43	430	morphology	wing angle 39	0.84	[26]
bee	Am	social	SI	BS	43	430	morphology	wing cubital vein B	0.53	[26]
bee	Am	social	SI	BS	43	430	morphology	wing cubital vein A	0.42	[26]
bee	Am	social	SI	BS	43	430	morphology	basitarsis width	0.89	[26]
bee	Am	social	SI	BS	43	430	morphology	distance between wax mirrors	0.85	[26]
bee	Am	social	SI	BS	30	300	morphology	forewing length	0.89	[26]
bee	Am	social	SI	BS	30	300	morphology	forewing width	0.73	[26]
bee	Am	social	SI	BS	30	300	morphology	number of hamuli	0.69	[26]
bee	Am	social	SI	BS	30	300	morphology	wing angle 29	0.64	[26]
bee	Am	social	SI	BS	30	300	morphology	wing angle 30	0.68	[26]
bee	Am	social	SI	BS	30	300	morphology	wing angle 31	0.87	[26]
bee	Am	social	SI	BS	30	300	morphology	wing angle 33	0.50	[26]
bee	Am	social	SI	BS	30	300	morphology	wing angle 34	0.66	[26]
bee	Am	social	SI	BS	30	300	morphology	wing angle 35	0.78	[26]
bee	Am	social	SI	BS	30	300	morphology	wing angle 36	0.43	[26]
bee	Am	social	SI	BS	30	300	morphology	wing angle 38	0.99	[26]
bee	Am	social	SI	BS	30	300	morphology	wing angle 39	0.73	[26]
bee	Am	social	SI	BS	30	300	morphology	wing cubital vein B	1.00	[26]
bee	Am	social	SI	BS	30	300	morphology	wing cubital vein A	0.94	[26]
bee	Am	social	SI	BS	30	300	morphology	third sternum length	0.73	[26]
bee	Am	social	SI	BS	30	300	morphology	wax mirror length	0.52	[26]
bee	Am	social	SI	BS	30	300	morphology	wax mirror width	0.92	[26]
bee	Am	social	SI	BS	20	200	morphology	number of hamuli	0.39	[26]
bee	Am	social	SI	BS	20	200	morphology	wing angle 29	0.25	[26]
bee	Am	social	SI	BS	20	200	morphology	wing angle 30	0.02	[26]
bee	Am	social	SI	BS	20	200	morphology	wing angle 31	0.15	[26]
bee	Am	social	SI	BS	20	200	morphology	wing angle 32	0.30	[26]
bee	Am	social	SI	BS	20	200	morphology	wing angle 33	0.98	[26]
bee	Am	social	SI	BS	20	200	morphology	wing angle 34	0.79	[26]
bee	Am	social	SI	BS	20	200	morphology	wing angle 36	0.77	[26]

bee	Am	social	SI	BS	20	200	morphology	wing angle 38	0.47	[26]
bee	Am	social	SI	BS	20	200	morphology	wing angle 39	0.40	[26]
bee	Am	social	SI	BS	20	200	morphology	wing cubital vein A	0.20	[26]
bee	Am	social	SI	BS	20	200	morphology	basitarsis length	1.00	[26]
bee	Am	social	SI	BS	20	200	morphology	basitarsis width	0.96	[26]
bee	Am	social	SI	BS	20	200	morphology	distance between wax mirrors	0.81	[26]
bee	Am	social	SI	BS	12	120	morphology	number of hamuli	0.87	[26]
bee	Am	social	SI	BS	12	120	morphology	wing angle 29	0.41	[26]
bee	Am	social	SI	BS	12	120	morphology	wing angle 30	0.84	[26]
bee	Am	social	SI	BS	12	120	morphology	wing angle 31	0.51	[26]
bee	Am	social	SI	BS	12	120	morphology	wing angle 33	0.43	[26]
bee	Am	social	SI	BS	12	120	morphology	wing angle 35	0.36	[26]
bee	Am	social	SI	BS	12	120	morphology	wing cubital vein A	0.94	[26]
bee	Am	social	SI	BS	12	120	morphology	third sternum length	0.66	[26]
bee	Am	social	SI	BS	12	120	morphology	wax mirror length	0.96	[26]
bee	Am	social	SI	BS	12	120	morphology	distance between wax mirrors	0.44	[26]
bee	Am	social	SI	BS	12	120	morphology	number of hamuli	0.48	[26]
bee	Am	social	SI	BS	12	120	morphology	wing angle 31	0.91	[26]
bee	Am	social	SI	BS	12	120	morphology	wing angle 33	0.19	[26]
bee	Am	social	SI	BS	12	120	morphology	wing angle 34	0.31	[26]
bee	Am	social	SI	BS	12	120	morphology	wing angle 35	0.51	[26]
bee	Am	social	SI	BS	12	120	morphology	wing angle 36	0.27	[26]
bee	Am	social	SI	BS	12	120	morphology	wing angle 38	0.75	[26]
bee	Am	social	SI	BS	12	120	morphology	wing angle 39	1.00	[26]
bee	Am	social	SI	BS	12	120	morphology	wing cubital vein A	0.66	[26]
bee	Am	social	SI	BS	12	120	morphology	tibia length	0.34	[26]
bee	Am	social	SI	BS	12	120	morphology	femur length	0.35	[26]
bee	Am	social	SI	BS	12	120	morphology	basitarsis width	0.51	[26]
bee	Am	social	SI	BS	12	120	morphology	wax mirror length	0.22	[26]
bee	Am	social	SI	BS	12	120	morphology	wax mirror width	0.62	[26]
bee	Am	social	SI	BS	12	120	morphology	distance between wax mirrors	0.97	[26]
bee	Am	social	SI	BS	26	180	metabolism	duration of uncapped stage	0.41	[27]
bee	Am	social	SI	BS	26	180	metabolism	duration of postcapping stage	0.61	[27]
bee	Am	social	SI	BS	26	180	metabolism	development	0.52	[27]

wasp	Ape	solitary	SI	NS	-	766	morphology	body size	0.39	[28]
wasp	Ape	solitary	SI	NS	-	766	morphology	body size	0.37	[28]
wasp	Ape	solitary	SI	NS	-	766	morphology	male size	0.77	[28]
wasp	Ape	solitary	PO	NS	37	-	morphology	body size	0.23	[28]
bee	Am	social	PO	NS	20	600	morphology	queen color patterns	0.21	[29]
bee	Am	social	PO	NS	10	300	morphology	queen color patterns	0.23	[29]
bee	Am	social	PO	NS	20	600	morphology	worker color patterns	0.31	[29]
bee	Am	social	PO	NS	20	600	morphology	drone color patterns	0.39	[29]
bee	Am	social	SI	BS	30	750	fertility	number of ovarioles	0.37	[30]
bee	Am	social	SI	BS	30	750	fertility	number of ovarioles	0.36	[30]
bee	Am	social	SI	BS	30	660	fertility	number of ovarioles	0.37	[30]
bee	Am	social	SI	BS	2864	-	productivity	honey production	0.24	[31]
bee	Am	social	SI	BS	2832	-	productivity	quietness (agressiveness and calmness)	0.08	[31]
bee	Am	social	SI	BS	2721	-	productivity	swarming	0.08	[31]
bee	Am	social	SI	BS	1775	-	productivity	number of combs covered with bees after hibernation	0.19	[31]
bee	Am	social	SI	BS	1714	-	productivity	number of combs covered with bees at the beginning of honey production	0.21	[31]
bee	Am	social	SI	BS	1662	-	productivity	number of combs covered with bees at the beginning of main honey flow	0.18	[31]
bee	Am	social	PO	NS	12	107	metabolism	duration of queen postcapping stage	0.31	[32]
bee	Am	social	PO	BS	12	107	metabolism	duration of queen postcapping stage	0.22	[32]
bee	Mro	solitary	PO	NS	25	-	morphology	body size	0.24	[33]
bee	Mro	solitary	PO	NS	19	-	morphology	male size	0.09	[33]
bee	Mro	solitary	SI	BS	-	312	morphology	body size	0.75	[33]
bee	Mro	solitary	SI	BS	-	769	morphology	male size	0.81	[33]
bee	Am	social	SI	BS	44	732	morphology	lateral femur area	0.64	[34]
bee	Am	social	SI	BS	44	732	morphology	lateral metatarsus area	0.94	[34]
bee	Am	social	SI	BS	44	732	morphology	hind leg area	0.95	[34]
bee	Am	social	SI	BS	44	732	morphology	surface of hindwing	0.68	[34]
bee	Am	social	SI	BS	44	732	morphology	cubital index	0.74	[34]
bee	Am	social	SI	BS	44	732	morphology	length of corbicula hairs	0.37	[34]
bee	Bo	social	SI	BS	20	400	productivity	allometry coefficient between glossa and wing lengths (foraging success)	0.69	[35]
bee	Bo	social	SI	BS	14	280	productivity	allometry coefficient between glossa and wing lengths (foraging success)	0.73	[35]
bee	Bh	social	SI	BS	17	340	productivity	allometry coefficient between glossa and wing lengths (foraging success)	0.51	[35]
wasp	Ad	solitary	GT	BS	-	-	fertility	number of eggs	0.47	[36]
wasp	Ad	solitary	GT	BS	-	-	morphology	ovipositor length	0.37	[36]

wasp	Ad	solitary	GT	BS	-	-	morphology	body size	0.2	[36]
wasp	Ad	solitary	GT	BS	-	-	fertility	time on a patch for oviposition	0.11	[36]
wasp	Ad	solitary	GT	BS	-	-	fertility	number of ovipositions per patch	0.09	[36]
wasp	Ad	solitary	GT	BS	-	-	fertility	oviposition rate	0.05	[36]
wasp	Ad	solitary	PO	BS	-	-	fertility	number of eggs	0.71	[36]
wasp	Ad	solitary	PO	BS	-	-	morphology	ovipositor length	0.54	[36]
wasp	Ad	solitary	PO	BS	-	-	morphology	body size	0.28	[36]
wasp	Ad	solitary	PO	BS	-	-	fertility	time on a patch for oviposition	0.09	[36]
wasp	Ad	solitary	PO	BS	-	-	fertility	number of ovipositions per patch	0.11	[36]
wasp	Ad	solitary	PO	BS	-	-	fertility	oviposition rate	0.03	[36]
bee	Am	social	SI	BS	16	-	morphology	sting length	0.52	[37]
bee	Am	social	SI	BS	16	-	morphology	length of sting stylet	0.28	[37]
bee	Am	social	SI	BS	16	-	morphology	length of the bulb of sting stylet	0.30	[37]
bee	Am	social	SI	BS	16	-	morphology	maximum width of bulb of sting stylet	0.17	[37]
bee	Am	social	SI	BS	16	-	morphology	height of the valve of right lancet	0.17	[37]
bee	Am	social	SI	BS	16	-	morphology	length of the lancet	0.74	[37]
bee	Am	social	SI	BS	16	-	morphology	number of tooth barb of sting	0.51	[37]
wasp	Tca	solitary	PO	NS	-	102	morphology	forewing length	0.19	[38]
wasp	Tca	solitary	PO	NS	-	102	morphology	forewing width	0.19	[38]
wasp	Tca	solitary	PO	NS	-	93	morphology	hindwing length	0.18	[38]
wasp	Tca	solitary	PO	NS	-	96	morphology	tibia length	0.09	[38]
wasp	Tca	solitary	PO	NS	-	102	morphology	asymmetry in trichiae	0.03	[38]
ant	Si	social	SI	-	-	-	metabolism	resistance to desiccation	0.42	[39]
bee	Am	social	SI	BS	28	-	defense	suppression of mite reproduction	0.38	[40]
bee	Am	social	SI	BS	28	-	defense	suppression of mite reproduction	0.06	[40]
bee	Am	social	SI	BS	28	-	defense	suppression of mite reproduction	0.46	[40]
bee	Am	social	SI	BS	28	-	defense	hygienic behavior	0.65	[40]
bee	Am	social	SI	BS	28	-	defense	physical damage to mites (total)	0	[40]
bee	Am	social	SI	BS	28	-	defense	physical damage to mites (dents in body)	0	[40]
bee	Am	social	SI	BS	28	-	defense	physical damage to mites (broken legs or bodies)	0.17	[40]
bee	Am	social	SI	BS	28	-	metabolism	duration of postcapping stage	0.89	[40]
bee	Am	social	SI	BS	28	-	defense	mites per 100 cells in brood	0.28	[40]
bee	Am	social	SI	BS	28	-	defense	final mite population	0.17	[40]
bee	Am	social	SI	BS	28	-	defense	mites on adult bees	0.01	[40]

bee	Am	social	-	BS	2494	-	productivity	honey production	0.41	[41]
bee	Am	social	-	BS	2494	-	productivity	swarming	0.73	[41]
bee	Am	social	-	BS	2494	-	communication	colony defense	0.49	[41]
bee	Am	social	PO	BS	45	-	defense	hygienic behavior towards V. destructor-infested brood cells	0.18	[42]
bee	Am	social	PO	BS	66	-	defense	hygienic behavior	0.36	[42]
wasp	Cg	solitary	SI	BS	16	720	communication	olfactory response (flight orientation)	0.45	[43]
bee	Am	social	-	-	150	-	productivity	summer honey production	0.64	[44]
bee	Am	social	-	-	150	-	productivity	fall honey production	0.51	[44]
bee	Am	social	-	-	150	-	productivity	honey production	0.30	[44]
ant	Lr	social	PO	NS	-	-	morphology	gyne size	0.88	[45]
ant	Lr	social	PO	NS	-	-	morphology	gyne size (macrogyenes)	0.62	[45]
ant	Lr	social	PO	NS	-	-	morphology	gyne size (microgyenes)	0.82	[45]
wasp	Tn	solitary	PO	BS	25	104	fertility	percentage of an egg mass parasitized	0.48	[46]
wasp	Tn	solitary	SI	NS	120	300	fertility	percentage of an egg mass parasitized	0	[46]
wasp	Tca	solitary	-	-	-	-	fertility	fecundity	0.10	[47]
ant	Ft	social	SI	BS	11	880	communication	cuticular hydrocarbon profile (recognition cues) - PC1	0.22	[48]
ant	Ft	social	SI	BS	11	880	communication	cuticular hydrocarbon profile (recognition cues) - PC1	0.03	[48]
ant	Ft	social	SI	BS	11	880	communication	cuticular hydrocarbon profile (recognition cues) - PC2	0.44	[48]
ant	Ft	social	SI	BS	11	880	communication	cuticular hydrocarbon profile (recognition cues) - PC2	0.16	[48]
wasp	Hp	solitary	SI	NS	-	30	fertility	sex ratio	0.32	[49]
wasp	Hp	solitary	SI	NS	-	30	fertility	sex ratio	0.32	[49]
wasp	Hp	solitary	SI	NS	-	15	fertility	sex ratio	0.20	[49]
wasp	Hp	solitary	SI	NS	-	15	fertility	sex ratio	0.18	[49]
wasp	Hp	solitary	SI	NS	-	30	fertility	sex changing point	0.23	[49]
wasp	Hp	solitary	SI	NS	-	30	fertility	sex changing point	0.34	[49]
wasp	Hp	solitary	SI	NS	-	15	fertility	sex changing point	0.21	[49]
wasp	Hp	solitary	SI	NS	-	15	fertility	sex changing point	0.24	[49]
wasp	Hp	solitary	SI	NS	-	30	fertility	sex changing range	0.10	[49]
wasp	Hp	solitary	SI	NS	-	15	fertility	sex changing range	0.05	[49]
wasp	Hp	solitary	SI	NS	-	36	morphology	body size	0.13	[49]
wasp	Hp	solitary	SI	NS	-	26	morphology	body size	0.22	[49]
wasp	Hp	solitary	SI	NS	-	23	morphology	body size	0.11	[49]
wasp	Hp	solitary	SI	NS	-	23	morphology	body size	0.18	[49]
wasp	Hp	solitary	SI	NS	-	35	fertility	offspring number	0.51	[49]

wasp	Hp	solitary	SI	NS	-	18	fertility	offspring number	0.54	[49]
wasp	Hp	solitary	SI	NS	-	18	fertility	offspring number	0.59	[49]
wasp	Cg	solitary	RE	NS	40	1240	communication	olfactory response (flight orientation)	0.31	[50]
wasp	Cg	solitary	RE	NS	40	1240	communication	olfactory response (flight orientation)	0.18	[50]
wasp	Cg	solitary	RE	NS	40	1240	communication	olfactory response (flight orientation)	0.25	[50]
wasp	Cg	solitary	RE	NS	40	1240	communication	olfactory response (landing success)	0.08	[50]
wasp	Cg	solitary	RE	NS	40	1240	communication	olfactory response (landing success)	0.35	[50]
wasp	Cg	solitary	RE	NS	40	1240	communication	olfactory response (landing success)	0.22	[50]
ant	Ft	social	SI	BS	5	100	morphology	gyne size	0.51	[51]
ant	Ft	social	SI	BS	4	104	morphology	gyne size	0	[51]
ant	Ft	social	SI	BS	7	280	morphology	body size	0.09	[51]
ant	Ln	social	SI	BS	12	-	morphology	body size	0.11	[52]
ant	Ln	social	SI	BS	19	-	morphology	body size	0.03	[52]
ant	Ln	social	SI	BS	12	-	morphology	head width	0.42	[52]
ant	Ln	social	SI	BS	19	-	morphology	head width	0.28	[52]
bee	Am	social	SI	BS	7	28	behavior	estimated number of matings (k)	0.45	[53]
bee	Am	social	SI	BS	7	28	behavior	effective number of matings (me)	0.26	[53]
ant	Fs	social	SI	BS	6	300	morphology	body size	0.26	[54]
bee	Bt	social	SI	NS	14	285	morphology	male size	0.54	[55]
bee	Bt	social	SI	NS	14	285	morphology	individual sperm length	0.20	[55]
bee	Bt	social	SI	NS	14	285	morphology	mean sperm length	0.43	[55]
bee	Ms	social	RE	NS	10	-	productivity	colony weight gain	0.16	[56]
ant	Tc	social	SI	BS	341	3828	morphology	body size	0.37	[57]
ant	Tc	social	SI	BS	323	2101	morphology	gyne size	0.74	[57]
ant	Tc	social	SI	BS	135	639	morphology	male size	0.99	[57]
ant	Tc	social	SI	BS	355	11464	fertility	caste ratio	0.65	[57]
ant	Tc	social	SI	BS	341	3828	morphology	body size	0.19	[57]
ant	Tc	social	SI	BS	323	2101	morphology	gyne size	0.47	[57]
ant	Tc	social	SI	BS	135	639	morphology	male size	0.11	[57]
ant	Tc	social	SI	BS	355	11464	fertility	caste ratio	0.52	[57]
ant	Tc	social	SI	BS	267	4400	fertility	sex ratio	0.35	[57]
ant	Tc	social	SI	BS	341	3828	morphology	body size	0.12	[57]
ant	Tc	social	SI	BS	323	2101	morphology	gyne size	0.43	[57]
ant	Tc	social	SI	BS	135	639	morphology	male size	0.37	[57]

ant	Tc	social	SI	BS	355	11464	fertility	caste ratio	0.49	[57]
ant	Tc	social	SI	BS	267	4400	fertility	sex ratio	0.48	[57]
bee	Am	social	SI	BS	-	114	morphology	proboscis length	0.64	[58]
bee	Am	social	SI	BS	-	114	morphology	femur length	0.50	[58]
bee	Am	social	SI	BS	-	114	morphology	tibia length	0.46	[58]
bee	Am	social	SI	BS	-	114	morphology	metatarsus length	0.43	[58]
bee	Am	social	SI	BS	-	114	morphology	metatarsus width	0.34	[58]
bee	Am	social	SI	BS	-	114	morphology	hind leg length	0.51	[58]
bee	Am	social	SI	BS	-	114	morphology	forewing length	0.42	[58]
bee	Am	social	SI	BS	-	114	morphology	forewing width	0.90	[58]
bee	Am	social	SI	BS	-	114	morphology	hind leg length	0.94	[58]
bee	Am	social	SI	BS	-	114	morphology	hind leg width	0.72	[58]
bee	Am	social	SI	BS	-	114	morphology	cubital index	0.50	[58]
bee	Am	social	SI	BS	-	114	morphology	wing cubital vein A	0.61	[58]
bee	Am	social	SI	BS	-	114	morphology	wing cubital vein B	0.20	[58]
bee	Am	social	SI	BS	-	114	morphology	number of hamuli	0.45	[58]
bee	Am	social	GT	BS	5	-	metabolism	maturation rate of drones (age at the onset of flight)	0.02	[59]
bee	Am	social	GT	BS	5	-	metabolism	maturation rate of drones (age at the onset of flight)	0.08	[59]
bee	Am	social	GT	BS	5	-	metabolism	maturation rate of drones (age at the onset of flight)	0.03	[59]
bee	Am	social	GT	BS	5	-	metabolism	maturation rate of drones (age at the onset of flight)	0.12	[59]
bee	Am	social	GT	BS	5	212	metabolism	maturation rate of drones (initial locomotor activity - light)	0.05	[59]
bee	Am	social	GT	BS	5	212	metabolism	maturation rate of drones (initial locomotor activity - light)	0.11	[59]
bee	Am	social	GT	BS	5	212	metabolism	maturation rate of drones (initial locomotor activity - dark)	0.02	[59]
bee	Am	social	GT	BS	5	212	metabolism	maturation rate of drones (initial locomotor activity - dark)	0.05	[59]
bee	Am	social	SI	BS	19	-	productivity	sunflower pollen collection	0.26	[60]
bee	Am	social	PO	NS	-	-	defense	immune gene expression	0.40	[61]
bee	Am	social	PO	NS	-	-	defense	immune gene expression	0.35	[61]
bee	Am	social	SI	BS	53	636	defense	immune gene expression	0.72	[61]
bee	Am	social	SI	BS	36	432	defense	immune gene expression	0.25	[61]
ant	Eb	social	SI	BS	5	991	morphology	body size	0.15	[62]
wasp	Nv	solitary	SI	BS	-	1005	behavior	receptivity at first courtship	0.10	[63]
wasp	Nv	solitary	SI	BS	-	1005	behavior	receptivity at first courtship	0.55	[63]
wasp	Nv	solitary	SI	BS	-	1005	behavior	receptivity within 10 min	0.07	[63]
wasp	Nv	solitary	SI	BS	-	1005	behavior	receptivity within 10 min	0.82	[63]

wasp	Nv	solitary	SI	BS	-	1005	behavior	courtship duration	0.29	[63]
wasp	Nv	solitary	SI	BS	-	1005	behavior	courtship duration	0.22	[63]
wasp	Nv	solitary	SI	BS	-	1005	behavior	copulation duration	0.15	[63]
wasp	Nv	solitary	SI	BS	-	1005	behavior	copulation duration	0.54	[63]
ant	Cc	social	SI	BS	12	480	morphology	body size	0.10	[64]
bee	Am	social	PO	NS	-	16	defense	hygienic behavior	0.63	[65]
bee	Am	social	PO	NS	-	16	defense	hygienic behavior	0.45	[65]
bee	Am	social	PO	NS	-	16	defense	hygienic behavior	0.44	[65]
wasp	Nv	solitary	GT	BS	171	1648	fertility	sex ratio mutation (single foundress)	0.0016	[66]
wasp	Nv	solitary	GT	BS	35	301	fertility	sex ratio mutation (two foundress)	0.0008	[66]
wasp	Nv	solitary	GT	BS	-	213	fertility	sex ratio (single foundress)	0.02	[66]
wasp	Nv	solitary	GT	BS	-	40	fertility	two ratio (single foundress)	0.05	[66]
wasp	Vm	social	SI	BS	-	-	morphology	thorax width	0	[67]
wasp	Vm	social	SI	BS	-	-	morphology	thorax length	0.04	[67]
wasp	Vm	social	SI	BS	-	-	morphology	head to thorax length	0.03	[67]
wasp	Vm	social	SI	BS	-	-	morphology	third tergum length	0.07	[67]
wasp	Vm	social	SI	BS	-	-	morphology	gaster length	0.02	[67]
wasp	Vm	social	SI	BS	-	-	morphology	body length	0	[67]
wasp	Vm	social	SI	BS	-	-	morphology	body size	0.06	[67]
wasp	Vm	social	SI	BS	-	-	morphology	gaster length	0.06	[67]
wasp	Vm	social	SI	BS	-	-	morphology	body size	0.02	[67]
wasp	Vm	social	PO	NS	-	-	morphology	gyne thorax width	0.2	[67]
wasp	Vm	social	PO	NS	-	-	morphology	gyne thorax length	0.05	[67]
wasp	Vm	social	PO	NS	-	-	morphology	gyne head to thorax length	0.02	[67]
wasp	Vm	social	PO	NS	-	-	morphology	gyne gaster length	0.13	[67]
wasp	Vm	social	PO	NS	-	-	morphology	gyne size	0.18	[67]
wasp	Vm	social	PO	NS	-	-	morphology	gyne thorax width	0.46	[67]
wasp	Vm	social	PO	NS	-	-	morphology	gyne thorax length	0.04	[67]
wasp	Vm	social	PO	NS	-	-	morphology	gyne head to thorax length	0.06	[67]
wasp	Vm	social	PO	NS	-	-	morphology	gyne third tergum length	0.12	[67]
wasp	Vm	social	PO	NS	-	-	morphology	gyne gaster length	0.2	[67]
wasp	Vm	social	PO	NS	-	-	morphology	gyne body length	0.38	[67]
wasp	Vm	social	PO	NS	-	-	morphology	thorax width	0.05	[67]
wasp	Vm	social	PO	NS	-	-	morphology	thorax length	0.02	[67]

wasp	Vm	social	PO	NS	-	-	morphology	thorax length	0.45	[67]
wasp	Vm	social	PO	NS	-	-	morphology	third tergum length	0.23	[67]
wasp	Vm	social	PO	NS	-	-	morphology	gaster length	0.66	[67]
wasp	Vm	social	PO	NS	-	-	morphology	body length	0.59	[67]
wasp	Vm	social	PO	NS	5	80	morphology	gyne head to thorax length	0.17	[67]
wasp	Vm	social	PO	NS	5	80	morphology	gyne third tergum length	0.99	[67]
wasp	Vm	social	PO	NS	5	80	morphology	gyne gaster length	0.33	[67]
wasp	Vm	social	PO	NS	5	80	morphology	gyne body length	0.49	[67]
bee	Am	social	PO	-	-	-	productivity	avocado foraging	0.22	[68]
ant	Ao	social	SI	BS	3	150	defense	large worker relative diameter of metapleural gland bulla	0.61	[69]
ant	Ae	social	SI	BS	5	235	defense	small worker relative diameter of metapleural gland bulla	0.86	[69]
ant	Ao	social	SI	BS	5	235	defense	large worker relative diameter of metapleural gland bulla	0.55	[69]
ant	Ae	social	SI	BS	5	470	defense	gyne relative diameter of metapleural gland bulla	0.25	[69]
bee	Am	social	SI	BS	6	903	behavior	learning behavior (PER)	0.22	[70]
bee	Am	social	SI	BS	6	903	behavior	olfactory learning (resistance of the conditioned response to extinction)	0.13	[70]
wasp	Pd	social	PO	BS	23	-	communication	quality signal (facial pattern brokenness)	0.64	[71]
wasp	Pd	social	PO	BS	23	-	morphology	proportion of black pigment in clypeus	0.58	[71]
bee	Am	social	PO	NS	88	-	defense	grooming behavior	0.49	[72]
bee	Am	social	PO	NS	88	-	defense	grooming behavior	0.18	[72]
bee	Am	social	PO	NS	88	-	defense	grooming behavior	0.16	[72]
bee	Am	social	SI	BS	40	-	defense	hygienic behavior	0.10	[73]
bee	Am	social	SI	BS	40	-	defense	hygienic behavior (48h)	0.11	[73]
bee	Am	social	SI	BS	40	-	defense	hygienic behavior (72h)	0.11	[73]
bee	Am	social	SI	BS	40	-	defense	hygienic behavior	0.10	[73]
bee	Am	social	SI	BS	40	-	defense	hygienic behavior (48h)	0.11	[73]
bee	Am	social	SI	BS	40	-	defense	hygienic behavior (72h)	0.12	[73]
bee	Am	social	SI	BS	40	-	defense	hygienic behavior	0.28	[73]
bee	Am	social	SI	BS	40	-	defense	hygienic behavior (48h)	0.15	[73]
bee	Am	social	SI	BS	40	-	defense	hygienic behavior (72h)	0.24	[73]
bee	Am	social	SI	BS	40	-	defense	hygienic behavior	0.23	[73]
bee	Am	social	SI	BS	40	-	defense	hygienic behavior (48h)	0.29	[73]
bee	Am	social	SI	BS	40	-	defense	hygienic behavior (72h)	0.27	[73]
bee	Am	social	PO	BS	174	-	productivity	royal jelly production per colony	0.06	[74]
bee	Am	social	PO	BS	175	-	productivity	royal jelly production per cup	0.08	[74]

bee	Am	social	PO	BS	176	-	productivity	larvae acceptance	0.01	[74]
bee	Am	social	PO	BS	177	-	productivity	royal jelly production per colony	0.01	[74]
bee	Am	social	PO	BS	178	-	productivity	royal jelly production per cup	0.01	[74]
bee	Am	social	PO	BS	179	-	productivity	larvae acceptance (royal jelly production)	0	[74]
wasp	Ck	solitary	PO	NS	-	44	morphology	body size	0.70	[75]
bee	Am	social	SI	BS	767	-	defense	hygienic behavior	0.17	[76]
bee	Am	social	SI	BS	835	-	defense	hygienic behavior (48h)	0.25	[76]
ant	Ac	social	SI	BS	5	250	morphology	male size	0.66	[77]
ant	Ac	social	SI	BS	5	250	morphology	male head width	0.61	[77]
ant	Ac	social	SI	BS	5	250	morphology	mesosoma mass	0.08	[77]
ant	Ac	social	SI	BS	4	160	morphology	mesosoma width	0.04	[77]
ant	Ac	social	SI	BS	5	250	morphology	mean sperm length	0.36	[77]
ant	Ac	social	SI	BS	5	250	fertility	sperm number	0.47	[77]
ant	Ac	social	SI	BS	5	250	morphology	accessory gland size	0.72	[77]
ant	Ac	social	SI	BS	4	160	morphology	accessory gland size	0.13	[77]
ant	Ac	social	SI	BS	5	170	morphology	accessory testes size	0.07	[77]
ant	Ac	social	SI	BS	4	160	morphology	accessory testes size	0.25	[77]
ant	Ac	social	SI	BS	5	200	fertility	sperm viability Hayes treatment 2009	0.04	[77]
ant	Ac	social	SI	BS	4	80	fertility	sperm viability Hayes treatment 2008	0.17	[77]
bee	Am	social	SI	BS	24	480	fertility	number of ovarioles	0.19	[78]
bee	Am	social	SI	BS	24	480	fertility	number of ovarioles	0.57	[78]
bee	Am	social	GT	BS	18	-	productivity	honey production	0.72	[79]
bee	Am	social	GT	BS	18	-	productivity	swarming	0.33	[79]
bee	Am	social	GT	BS	18	-	productivity	aggressiveness	0.11	[79]
bee	Am	social	GT	BS	18	-	productivity	honey robbing	0.35	[79]
bee	Am	social	GT	BS	18	-	productivity	honey production	0.78	[79]
bee	Am	social	GT	BS	18	-	productivity	swarming	0.28	[79]
bee	Am	social	GT	BS	18	-	productivity	aggressiveness	0.04	[79]
bee	Am	social	GT	BS	18	-	productivity	honey robbing	0.38	[79]
bee	Am	social	GT	BS	18	-	productivity	honey production	0.74	[79]
bee	Am	social	GT	BS	18	-	productivity	swarming	0.33	[79]
bee	Am	social	GT	BS	18	-	productivity	aggressiveness	0.89	[79]
bee	Am	social	GT	BS	18	-	productivity	honey robbing	0.51	[79]
bee	Am	social	GT	BS	18	-	productivity	honey production	0.81	[79]

bee	Am	social	GT	BS	18	-	productivity	swarming	0.11	[79]
bee	Am	social	GT	BS	18	-	productivity	aggressiveness	0.21	[79]
bee	Am	social	GT	BS	18	-	productivity	honey robbing	0.38	[79]
bee	Am	social	GT	BS	18	-	productivity	honey production	0.80	[79]
bee	Am	social	GT	BS	18	-	productivity	swarming	0.33	[79]
bee	Am	social	GT	BS	18	-	productivity	aggressiveness	0.33	[79]
bee	Am	social	GT	BS	18	-	productivity	honey robbing	0.42	[79]
bee	Am	social	GT	BS	18	-	productivity	honey production	0.75	[79]
bee	Am	social	GT	BS	18	-	productivity	swarming	0.33	[79]
bee	Am	social	GT	BS	18	-	productivity	aggressiveness	0.28	[79]
bee	Am	social	GT	BS	18	-	productivity	honey robbing	0.32	[79]
bee	Am	social	GT	BS	18	-	productivity	spring number of bees	0.54	[79]
bee	Am	social	GT	BS	18	-	productivity	spring brood production	0.80	[79]
bee	Am	social	GT	BS	18	-	productivity	spring honey production	0.60	[79]
bee	Am	social	GT	BS	18	-	productivity	spring pollen production	0.12	[79]
bee	Am	social	GT	BS	18	-	productivity	spring number of bees	0.08	[79]
bee	Am	social	GT	BS	18	-	productivity	spring brood production	0.4	[79]
bee	Am	social	GT	BS	18	-	productivity	spring honey production	0.04	[79]
bee	Am	social	GT	BS	18	-	productivity	spring pollen production	0.33	[79]
bee	Am	social	GT	BS	18	-	productivity	spring number of bees	0.77	[79]
bee	Am	social	GT	BS	18	-	productivity	spring brood production	0.49	[79]
bee	Am	social	GT	BS	18	-	productivity	spring honey production	0.11	[79]
bee	Am	social	GT	BS	18	-	productivity	spring pollen production	0.33	[79]
bee	Am	social	GT	BS	18	-	productivity	spring number of bees	0.33	[79]
bee	Am	social	GT	BS	18	-	productivity	spring brood production	0.65	[79]
bee	Am	social	GT	BS	18	-	productivity	spring honey production	0.68	[79]
bee	Am	social	GT	BS	18	-	productivity	spring pollen production	0.31	[79]
bee	Am	social	GT	BS	18	-	productivity	spring number of bees	0.41	[79]
bee	Am	social	GT	BS	18	-	productivity	spring brood production	0.56	[79]
bee	Am	social	GT	BS	18	-	productivity	spring honey production	0.14	[79]
bee	Am	social	GT	BS	18	-	productivity	spring pollen production	0.5	[79]
bee	Am	social	GT	BS	18	-	productivity	spring number of bees	0.12	[79]
bee	Am	social	GT	BS	18	-	productivity	spring brood production	0.4	[79]
bee	Am	social	GT	BS	18	-	productivity	spring honey production	0.19	[79]

bee	Am	social	GT	BS	18	-	productivity	spring pollen production	0.57	[79]
bee	Am	social	GT	BS	18	-	productivity	fall number of bees	0.54	[79]
bee	Am	social	GT	BS	18	-	productivity	fall brood production	0.42	[79]
bee	Am	social	GT	BS	18	-	productivity	fall honey production	0.11	[79]
bee	Am	social	GT	BS	18	-	productivity	fall pollen production	0.11	[79]
bee	Am	social	GT	BS	18	-	productivity	fall number of bees	0.36	[79]
bee	Am	social	GT	BS	18	-	productivity	fall brood production	0.42	[79]
bee	Am	social	GT	BS	18	-	productivity	fall honey production	0.55	[79]
bee	Am	social	GT	BS	18	-	productivity	fall pollen production	0.04	[79]
bee	Am	social	GT	BS	18	-	productivity	fall number of bees	0.50	[79]
bee	Am	social	GT	BS	18	-	productivity	fall brood production	0.43	[79]
bee	Am	social	GT	BS	18	-	productivity	fall honey production	0.37	[79]
bee	Am	social	GT	BS	18	-	productivity	fall pollen production	0.10	[79]
bee	Am	social	GT	BS	18	-	productivity	fall number of bees	0.69	[79]
bee	Am	social	GT	BS	18	-	productivity	fall brood production	0.54	[79]
bee	Am	social	GT	BS	18	-	productivity	fall honey production	0.39	[79]
bee	Am	social	GT	BS	18	-	productivity	fall pollen production	0.33	[79]
bee	Am	social	GT	BS	18	-	productivity	fall number of bees	0.57	[79]
bee	Am	social	GT	BS	18	-	productivity	fall brood production	0.51	[79]
bee	Am	social	GT	BS	18	-	productivity	fall honey production	0.67	[79]
bee	Am	social	GT	BS	18	-	productivity	fall pollen production	0.46	[79]
bee	Am	social	GT	BS	18	-	productivity	fall number of bees	0.6	[79]
bee	Am	social	GT	BS	18	-	productivity	fall brood production	0.67	[79]
bee	Am	social	GT	BS	18	-	productivity	fall honey production	0.18	[79]
bee	Am	social	GT	BS	18	-	productivity	fall pollen production	0.33	[79]
wasp	Ck	solitary	SI	BS	-	144	defense	number of soldier clones in male brood (3rd instar host)	0.85	[80]
wasp	Cf	solitary	SI	BS	-	89	defense	number of soldier clones in female brood (3rd instar host)	0.66	[80]
wasp	Cf	solitary	SI	BS	-	183	defense	number of soldier clones in male brood (6th instar host)	0.75	[80]
wasp	Cf	solitary	SI	BS	-	124	defense	number of soldier clones in female brood (6th instar host)	0.32	[80]
bee	Am	social	PO	NS	1088	-	productivity	honey production	0.27	[81]
bee	Am	social	PO	NS	1088	-	productivity	gentleness	0.08	[81]
bee	Am	social	PO	NS	10	-	productivity	honey production	0.36	[82]
bee	Am	social	PO	NS	10	-	productivity	propolis production	0.87	[82]
bee	Am	social	PO	NS	10	-	communication	time to first sting	0.86	[82]

bee	Am	social	PO	NS	10	-	communication	number of stings in target	0.71	[82]
bee	Am	social	PO	NS	10	-	communication	time for clustering	0.84	[82]
bee	Am	social	PO	NS	10	-	defense	hygienic behavior	0.52	[82]
bee	Am	social	PO	BS	110	-	productivity	honey production	0.16	[83]
bee	Am	social	PO	BS	110	-	productivity	syrup collection rate	0.23	[83]
bee	Am	social	PO	BS	110	-	productivity	propolis production	0.66	[83]
bee	Am	social	PO	BS	110	-	defense	hygienic behavior	0.52	[83]
bee	Am	social	PO	BS	110	-	defense	mites on adult bees	0.13	[83]
bee	Am	social	PO	NS	50	-	defense	hygienic behavior	0.58	[84]
bee	Am	social	PO	NS	50	-	defense	mites on adult bees	0.64	[84]
bee	Am	social	PO	NS	50	-	defense	mites per 100 cells in brood	0.56	[84]
bee	Am	social	PO	NS	50	-	defense	total reproduction rate of the mite <i>Varroa destructor</i>	0.63	[84]
bee	Am	social	PO	NS	50	-	defense	effective reproduction rate of the mite <i>Varroa destructor</i>	0.61	[84]
bee	Am	social	PO	NS	40	-	productivity	brood production	0.64	[85]
bee	Am	social	PO	NS	40	-	productivity	colony population	0.61	[85]
bee	Am	social	PO	NS	40	-	productivity	brood viability	0.36	[85]
bee	Am	social	PO	NS	40	-	defense	hygienic behavior	0.72	[85]
bee	Am	social	PO	NS	40	-	productivity	honey production	0.31	[85]
bee	Am	social	SI	BS	2	309	defense	innate immune response	0	[86]
bee	Mq	social	PO	NS	-	-	productivity	honey pot number	0.53	[87]
bee	Mq	social	PO	NS	-	-	productivity	honey pot width	0.4	[87]
bee	Mq	social	PO	NS	-	-	productivity	honey pot volume	0.41	[87]
bee	Mq	social	PO	NS	-	-	productivity	honey pot height	0.35	[87]
bee	Mq	social	PO	NS	-	-	productivity	pollen pot number	0.36	[87]
bee	Mq	social	PO	NS	-	-	productivity	pollen pot height	0.51	[87]
bee	Mq	social	PO	NS	-	-	productivity	pollen pot diameter	0.39	[87]
bee	Mq	social	PO	NS	-	-	productivity	honey production	0.5	[87]
bee	Am	social	PO	NS	14650	-	productivity	honey production	0.70	[88]
bee	Am	social	PO	NS	-	-	productivity	gentleness	0.41	[88]
bee	Am	social	PO	NS	-	-	productivity	calmness	0.43	[88]
bee	Am	social	PO	NS	-	-	productivity	swarming	0.36	[88]
bee	Am	social	PO	NS	-	-	productivity	honey production	0.36	[88]
bee	Am	social	PO	NS	-	-	productivity	gentleness	0.14	[88]
bee	Am	social	PO	NS	-	-	productivity	calmness	0.07	[88]

bee	Am	social	PO	NS	-	-	productivity	swarming	0.25	[88]
bee	Am	social	PO	NS	-	-	productivity	honey production	0.27	[88]
bee	Am	social	PO	NS	-	-	productivity	gentleness	0.37	[88]
bee	Am	social	PO	NS	-	-	productivity	calmness	0.38	[88]
bee	Am	social	PO	NS	-	-	productivity	swarming	0.06	[88]

^a Studies with heritability estimates excluded from the analyses: [6], [12], [14], [17], [18], [26], [33], [34], [38], [40], [57], [67], [77], [89], [90].

^b Studied species - Ac: *Atta colombica*, Ad: *Anagrus delicatus*, Ae: *Acromyrmex echinator*, Am: *Apis mellifera*, Ao: *Acromyrmex octospinosus*, Ape: *Aphidius ervi*, Bh: *Bombus huntii*, Bo: *Bombus occidentalis*, Br: *Bombus rufocinctus*, Bt: *Bombus terrestris*, Cc: *Cataglyphis cursor*, Cf: *Copidosoma floridanum*, Cg: *Cotesia glomerata*, Ck: *Copidosoma koehleri*, Eb: *Eciton burchellii*, Fs: *Formica selysi*, Ft: *Formica truncorum*, Hp: *Heterospilus prosopidis*, Ln: *Lasius niger*, Lr: *Leptothorax rugatulus*, Mq: *Melipona quadrifasciata*, Mr: *Muscidifurax raptorellus*, Mro: *Megachile rotundata*, Ms: *Melipona scutellaris*, Ol: *Osmia lignaria*, Nv: *Nasonia vitripennis*, Pd: *Polistes dominulus*, Si: *Solenopsis invicta*, Tc: *Temnothorax curvispinosus*, Tca: *Trichogramma carverae*, Tn: *Trichogramma nubilale*, Vm: *Vespula maculifrons*.

^c Experimental design - DC: diallel cross, GT: ratio between genetic variance and phenotypic variance, PO: parent-offspring regression, RE: realized heritability (breeder's equation), SI: sibling analysis.

^d Estimation type - NS: narrow-sense estimate, BS: broad-sense estimate.

Table S2. Dataset of genetic correlations between honeybee traits, with information on traits category, correlated traits, genetic correlation coefficient and reference. Genetic correlation coefficients below -1 or above 1 were excluded from the analysis (see the footnote^a for the list of studies with excluded genetic correlation coefficients, as well as the full csv dataset).

Trait category	Correlated traits	Genetic correlation coefficient (r)	Reference
defense - longevity	longevity and response to alarm pheromone	0.72	[5]
hoarding - comb cell size	hoarding behavior and comb cell size	0.08	[6]
defense - hoarding	hoarding behavior and time to react to alarm pheromone (in laboratory)	-0.28	[6]
defense - hoarding	hoarding behavior and time to react to targets	0.80	[6]
defense - comb cell size	comb cell size and time to react to alarm pheromone (in laboratory)	0.45	[6]
defense - comb cell size	comb cell size and time to react to targets	0.84	[6]
defense traits	number of stings in target and reaction to alarm pheromone (nbees after 30s)	0.40	[6]
defense traits	number of stings in target and reaction to alarm pheromone (nbees after 30s)	-0.58	[6]
defense traits	number of stings in target and reaction to alarm pheromone (nbees after 60s)	0.39	[6]
defense traits	number of stings in target and reaction to alarm pheromone (nbees after 60s)	-0.66	[6]
defense traits	number of stings in target and reaction to alarm pheromone (nbees after 90s)	-0.26	[6]
defense traits	time to react to alarm pheromones (in laboratory) and time to react to targets	-0.82	[6]
defense traits	time to react to alarm pheromones (in laboratory) and time to react to targets	0.36	[6]
sting pheromones	sting pheromones (butyl acetate and isopentyl acetate)	0.99	[17]
sting pheromones	sting pheromones (butyl acetate and hexyl acetate)	0.99	[17]
sting pheromones	sting pheromones (butyl acetate and 1-hexanol)	0.63	[17]
sting pheromones	sting pheromones (butyl acetate and 2-heptyl acetate)	0.81	[17]
sting pheromones	sting pheromones (butyl acetate and 2-heptanol)	0.90	[17]
sting pheromones	sting pheromones (butyl acetate and octyl acetate)	0.04	[17]
sting pheromones	sting pheromones (butyl acetate and 2-nonanol)	0.63	[17]
sting pheromones	sting pheromones (isopentyl acetate and hexyl acetate)	0.95	[17]
sting pheromones	sting pheromones (isopentyl acetate and octyl acetate)	0.46	[17]

sting pheromones	sting pheromones (hexyl acetate and 1-hexanol)	0.70	[17]
sting pheromones	sting pheromones (hexyl acetate and 2-heptyl acetate)	0.89	[17]
sting pheromones	sting pheromones (hexyl acetate and octyl acetate)	0.15	[17]
sting pheromones	sting pheromones (hexyl acetate and 2-nonanol)	0.71	[17]
sting pheromones	sting pheromones (1-hexanol and octyl acetate)	0.51	[17]
sting pheromones	sting pheromones (2-heptyl acetate and octyl acetate)	-0.07	[17]
sting pheromones	sting pheromones (2-heptanol and octyl acetate)	0.53	[17]
sting pheromones	sting pheromones (octyl acetate and 2-nonyl acetate)	-0.09	[17]
sting pheromones	sting pheromones (octyl acetate and 2-nonanol)	-0.27	[17]
sting - mandibular pheromones	sting pheromone (butyl acetate) and mandibular gland pheromone (2-heptatone)	-0.14	[17]
sting - mandibular pheromones	sting pheromone (isopentyl acetate) and mandibular gland pheromone (2-heptatone)	0.16	[17]
sting - mandibular pheromones	sting pheromone (hexyl acetate) and mandibular gland pheromone (2-heptatone)	-0.10	[17]
sting - mandibular pheromones	sting pheromone (2-heptyl acetate) and mandibular gland pheromone (2-heptatone)	-0.37	[17]
sting - mandibular pheromones	sting pheromone (2-heptanol) and mandibular gland pheromone (2-heptatone)	-0.35	[17]
sting - mandibular pheromones	sting pheromone (2-nonyl acetate) and mandibular gland pheromone (2-heptatone)	-0.46	[17]
sting - mandibular pheromones	sting pheromone (2-nonanol) and mandibular gland pheromone (2-heptatone)	-0.92	[17]
honey production - colony agressiveness	honey production and colony quietness	0.07	[31]
honey production - swarming colony aggressiveness - swarming	honey production and swarming quietness and swarming	0.15	[31]
		-0.17	[31]
hind leg morphology	lateral tibia area and lateral femur area	0.16	[34]
hind leg morphology	lateral tibia area and lateral metatarsus area	0.22	[34]
hind leg morphology	lateral tibia area and hind leg area	0.80	[34]
hind leg - wing morphology	lateral tibia area and surface of hindwing	0.56	[34]
hind leg - wing morphology	lateral tibia area and surface of forewing	0.36	[34]
hind leg - wing morphology	lateral tibia area and total surface of wings	0.48	[34]
hind leg - wing morphology	lateral tibia area and cubital index	-0.14	[34]
hind leg morphology	lateral tibia area and length of corbicula hairs	0.08	[34]
hind leg - abdomen morphology	lateral tibia area and length of hairs in tergite 5	-0.19	[34]
hind leg morphology	lateral femur area and lateral metatarsus area	0.02	[34]
hind leg morphology	lateral femur area and hind leg area	0.58	[34]
hind leg - wing morphology	lateral femur area and surface of hindwing	0.01	[34]
hind leg - wing morphology	lateral femur area and surface of forewing	0.20	[34]
hind leg - wing morphology	lateral femur area and total surface of wings	0.15	[34]
hind leg - wing morphology	lateral femur area and cubital index	0.09	[34]

hind leg morphology	lateral femur area and length of corbicula hairs	0.13 [34]
hind leg - abdomen morphology	lateral femur area and length of hairs in tergite 5	0.66 [34]
hind leg morphology	lateral metatarsus area and hind leg area	0.65 [34]
hind leg - wing morphology	lateral metatarsus area and surface of hindwing	0.55 [34]
hind leg - wing morphology	lateral metatarsus area and surface of forewing	0.60 [34]
hind leg - wing morphology	lateral metatarsus area and total surface of wings	0.65 [34]
hind leg - wing morphology	lateral metatarsus area and cubital index	-0.32 [34]
hind leg morphology	lateral metatarsus area and length of corbicula hairs	0.06 [34]
hind leg - abdomen morphology	lateral metatarsus area and length of hairs in tergite 5	0.30 [34]
hind leg - wing morphology	hind leg area and surface of hindwing	0.58 [34]
hind leg - wing morphology	hind leg area and surface of forewing	0.55 [34]
hind leg - wing morphology	hind leg metatarsus area and total surface of wings	0.62 [34]
hind leg - wing morphology	hind leg metatarsus area and cubital index	-0.17 [34]
hind leg morphology	hind leg metatarsus area and length of corbicula hairs	0.13 [34]
hind leg - abdomen morphology	hind leg metatarsus area and length of hairs in tergite 5	0.17 [34]
wing morphology	surface of hindwing and surface of forewing	0.58 [34]
wing morphology	surface of hindwing and total surface of wings	0.81 [34]
wing morphology	surface of hindwing and cubital index	-0.36 [34]
hind leg - wing morphology	surface of hindwing and length of corbicula hairs	0.16 [34]
hindwing - abdomen morphology	surface of hindwing and length of hairs in tergite 5	0.16 [34]
wing morphology	surface of forewing and total surface of wings	-0.95 [34]
wing morphology	surface of forewing and cubital index	-0.19 [34]
hind leg - wing morphology	surface of forewing and length of corbicula hairs	0.06 [34]
hindwing - abdomen morphology	surface of forewing and length of hairs in tergite 5	-0.07 [34]
wing morphology	total surface of wings and cubital index	-0.28 [34]
hind leg - wing morphology	total surface of wings and length of corbicula hairs	0.11 [34]
hindwing - abdomen morphology	total surface of wings and length of hairs in tergite 5	0.03 [34]
hind leg - wing morphology	cubital index and length of corbicula hairs	0.21 [34]
hindwing - abdomen morphology	cubital index and length of hairs in tergite 5	-0.27 [34]
hind leg - abdomen morphology	length of corbicula hairs and length of hairs in tergite 5	-0.28 [34]
hygienic behavior traits	hygienic behavior in 24h and 48h	0.49 [73]
hygienic behavior traits	hygienic behavior in 24h and 72h	0.4 [73]
hygienic behavior traits	hygienic behavior in 48h and 72h	0.47 [73]
royal jelly production traits	royal jelly production per colony and larvae acceptance	0.42 [74]

royal jelly production traits	royal jelly production per cup and larvae acceptance	0.06	[74]
royal jelly production traits	royal jelly production per colony and per cup	0.29	[74]
hygienic behavior traits	hygienic behavior in 24h and 48h	0.98	[76]
honey production - colony aggressiveness	honey production and gentleness	-0.50	[81]
honey production - hoarding	honey production and syrup collection rate	0.48	[83]
honey production - propolis production	honey production and propolis production	0.58	[83]
honey production - hygienic behavior	honey production and hygienic behavior	-0.34	[83]
honey production - mite presence	honey production and mites on adult bees	-0.52	[83]
hoarding - propolis production	syrup collection rate and propolis production	0.67	[83]
hygienic behavior - hoarding	syrup collection rate and hygienic behavior	-0.24	[83]
hoarding - mite presence	syrup collection rate and mites on adult bees	-0.66	[83]
hygienic behavior - propolis production	propolis production and hygienic behavior	0.15	[83]
propolis production - mite presence	propolis production and mites on adult bees	0.49	[83]
hygienic behavior - mite presence	hygienic behavior and mites on adult bees	-0.82	[83]
hygienic behavior - mite presence	hygienic behavior in 24h and mites in adult bees	-0.02	[84]
hygienic behavior - mite presence	hygienic behavior in 24h and mites in brood	-0.01	[84]
hygienic behavior - mite presence	hygienic behavior in 24h and total mite reproduction rate	-0.48	[84]
hygienic behavior - mite presence	hygienic behavior in 24h and effective mite reproduction rate	0.01	[84]
mite presence traits	mites on adult bees and mites in brood	0.02	[84]
mite presence traits	mites on adult bees and total mite reproduction rate	-0.19	[84]
mite presence traits	mites on adult bees and effective mite reproduction rate	0.21	[84]
mite presence traits	mites in brood and total mite reproduction rate	0.46	[84]
mite presence traits	mites in brood and effective mite reproduction rate	0.06	[84]
mite presence traits	total mite reproduction rate and effective mite reproduction rate	0.43	[84]
honey production - colony aggressiveness	honey production and gentleness	-0.07	[88]
honey production - colony aggressiveness	honey production and calmness	-0.03	[88]
honey production - swarming	honey production and swarming	-0.82	[88]
colony aggressiveness traits	gentleness and calmness	0.91	[88]
colony aggressiveness traits	gentleness and swarming	0.65	[88]
colony aggressiveness - swarming	calmness and swarming	0.37	[88]

^a Studies with excluded genetic correlation coefficients: [1], [17].

Fig. S1. Phylogenetic tree containing all Hymenoptera species analyzed in this study.

Fig. S2. Broad and narrow-sense heritability estimates across the Hymenoptera. While median values are represented by the lines inside boxes, which span the first and third quartiles, sample sizes are provided in brackets. Heritability estimates were normalized (arcsin-transformed) and detrended by removing the effect of trait category. Estimate type (broad or narrow-sense) and a variance structure did not improve the model's likelihood, even though variance seemed higher for broad-sense estimates.

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Competitive males have higher quality sperm in a monogamous social bee

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Chapter 2.

Reproductive biology of stingless bees: identifying male quality traits and sexual selection episodes

Resumo

Introdução: O sucesso reprodutivo é determinado pela interação entre o sucesso de cópula e o sucesso de fertilização. Em espécies de insetos sociais com razão sexual enviesada para machos e rainhas monogâmicas, machos estão sob forte seleção sexual pré-copulatória, visto que devem competir com centenas de outros machos por uma oportunidade única de acasalamento. A qualidade do ejaculado também está sob seleção, já que rainhas são longevas e armazenam o esperma por toda a vida, de forma que ejaculados de alta qualidade permitem o desenvolvimento de colônias maiores e mais longevas, que por sua vez devem produzir mais rainhas (o único ganho direto em fitness para machos haplodiploides). Considerando a ação da seleção sexual pré e pós-copulatória nos caracteres dos machos, três cenários podem ser esperados: uma associação positiva, negativa ou nula entre a habilidade de acasalamento e a habilidade de fertilização do macho. Neste trabalho, exploramos estes três cenários na abelha sem ferrão *Scaptotrigona aff depilis*, em que machos se reúnem em grandes agregações e rainhas se acasalam com um único macho. A habilidade de acasalamento do macho foi avaliada por sua capacidade de atingir e persistir em uma agregação, enquanto viabilidade espermática, número e morfologia dos espermatozoides foram utilizados como indicadores de qualidade espermática.

Resultados: A viabilidade espermática foi associada à persistência do macho na agregação, e machos que persistiram por mais tempo apresentaram espermatozoides mais curtos e maior variação no comprimento do espermatozoide do que machos recém-chegados. No entanto, os caracteres espermáticos de machos que atingiram uma agregação não diferiram dos caracteres espermáticos de machos coletados no interior da colônia. Além disso, machos que permaneceram por mais tempo em agregações eram menores do que machos recém-chegados. O tamanho do macho e a viabilidade espermática não foram correlacionados, sugerindo que o padrão observado não foi devido a um trade-off na alocação de recursos.

Conclusões: A persistência dos machos na agregação parece selecionar machos mais competitivos com maior qualidade espermática. Nosso trabalho é o primeiro a revelar uma associação entre a habilidade competitiva do macho e o sucesso de fertilização em um inseto social monogâmico. Esse resultado traz evidências importantes sobre a evolução de caracteres de machos em insetos sociais e mecanismos gerais de seleção sexual.

Abstract

Background: Reproductive success is determined by the interplay between mating and fertilization success. In social insect species with male-biased sex ratios and queen monogamy, males face particularly strong pre-copulatory sexual selection since they must compete with thousands of other males for a unique mating opportunity. Ejaculate quality is also expected to be under selection, because queens are long-lived and store sperm for life, so males with higher quality ejaculates are expected to provide queens with larger and longer-lived colonies, which in turn may produce more daughter queens (the only direct fitness gains of haplodiploid males). Considering the action of pre and post-copulatory sexual selection on male traits, three scenarios might thus be expected: positive, negative or no association between male mating ability and fertilization success. Here we explored these scenarios in the stingless bee *Scaptotrigona aff. depilis*, where males gather in large aggregations and queens mate with a single male. Male mating ability was assessed through the capacity of a male to reach an aggregation and persist on it; while sperm viability, sperm number, and sperm morphology were used as proxies for sperm quality.

Results: Sperm viability was associated with persistence time in the aggregation, and males that persisted longer presented shorter spermatozoa and higher variation in sperm length than recently arrived males. However, sperm traits of males that reached aggregations did not differ from those of males collected inside their colonies. In addition, males that persisted longer in aggregations were smaller than other males. Male size and sperm viability were not correlated, suggesting that the observed patterns were not due to trade-offs in male resource allocation.

Conclusions: Persistence in male aggregations thus seems to select more competitive males with higher quality sperm. Our work is the first one to reveal an association between male competitive ability and fertilization success in a monogamous social insect. This finding sheds important light on the evolution of male traits in social insects and the general mechanisms of sexual selection

Introduction

Reproductive success is determined by the interplay between individual mating and fertilization success. While mating success is subject to pre-copulatory sexual selection, via male-male competition and female choice [1,2], fertilization success is usually related to post-copulatory sexual selection in species with multiple mating, through sperm competition [3] and cryptic female choice [4]. Considering these distinct selective episodes on males, attempts have been made to integrate pre and post-copulatory sexual selection, by understanding how males allocate limited resources into different traits [5,6] and what is the genetic basis of these trade-offs [7,8]. Pre- and post-copulatory sexual selection may exhibit synergistic or opposite effects, as pre-copulatory sexual selection outcomes may be reinforced by post-copulatory sexual selection [9,10], or in other cases, post-copulatory sexual selection may attenuate pre-copulatory sexual selection [8,11–13].

In monogamous species, selection for fertilization ability is expected to be more relaxed than in polygamous species, since the main selective pressure is to achieve copulation and insemination [14,15]. Here, male resource allocation should have been selected to invest into traits maximizing the chances of insemination, as sperm entering the female reproductive tract will not compete with sperm from other males. However, in monogamous species where egg fertilization does not occur immediately after mating, male fertilization ability may also be subject to selection. In these species, females normally possess sperm-storage organs, where sperm are kept viable until fertilization [16]. Both mating and fertilization abilities are thus expected to be under selection in males of

monogamous species with sperm-storage, making them particularly well suited to study post-copulatory sexual selection in the absence of sperm competition.

The social Hymenoptera (ants, bees and wasps) exhibit one of the longest sperm-storage periods in the animal kingdom, with queens mating once in life and storing sperm for several years, during which they continuously lay eggs [17–19]. In this group of haplodiploid insects, males are haploid because they originate from unfertilized eggs, whereas fertilized eggs give rise to diploid females. Since reproductive success is determined by the production of sexual offspring (males and daughter queens, also known as gynes), males only achieve direct fitness gains through the production of gynes [20]. As sexual offspring are only produced in strong and well established colonies, most sperm is used to produce sterile workers [21,22]. Male reproductive success is thus directly dependent on their fertilization capacity, which will translate into workers and later in gynes, so stored sperm is expected to be under selection for quantity and quality.

Stingless bees are an ideal model system to study the interplay between mating and fertilization success in the absence of sperm competition, given that they have extremely male-biased sex ratios (strong selection for mating success), and monandrous long-lived queens (strong selection for fertilization success) [18,23–27]. During reproductive events, hundreds to thousands of males gather in aggregations waiting for an opportunity to mate with a virgin queen [28,29]. This mating system results in intense male competition for mating, as males must find an aggregation, persist on it until a queen arrives, chase her on the flight, and finally achieve copulation and insemination. Whether the queen chooses a mate [30] remains unknown, but the extreme male-biased sex ratios imply that male competition is the predominant form of pre-copulatory sexual selection [2,18]. On the other hand, ejaculate quality is expected to be under selection, since queens live up to several years, mate only once in life, and maintain colonies that can harbor thousands of individuals [27,31,32]. Considering that selection is acting both before and after mating, three scenarios might be expected: 1) male competitive and fertilization abilities are

Figure 1. (a) Male aggregation of *Scaptotrigona* aff. *depilis*, above a colony of the same species. The individuals are paint-marked in the thorax. **(b)** Representation of the sampling designs performed to discriminate males with different competitive ability. In the first selection episode investigated, the capacity of males to reach an aggregation was analyzed (black arrows) and males collected inside the colonies were compared to males collected at aggregations. In the second selection episode, male persistence in the aggregations was analyzed (gray arrows), and new-coming males and males that persisted for three and five days were compared.

positively related, with competitive males also exhibiting high ejaculate quality; 2) male competitive and fertilization abilities are negatively related, with males exhibiting higher competitive ability or higher ejaculate quality, but not both; and 3) male competitive and fertilization abilities are uncorrelated.

Here we investigated the relationship between male competitive ability and fertilization success in the stingless bee *Scaptotrigona* aff. *depilis*, a species exhibiting large male aggregations [33] that are found throughout the year (Fig. 1a). Two contrasting hypotheses were explored: 1) male traits exposed to pre- and post-copulatory sexual selection are related (either positively, which predicts that more competitive males would exhibit higher quality sperm, or negatively, which predicts that competitive males would exhibit lower quality sperm, and males exhibiting high quality sperm would be less competitive); 2) pre-copulatory traits are unrelated to post-copulatory traits. To examine these hypotheses, we first identified a set of behavioral traits that successfully predicted male competitive ability (ability to reach and persist in an aggregation). We then employed a model-selection protocol to relate these traits to different indicators of ejaculate quality (sperm viability, sperm counts, and sperm length), thus effectively integrating mating and fertilization success. We also identified morphological traits under pre-copulatory sexual selection affecting male competitive ability and investigated potential trade-offs in male resource allocation into different traits. We discuss the implications of our findings for the evolution of male traits in social insects, the operation of sexual selection in this group, and the general theory on integrating pre and post-copulatory sexual selection.

Methods

Mating flights are rarely observed in stingless bees, making it difficult or impossible to compare males that mated successfully with males that failed to mate. Moreover, after mating, males lose their genitalia and die [18]. To account for these particular features,

male competitive ability was analyzed at two selection episodes before mating: 1) leaving the colony and reaching an aggregation, and 2) persisting in the aggregation (Fig. 1b). In the first selection episode, males collected inside colonies were compared to males from the same colonies that successfully reached an aggregation. In the second selection episode, the number of days spent at the aggregation was measured.

Morphological and sperm quality indicators were analyzed in all males. Since chemical and visual cues play important roles in the aggregation process [34], we expected that competitive males would present better sensory structures (larger eyes and longer antennae). In addition, as males showed conspicuous directional asymmetry in the compound eyes, we tested if the degree of asymmetry was associated with male performance. We also expected that competitive males would present larger body size, as larger sizes are related to higher mating success in other social insects [35–37]. To assess male fertilization ability, sperm viability, number, length, and length variation were measured. Males with higher fertilization ability were expected to exhibit higher sperm viability, which allows efficient sperm storage and fertilization [38–40]. Sperm number is also expected to be related to fertilization success, given that sufficient sperm are needed for long term fertilizations [19,41]. Since producing short sperm cells is less costly and allows the storage of higher amounts of sperm in the queen's spermathecae [18], we expected sperm length to be negatively associated to fertilization ability. Finally, as selection is expected to reduce sperm length around an optimal length [42], males with higher fertilization ability are also expected to exhibit lower sperm length variation.

Assessing mating success 1: from the colony to the aggregation

Five colonies of *Scaptotrigona* aff. *depilis* earlier inspected for male production were selected and kept in laboratory conditions (University of São Paulo, São Paulo, SP, Brazil). Each colony was maintained indoors, in a wooden box covered with glass lids and connected to the outside by a plastic tube, so the bees had free access to the environment. All brood combs with pre-emergent pupae were collected from the colonies and kept in individually

labeled Petri dishes placed inside an incubator ($28 \pm 1^\circ\text{C}$), with sugar water solution. Emerging males were paint-marked on the thorax (using UniPosca® pens) every second day, for four continuous days. Males from each colony were identified by the same color and were returned to the colony after marking. Since males were previously found to reach aggregations between 17 to 20 days after emergence (in our study location, Fig. S1), between 2 and 31 males were collected from each colony 15 days after marking (Table 1). These hive-collected males were kept in Petri dishes in the incubator and provided with sugar water solution *ad libitum*. Aggregations formed in the vicinity of the colonies were then daily inspected for marked males, which were collected for two continuous weeks. Four aggregations were found in the outside area of the laboratory and males were collected from all aggregations, to maximize sample size. Aggregation-collected males were kept in the incubator, following the same procedure described above, until sperm viability analyses were performed. In order to avoid great age differences among males from the two sampling groups (hive and aggregation), which could affect sperm viability measures (Fig. S2), males collected from the aggregations were analyzed together with the same number of hive-collected males. Males from three colonies were included in the analyses, since only two males of the remaining colonies were collected (Table 1).

Assessing mating success 2: persistence in aggregations

We then quantified persistence time in the aggregations, using different groups of males (all the previously marked males had died by the time we began assessing persistence time). We first selected a large aggregation and proceeded to mark all males on their thorax with the same color. The marking process continued throughout a full day, until only marked males were found in the aggregation. On the following day, new-coming males (with no paint marks) were marked with a different color, following the same procedure. This second group of males, which had a known arrival date, was then subsequently collected in the aggregation during the following five days. Males were collected haphazardly, using a net, and collection was always performed at the same interval time (1 to 3 pm) in the

Table 1. Comparing males collected inside colonies with males collected at aggregations. Data are presented for number of marked males, number of marked males collected inside the colonies 15 days after marking, and number and proportion of males collected at aggregations during the two weeks of inspection.

Colony	Number of marked males	Number of marked males collected inside the colonies	Number of marked males collected at aggregations	Number of marked males collected at aggregations (%)
1	507	31	51	10
2	461	30	32	7
3	362	30	24	6
4	110	26	2	2
5	77	2	1	1

defined days. Previous observations revealed that males show high fidelity to the visited aggregation, as less than 1% of the marked males were found in different aggregations. We followed this protocol to quantify persistence time in May 2014 (n = 233 marked males) and again in May 2015 (n = 68 marked males). In 2015, only small aggregations were found and males from two aggregations were followed (each aggregation marked with a different color, n = 30 and n = 38). Males were collected in the marking day (new-coming males – persistence of one day), after two days in the aggregation (males that persistence for three days), and after four days in the aggregation (males that persisted for five days). All males collected from the aggregations were kept inside an incubator ($28 \pm 1^\circ\text{C}$) in Petri dishes with sugar water solution *ad libitum* for one day, until dissection for sperm viability analyses were performed. Assays were previously performed in order to test whether male age affected sperm viability, as found for honey bee drones [40]. Emerging males from two colonies were collected and kept in individual wooden boxes, inside an incubator, and receiving sugar syrup and pollen *ad libitum*. Weekly, 10 males were sampled and sperm viability was analyzed, repeating this procedure for five weeks. As sperm viability was

found to decrease with male age (Fig. S2), and males were at least 17 days old when they arrived at the aggregation, we did not collect males that persisted more than five days in the aggregations (around three weeks old).

Measurement of male traits

To assess morphological traits (Table S1), males were kept frozen after dissections (see below). Male head and thorax were photographed using a stereomicroscope coupled with a camera (20x magnification) and measurements were made using the open source software ImageJ [43]. Male heads were placed over foam sheet material and covered with a glass lid. We used intertegular distance (the shortest distance between the bases of the tegulae) as an estimate of bee size [44], and also quantified maximum head length, total eye area (area of the left and right compound eyes in frontal view) and antennae length. Eye asymmetry was calculated as the difference between the left eye area and right eye area.

To assess ejaculate quality we measured sperm viability (relative proportion of live sperm cells), total sperm cell number and sperm length, following standard protocols [45]. Male's abdomens were pressed until the exposure of the endophallus and the two seminal vesicles, which were then removed with forceps and placed in 120µl of Hayes solution (9g of NaCl, 2g of CaCl₂, 0.2g of KCl, and 0.1g NaHCO₃, to 1l of deionized water, pH adjusted to 8.7). Vesicles were ruptured and emptied using a pin and the semen solution was gently homogenized with a pipette. We excluded samples if the male died before dissection or the vesicles ruptured inside the male's abdomen. To perform sperm viability analysis, 5µl of the semen solution was stained with 5µl of SYBR14 working solution (2µl of SYBR14 in 98µl of Hayes solution) and 2µl of propidium iodide (LIVE/DEAD® Sperm Viability Kit). Microscope slides were inspected in a fluorescence microscope at 40x magnification and 400 cells in each sample were analyzed, counting live (green), dead (red) and dying (both colors) spermatozoa. Double stained cells represented less than 5% of counts in all samples and were considered as live spermatozoa [19]. When comparing sperm viability of hive-collected and aggregation-collected males, we only included data from the first week

of sampling, because hive-collected males that remained in the incubator for two weeks were not comparable to freshly collected males from the aggregations. Sperm length was assessed by spreading 10 μ l of sperm solution in a microscope slide. Each sample was air dried and stained with 30 μ l of DAPI (4ng/ μ l to 100ng/ μ l) and photographs were taken at 20x magnification in a fluorescence microscope. For each male, 10 sperm cells were measured using the software ImageJ, and mean sperm length (from the tip of the head to the end of the tail) and the coefficient of variation (ratio of standard deviation to the mean) were calculated. Sperm counts were only performed for males collected in 2015, and were assessed by diluting 12 μ l of the semen solution in 988 μ l of Hayes solution. Five samples of 1 μ l of the diluted sperm were added to a microscope slide. Each sample was air-dried and stained with 5 μ l DAPI (4ng/ μ l) and all sperm heads from three samples from each male were counted at 40x magnification. Sperm counts were then multiplied by the dilution factor (x 10,000).

Statistical analyses

In order to identify which morphological traits were exposed to pre-copulatory sexual selection, male competitive ability was modeled in relation to the different morphological traits assessed. Since some morphological traits (head width, intertegular distance, eye area and left antennae length) were positively correlated, these variables were merged using Principal Components Analysis. The first component (PC1) explained 59.8% of the variation in male morphology in the sample of hive-collected and aggregation-collected males (scores were positively correlated to the size of morphological traits), and 75.6% of the variation in the sample of males with different persistence times (scores were negatively correlated with the size of morphological traits, and were thus multiplied by -1). The probability of reaching the aggregation (presence/absence in the aggregation) was analyzed fitting a generalized linear mixed effects model with binomial distribution, including a random effect for the colony of origin and an observation-level random effect to account for overdispersion. Colony of origin was not included as a fixed factor in our models, because

we were not interested in the particular effects of colony on male performance. Persistence time (number of days in the aggregation) was modeled fitting a generalized linear mixed effects model with Poisson distribution, with a random factor for aggregation identity. In both analyses, male size (PC1) and eye asymmetry were included as predictors in a full model. Predictors were scaled, by centering the mean on zero and dividing by the standard deviation, to aid model convergence. We used Likelihood Ratio Tests (LRT, $\alpha = 0.05$) to reduce the number of predictor to those significantly improving our model's log-likelihood. We compared full models with models where each predictor was excluded, and selected those which significantly (LRT $p < 0.05$) increased the model's log-likelihood (as described in [46]). We thus assessed the individual effect of all predictors accounting for other covariates, and tested all possible combination of predictors. Overdispersion was diagnosed by calculating the sum of squared Pearson residuals and comparing it to the residual degrees of freedom (using a χ^2 distribution to estimate the p-value) and by plotting residuals vs. fitted values (sperm length data) [49]. The significance of estimates in the final model was also tested using a Wald test.

Models relating male fertilization ability and male competitive ability were constructed to assess the relationship between traits exposed to pre- and post-copulatory sexual selection. We used the ability to reach and persist in an aggregation as a direct measure of male quality, given that the morphological traits influencing male competitive ability exhibited higher variation and only represent indirect measures (see below). Each sperm trait was modeled using linear mixed effects models, with the sperm trait as response variable and male competitive ability (sampling site or sampling time) as predictor (see Table 2 for details). Sperm viability was modeled as the proportion of live sperm cells, using a binomial distribution. When comparing males from the hives versus males from the aggregations, the number of days in the incubator was also included as fixed factor (interacting with sampling site), in order to account for a possible influence of the incubator on sperm viability. Sperm number was modeled as the number of sperm cells in each of the three samples from each male, using a Poisson distribution and a random effect for replicate.

Sperm length and sperm length variation were modeled using a linear mixed model. Random effects were included to account for the dependence among samples (samples from the same colony, aggregation or male) or to account for overdispersion when necessary (observation-level random effect). Model selection was performed using Likelihood Ratio Tests as described above.

Finally, we correlated the morphological and sperm measures selected in the best models in the previous analyses, to check for trade-offs in resource allocation, using Spearman's correlation. All analyses were performed in R, using the *lme4* [47] and *Hmisc* [48] packages. Plots were produced with the package *ggplot2* [49].

Results

During two weeks, about 5% of all marked males reached four different aggregations outdoors (Table 1). The number of marked males in each colony was positively correlated to the number of marked males collected at the aggregations ($r_s = 0.96$, $p = 0.008$, $n = 5$).

The probability of reaching an aggregation was not influenced by any male morphological trait, while the number of days that a male persisted at the aggregation was best predicted by male size (Table 3). Males that persisted longer at aggregations were usually smaller (Table 4, Fig. 2). Even though directional asymmetry was found in the male's compound eyes (Fig. 2), with left eyes being always larger than right eyes (except for one male), the degree of eye asymmetry did not affect male competitive ability.

No sperm trait affected the probability of a male reaching an aggregation, as in our comparisons of hive and aggregation-collected males the best-fitting models were the intercept-only models (Tables 5 and 6, Fig 3a-c). The time spent in the incubator did not affect sperm viability. On the other hand, sperm viability, length and length variation were associated with persistence time in the aggregations (Table 5, Fig. 3d-g). Males that

persisted longer at the aggregations exhibited shorter spermatozoa and higher sperm length variation. However, the differences in sperm viability between days were not significant (Table 6). Male persistence time in the aggregations was not associated with sperm number.

Table 2. Models relating male sperm quality to male competitive ability. Each trait was analyzed according to a particular probability distribution and random effects were included to account for dependence among samples or overdispersion.

Selection episode	Model	Response	Probability distribution	Predictors	Random effect
Colony - Aggregation	sperm viability	proportion of live to dead sperm cells	Binomial	sampling site and number of days in incubator	colony and male ^a
	sperm length	sperm length	Normal	sampling site	colony and male
	sperm length variation	sperm CV	Normal	sampling site	colony
Persistence time in the aggregation	sperm viability	proportion of live to dead sperm cells	Binomial	days at aggregation	aggregation and male ^a
	sperm number	number of sperm cells	Poisson	days at aggregation	aggregation and male
	sperm length	sperm length	Normal	days at aggregation	aggregation and male
	sperm length variation	sperm CV	Normal	days at aggregation	aggregation

^a observation level random effect to account for overdispersion

Table 3. Model selection table for models relating competitive ability and male morphology. The p-values and degrees of freedom refer to Likelihood Ratio Tests (using a χ^2 test statistic), in which the full model was compared to a reduced model without each of the predictor variables. Parameters estimates of best models are presented in Table 4.

Selection episode	N.obs	Response	Random effect	Starting model	Fixed effect removed	Degrees of freedom	χ^2	P-value
Colony - Aggregation	110	Probability of reaching an aggregation	colony and male ^a	male size + eye asymmetry	male size	1	0.65	0.42
					eye asymmetry	1	0.05	0.82
					male size	1	0.67	0.41
					asymmetry	1	0.07	0.79
Persistence time in the aggregation	65	Number of days at the aggregation	aggregation	male size + eye asymmetry	male size	1	4.56	0.03
					eye asymmetry	1	0.76	0.38
					male size	1	5.17	0.02
					asymmetry	1	1.38	0.24

^a observation level random effect to account for overdispersion.

Figure 2. Morphological traits related to male competitive ability (male's ability to reach and persist in aggregations). Neither male body size nor eye asymmetry influenced the male's ability to reach an aggregation (a-b). Males that persisted more days in the aggregations were smaller, but did not show different eye asymmetry (c-d). Median values are represented by the lines inside the boxes, which span the first and third quartiles, and points represent data outside 1.5 times the interquartile range above the upper quartile and below the lower quartile. Data from different colonies and aggregations were merged (see colony and aggregation effects in Figure S3).

Table 4. Best-fitting model for the relationship between competitive ability and male morphology. Males that persisted longer at the aggregation were smaller. Parameter estimates, standard errors and p-values are given.

Selection episode	Response	Predictor	Parameter estimate	SE	P-value
Persistence time in the aggregations	Number of days at the aggregation	male size	-1.48	0.65	0.02

Figure 3. *Scaptotrigona aff. depilis* male at an aggregation. (a-c) Sperm traits of males collected inside the colonies and males that reached aggregations. (d-g) Sperm traits of males with different persistence times in the aggregations (new-coming males and males that persisted for three or five days). Median values are represented by the lines inside the boxes, which span the first and third quartiles, and points represent data outside 1.5 times the interquartile range above the upper quartile and below the lower quartile. Data from different colonies and aggregations were merged (see colony and aggregation effects in Figure S4).

Correlation between male size (which affected competitive ability) and sperm traits revealed that body size was not significantly correlated to sperm viability ($r_s = 0.06$, $p = 0.49$, $n = 148$), but was marginally correlated to mean sperm length ($r_s = 0.16$, $p = 0.053$, $n = 153$) and negatively correlated to sperm length variation ($r_s = -0.17$, $p = 0.04$, $n = 153$).

Discussion

Our results reveal that male competitive ability in *Scaptotrigona* aff. *depilis* is associated with male size, but not by eye asymmetry. Sperm traits from males that reached aggregations did not differ from sperm traits of hive-collected males, whereas male persistence time in the aggregations was associated with variation in sperm viability, sperm length and sperm length variation. Males that persisted longer in the aggregations usually showed shorter sperm cells and higher sperm length variation. Overall, our findings suggest that aggregations select high quality males, as male competitive ability was positively related to ejaculate quality.

a) Identifying morphological traits under pre-copulatory sexual selection

Colonies that produced more males also presented a higher number of males in the aggregations, as found in honey bee drone [35]. This finding suggests that colony fitness may be enhanced by the number of males produced. However, male quality could also influence colony fitness by affecting male performance in the aggregations. Indeed, competitive males were found to be smaller, contradicting the initial predictions and previous studies with social insects, which found a positive association between male size and mating success [35–37]. This finding could be related to a trade-off between investment in body size and sperm traits, although no correlations between the assessed traits were found (see below). In swarming species, even though larger males show greater longevity, smaller males have higher mating success [50], which is attributed to agility in flight while

in the swarm [51]. Male contests, on the other hand, usually select for larger body size [18]. In our case, as fights do not occur among males in aggregations of *S. aff. depilis* (personal observation), investing in larger body sizes would not provide a competition advantage. In contrast, in *Melipona favosa* aggressive behaviors have been observed between males in the aggregation [28]. In addition, larger males may incur in higher energy consumption, which may hinder long distance flights during the search for an aggregation, or compromise the ability to persist longer in an aggregation [52]. Male size may also be subject to other forms of natural selection, as longevity, predator avoidance [53] or immune defense.

Most males exhibited some degree of directional asymmetry in the compound eyes (the left eyes always larger than the right eyes). Even though directional asymmetries can be related to male quality signals, as in birds [54] and crickets [55], the degree of eye asymmetry did not influence male competitive ability in our study. More studies are thus needed to understand the possible adaptive function of the marked eye asymmetry in stingless bee males and if the larger left eye may act as a compensation for a differential visual performance.

b) Relationship between male competitive and fertilization abilities

Even though males spent up to five days in the incubator, sperm viability did not vary with the number of days males were kept in the incubator. This indicates that short term male confinement is feasible for sperm viability analysis, which greatly facilitates experimental designs with large sample sizes. Sperm viability did not vary between hive-collected males and males that reached aggregations, but it was associated to male persistence time at the aggregation (including sperm viability as a predictor that significantly improved our model's log-likelihood, Table 5). Even though the effect sperm viability was weak (Table 6), males that persisted for three days tended to show higher sperm viability than new-coming males, while males that persisted for five days tended to show lower sperm viability. Future studies are thus needed to explore this effect in more detail, and understand why sperm viability increases at three days in the aggregation in spite of the negative effect of aging.

Table 5. Model selection table for different models testing the relationship between male competitive ability and fertilization success (sperm traits). The p-values and degrees of freedom refer to Likelihood Ratio Tests (using a χ^2 test statistic), in which the full model was compared to a reduced model without each of the predictor variables. Parameters estimates of best models are presented in Table 6.

Selection episode	N. obs	Response	Starting model	Fixed effect removed	Degrees of freedom	χ^2	P-value
Colony - Aggregation	47	Sperm viability	sampling site*days in incubator	interaction	1	0.05	0.82
	1070		sampling site + days in incubator	sampling site	1	0.29	0.59
	107		sampling site + days in incubator	days in incubator	1	0.004	0.95
			sampling site	sampling site	1	1.82	0.18
			days in incubator	days in incubator	1	1.54	0.21
		Sperm length	sampling site	sampling site	1	0.01	0.91
		Sperm length variation	sampling site	sampling site	1	0.12	0.73
Persistence time in the aggregation	60	Sperm viability	days at aggregation	days at aggregation	2	7.28	0.03
	90	Sperm number	days at aggregation	days at aggregation	2	3.77	0.15
	609	Sperm length	days at aggregation	days at aggregation	2	12.70	< 0.01
	61	Sperm length variation	days at aggregation	days at aggregation	2	16.07	< 0.001

Table 6. Best-fitting models describing the relationship between male competitive ability and fertilization success (sperm traits). For each predictor, parameter estimates, standard errors and p-values are given.

Selection episode	Response	Predictor	Parameter estimate	SE	P-value
Persistence time in the aggregation	Sperm viability	Days at aggregation	3 days 0.26	0.18	0.15
			5 days -0.24	0.18	0.18
	Sperm length	Days at aggregation	3 days -3.36	0.98	< 0.01
			5 days -3.02	0.99	< 0.01
	Sperm length variation	Days at aggregation	3 days 0.03	0.08	< 0.001
			5 days 0.03	0.08	< 0.01

Although a virgin queen mating with males that stay five or more days in the aggregation would lower her fertilization success, it remains unclear if the mating ability of males also changes through time, perhaps decreasing the chances of an older male mating with a queen.

On the other hand, sperm number did not affect male persistence time at the aggregation. Observed sperm number was in agreement with previous sperm count estimates in *Scaptotrigona* queen spermathecae [56], suggesting that all sperm is transferred from the male to the queen during mating. This result suggests that sperm traits other than sperm number may influence male fertilization ability. Sperm viability for instance may be more relevant for fertilization potential, as sperm cells must be live to provide successful egg fertilization and higher amounts of sperm may compromise viability.

Sperm length was negatively related to male persistence time in the aggregation. The production of short sperm cells is expected to be less costly [57], which could allow the production of a higher number of sperm cells or the reduction of the resources allocated to sperm production. Also, shorter cells enable a higher amount of sperm to be stored in queen's spermathecae, resulting in higher long term fertilization potential if spermathecae volume is constrained [18].

Even though males that persisted longer in the aggregations presented shorter sperm cells, sperm length was also more variable, contradicting our initial expectations. This suggests no selective pressure acting to reduce sperm length variation, as described in polyandrous social species [42]. Previous work with *Bombus terrestris* revealed that sperm stored in queen's spermathecae was less variable than sperm from male ejaculates, suggesting that during the storing process variance is removed by selecting a certain sperm length [58]. Hence, active (queen controlled) or passive (sperm related) processes occurring after mating may reduce variance in sperm length.

c) Resource allocation trade-offs and concluding remarks

The fact that smaller males with higher sperm quality were selected in the aggregations suggests a trade-off between resource allocation in size and sperm quality, as found in leafcutter ant males [59]. However, we did not find a significant negative correlation between male size, sperm viability or sperm length. Our results thus fail to support trade-offs in resource allocation among these traits and suggest that two distinct selection episodes occur in male aggregations: one selecting smaller males, and the other selecting males with higher quality sperm.

Although no morphological or ejaculate difference was found between hive-collected and aggregation-collected males, we highlight that the studied colonies were very close to the aggregations (less than 30m), which may have influenced our assays. On the other hand, male persistence in aggregations seems to select more competitive and fertile males. From the males that reach an aggregation, only the fittest ones are able to find shelter at night, avoid predators, cope with depleting energy reserves, and then return to the aggregation on the following days. Our results thus suggest that selective pressures acting on male aggregations may shape the evolution of male traits [18,35,60,61]. As competitive males exhibited higher ejaculate quality, male aggregations may act as an indirect form of mate selection for queens, enhancing male competition through male persistence [62]. Similar processes were described for male swarms in paper wasps, in which endurance competition acts through male display duration [60]. This may be advantageous for the female when there is low opportunity for direct mate choice [62]. However, the mechanisms underlying direct female choice remain to be investigated [30].

Our results bring evidence of sexual selection acting on stingless bee males and reveal that competitive males exhibit higher fertilization potential, corroborating the hypothesis that male competitive and fertilization abilities are directly related. Male competition and long term sperm storage thus seem strong selective pressures shaping male traits in stingless bees. More generally, our findings reveal that ejaculate traits can be under post-

copulatory sexual selection even in the absence of sperm competition. In contrast to our results, most studies relating male traits under pre- and post-copulatory sexual selection reveal trade-offs [13], although positive correlations have also been found [9,10]. Models describing the evolution of sexual traits present two distinct scenarios according to the different mechanisms of male-male competition. Where contest competition occurs, males usually exhibit high investment in pre-copulatory sexual traits (weapons or ornaments), resulting in high female monopolization, and trade-offs between pre- and post-copulatory sexual traits are expected [63,64]. On the other hand, in cases of scramble competition in polygynous species, the probability of female monopolization is low, and positive covariation is expected between pre and post-copulatory sexual traits [63]. Stingless bee mating system is in agreement with the second type, as it exhibits scramble competition features [18] and a positive relation between mating and fertilization abilities, but under monogamy. Even though males do not remate in this group, the mating system is marked by mate search and low opportunity for mate monopolization. Our findings are thus consistent with the theoretical expectations of scramble competition models in polygynous species and highlight that mechanisms other than sperm competition, such as long term sperm storage, may lead to a positive association between competition and fertilization ability.

Conclusions

Stingless bee male aggregations seem to select more competitive males with higher quality sperm. Strong male competition and long term sperm storage are likely selective pressures shaping male traits in this group, showing that sperm quality may be under selection even in the absence of sperm competition. Our work is the first one to reveal an association between male competitive ability and fertilization success in a monogamous social insect. This finding sheds important light on the evolution of male traits in social insects and the general mechanisms of sexual selection.

List of abbreviations

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Additional file 1: Table S1. Male traits assessed in this study (DOCX).

Additional file 2: Figure S1. Arrival of mature *Scaptotrigona* aff. *depilis* males in aggregations, according to male age (days after emergence). Emerging males from three colonies were paint-marked in the thorax (each colony identified with a different color) and returned to their origin colonies (number of marked males: blue 206, beige 162, and green 60). Male aggregations were then inspected daily for marked males, which were counted when observed (TIFF).

Additional file 3: Figure S2. Aging effect on male sperm viability (proportion of live to dead sperm cells), for males of two different colonies (each color represents a colony). Between nine and ten males per colony were analyzed weekly, from one week old to five weeks old. Median values are represented by the lines inside the boxes, which span the first and third quartiles, and points represent outliers (TIFF).

Additional file 4: Figure S3. Morphological traits related to male competitive ability (male's ability to reach and persist in aggregations). Neither male body size nor eye asymmetry

influenced the male's ability to reach an aggregation (a-b, each color represents a colony). Males that persisted more days in the aggregations were smaller, but did not show different eye asymmetry (c-d). Each color represents an aggregation (one aggregation was used in 2014 and two aggregations were used in 2015). Median values are represented by the lines inside the boxes, which span the first and third quartiles, and points represent data outside 1.5 times the interquartile range above the upper quartile and below the lower quartile (TIFF).

Additional file 5: Figure S4. (a-c) Sperm traits of males collected inside the colonies and males that reached aggregations. Each color represents a colony. (d-g) Sperm traits of males with different persistence times in the aggregations (new-coming males and males that persisted for three or five days). Each color represents an aggregation (one aggregation was used in 2014 and two aggregations were used in 2015). Median values are represented by the lines inside the boxes, which span the first and third quartiles, and points represent data outside 1.5 times the interquartile range above the upper quartile and below the lower quartile (TIFF).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SK and RJ designed the study. SK and HM performed the mark and recapture of male bees, sperm analyses, and morphological measurements. SK and RJ analyzed the data. SK wrote the first manuscript draft and all authors participated in the improvement of the manuscript and approved the final version.

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Supplementary Material – Chapter 2

Table S1. Male traits assessed in this study. Morphological traits were measured to identify predictors of male competitive ability and sperm quality traits were measured to test the relationship between male fertilization success and competitive ability.

Trait type	Variable	Definition
Behavioral traits (male competitive ability)	success in reaching an aggregation	marked males collected in an aggregation and collected inside the colonies (binomial variable)
	persistence time in the aggregation	number of days a male persisted in the aggregation (categorical variable: new-coming males, males persisting three and five days in the aggregation)
Morphological traits	intertegular span	shortest distance between the bases of the tegulae
	head width	maximum head length (frontal view)
	eye area	area of the left and right eyes (frontal view)
	antennae length	length of the left and right antennae
	eye asymmetry	difference between left and right eye area
	male size (PC1)	first PCA score (PCA included intertegular span, head width, eye area and left antennae length)
Sperm quality traits	sperm viability	proportion of live sperm cells
	sperm count	estimated number of sperm cells in the seminal vesicles
	sperm length	length of sperm cell, from head to tail
	sperm length variation	coefficient of variation of sperm cells length (sd/mean)

Figure S1. Arrival of mature *Scaptotrigona* aff. *depilis* males in aggregations, according to male age (days after emergence). Emerging males from three colonies were paint-marked in the thorax (each colony identified with a different color) and returned to their origin colonies (number of marked males: blue 206, beige 162, and green 60). Male aggregations were then inspected daily for marked males, which were counted when observed.

Figure S2. Aging effect on male sperm viability (proportion of live to dead sperm cells), for males of two different colonies (each color represents a colony). Between nine and ten males per colony were analyzed weekly, from one week old to five weeks old. Median values are represented by the lines inside the boxes, which span the first and third quartiles, and points represent outliers.

Figure S3. Morphological traits related to male competitive ability (male's ability to reach and persist in aggregations). Neither male body size nor eye asymmetry influenced the male's ability to reach an aggregation (a-b, each color represents a colony). Males that persisted more days in the aggregations were smaller, but did not show different eye asymmetry (c-d). Each color represents an aggregation (one aggregation was used in 2014 and two aggregations were used in 2015). Median values are represented by the lines inside the boxes, which span the first and third quartiles, and points represent data outside 1.5 times the interquartile range above the upper quartile and below the lower quartile.

Figure S4. (a-c) Sperm traits of males collected inside the colonies and males that reached aggregations. Each color represents a colony. (d-g) Sperm traits of males with different persistence times in the aggregations (new-coming males and males that persisted for three or five days). Each color represents an aggregation (one aggregation was used in 2014 and two aggregations were used in 2015). Median values are represented by the lines inside the boxes, which span the first and third quartiles, and points represent data outside 1.5 times the interquartile range above the upper quartile and below the lower quartile.

**When size matters: heritability of male traits reveals selection for sperm length in
a monogamous stingless bee (*Scaptotrigona* aff. *depilis*)**

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Chapter 3.

Quantitative genetic parameters of stingless bee male traits important for bee breeding

Resumo

A atuação da seleção sexual em machos de insetos sociais ainda é um mistério. Em machos haploides dos Hymenoptera sociais, o sucesso reprodutivo depende da produção de novas rainhas, o que requer um grande investimento em operárias previamente à reprodução da colônia. Como rainhas acasalam uma única vez em sua vida e armazenam o esperma para utilizar em fertilizações ao longo da vida, é esperada uma forte seleção em direção ao aumento da qualidade e quantidade do esperma. A fim de avaliar como a seleção atua na evolução dos caracteres dos machos na abelha sem ferrão *Scaptotrigona aff. depilis*, estimamos a herdabilidade em quatro caracteres (viabilidade espermática, número de espermatozoides, comprimento do espermatozoide e tamanho do macho) e suas respectivas correlações genéticas. Utilizamos um modelo misto usando Máxima Verossimilhança Restrita, com machos coletados em seis colônias e em duas gerações consecutivas ($n = 172$). A herdabilidade para o comprimento do espermatozoide foi menor do que a herdabilidade para os demais caracteres, sugerindo que o comprimento do espermatozoide está sob maior pressão seletiva. As correlações genéticas entre o tamanho do macho e o número de espermatozoides, e entre o tamanho do macho e o comprimento do espermatozoide foram positivas, embora não significativamente maiores que zero. Nossos resultados confirmam resultados anteriores, mostrando que agregações de machos selecionam machos com espermatozoides mais curtos, o que pode ser uma adaptação no sentido de maximizar o armazenamento de esperma, visto que células menores ocupam menor volume. Esse é o primeiro estudo de genética quantitativa utilizando Máxima Verossimilhança Restrita para avaliar a herdabilidade e correlações genéticas em machos de insetos sociais. Em geral, nossos resultados revelam que caracteres do ejaculado podem estar sob seleção, mesmo na ausência de competição espermática.

Abstract

The operation of sexual selection on social insect males remains a puzzle. In the haploid social Hymenoptera males, fitness depends on the production of new queens, which requires a large investment in sterile workers prior to colony reproduction. Since queens mate only once in life and store sperm for use in fertilization throughout their lives, selection for sperm quality and amount is expected to be particularly strong. In order to assess how selection acts on male trait evolution in the stingless bee *Scaptotrigona aff. depilis*, we estimated heritability for four traits (sperm viability, sperm counts, sperm length, and male size), and its respective genetic correlations. We employed a mixed model using Restricted Maximum Likelihood, with males sampled from six colonies and two consecutive generations ($n = 172$). Heritability for sperm length was lower than heritability estimates for other traits, suggesting that sperm length is under stronger selection. Genetic correlations between male size and sperm counts, and male size and sperm length were positive, albeit not significantly greater than zero. Our results agree with previous findings showing that male aggregations select males with shorter sperm cells, which may be an adaptation to maximize sperm storage since shorter cells occupy less volume. Ours is the first quantitative genetic study employing Restricted Maximum Likelihood to assess heritability and genetic correlations in social insect male traits. Overall, our findings reveal that sperm traits can be under selection even in the absence of sperm competition.

Introduction

The evolution of social insect male traits remains poorly understood, as few studies have assessed how sexual selection operates in this group (Abell et al. 1999, Wiernasz et al. 2001, Couvillon et al. 2010, Jaffé et al. 2012, Koffler et al. 2016). Earlier attempts have been made to understand how competition for mating shapes male morphology (Abell et al. 1999, Wiernasz et al. 2001, Couvillon et al. 2010). However, ejaculate evolution is expected to be under more specific selective pressures. Males of the haplodiploid social Hymenoptera (ants, bees and wasps) originate from unfertilized eggs, so to achieve fitness gains these haploid males must sire reproductive females (queens). This implies that most sperm are used for the production of sterile worker offspring, and that male fitness gains only arrive when colonies are large enough to start producing queens. Male fitness gains can also be realized through the production of males by unmated workers, albeit worker reproduction is rare or absent in some groups (Seeley 1985, Tóth et al. 2002), and males usually appear in large and well established colonies (Oster & Wilson, 1978). As Hymenoptera queens mate only once in life, and store sperm in a specialized organ (the spermatheca) for use in egg fertilization throughout their lives, ejaculate quality is expected to be under strong selection (Boomsma et al. 2005). Specifically, a large number of stored sperm, high sperm viability, and high sperm longevity would ensure long term sperm storage and increased fertilizations (Al-Lawati et al. 2009). In addition, selection is also expected to shape more competitive ejaculates in species where queens mate with multiple males (den Boer et al. 2010, Fitzpatrick and Baer 2011), although selective pressures resulting from sperm competition are likely to differ from those other forms of selection. Studies aiming to

disentangle these selective pressures and assess the effect of long-term limited storage on male ejaculate should thus focus on singly-mating (monandrous) species (Simmons 2001).

Whereas selection acts on traits that exhibit some degree of additive genetic variance, the ratio between genetic and environmental variance determines how selection operates (Lynch and Walsh 1998). In addition, genetic correlations between traits may favor or constrain selection on each trait (Cheverud 1996). Previous studies using different animal groups revealed that fitness-related traits show lower heritability estimates (rate between additive genetic variance and total phenotypic variance) (Mousseau and Roff 1987, Postma 2014, Koffler et al. 2017), which was interpreted as a reduction in additive genetic variance due to fixation of optimum condition traits (Fisher 1930). However, when calculating coefficients of variation (standardizing the variance by trait mean), the additive coefficient of genetic variance (CVa) revealed that low-heritability traits did not always show low additive genetic variance, but that residual variance may also be high (CVr), thus reducing heritability estimates (Houle 1992, Merilä and Sheldon 2000).

Aiming to assess the operation of selection on male traits in a monandrous social insect, here we studied the stingless bee *Scaptotrigona* aff. *depilis*. This species exhibits large and long-lived colonies, and male aggregations with high male-male competition are formed during reproductive events (Engels and Engels 1984, Mueller et al. 2012). Behavioral studies revealed that male traits involved in copula are positively related to traits related with fertilization (Koffler et al. 2016). Specifically, competitive males that persisted longer in these aggregations were smaller, and showed higher sperm viability, shorter sperm length, and higher sperm length variation. However, these traits were not correlated, raising questions about the genetic architecture of male traits. Based on these previous findings, our aim was to assess how selection acts upon different male traits and how their expression is genetically correlated. Additionally, *S. aff. depilis* has key economic value in the beekeeping and pollination industries (Cortopassi-Laurino et al. 2006, Slaa et al. 2006). However, knowledge gaps on the reproductive biology of this stingless bee still constrain

breeding initiatives. Our work thus provides important insights for stingless bee breeding programs, especially regarding male selection for natural or instrumental insemination of queens in order to produce long-lived and healthy colonies.

Materials & Methods

Experimental design

Because heritability estimation using mixed models allows combining different breeding designs, it generally results in more accurate estimates (Wilson et al. 2010, Postma 2014). By feeding the models with varying degrees of genetic relatedness between individuals, this approach facilitates the inclusion of individuals scattered across complex pedigrees. This is especially well suited for studying social insect males, since they are haploid and do not produce direct male offspring. To obtain a gradient of relatedness coefficients from individuals sampled from different colonies and generations, we gathered six colonies of *Scaptotrigona* aff. *depilis*. Colonies were kept for 18 months during which their queens were removed to stimulate queen supersedure (and generate a second generation of offspring). Brood combs were removed from the colonies and cells were opened for mature bee emergence. All males emerging were collected and transferred to wooden boxes containing recently emerged workers from the same colony, pollen, and sugar syrup. These wooden boxes were kept inside an incubator at 28°C ($\pm 1^\circ\text{C}$), and food was replaced three times per week. Male sampling for the first generation was performed between May and June 2016, and between August 2016 and February 2017 for the second generation.

Male traits

Males were dissected 14 to 16 days after emergence, allowing male maturation and semen transfer from the testicles to the vesicles (Meneses et al. 2014). Between 9 and 20 males were sampled from each colony, in each generation ($n = 172$). To assess ejaculate quality we

measured sperm viability (relative proportion of live sperm cells), total sperm cell number and sperm length, following the protocols adapted to stingless bees (Meneses et al. 2014). Male's abdomen was pressed until the exposure of the endophallus and the two seminal vesicles, which were then removed with forceps and placed in 120µl of Hayes solution (9g of NaCl, 2g of CaCl₂, 0.2g of KCl, and 0.1g NaHCO₃, to 1l of deionized water, pH adjusted to 8.7). Vesicles were ruptured and emptied using a pin and the semen solution was gently homogenized with a pipette. We excluded samples where the male died before dissection or the vesicles ruptured inside the male's abdomen. For sperm viability analysis, 5µl of the semen solution was stained with 5µl of SYBR14 working solution (2µl of SYBR14 in 98µl of Hayes solution) and 2µl of propidium iodide (LIVE/DEAD® Sperm Viability Kit). Microscope slides were inspected in a fluorescence microscope at 40x magnification and 400 cells in each sample were analyzed, counting live (green), dead (red) and dying (both colors) spermatozoa. Double stained cells represented less than 5% of counts in all samples and were considered as live spermatozoa (den Boer et al. 2009). Sperm counts were assessed by diluting 12µl of the semen solution in 988µl of Hayes solution. Five samples of 1µl of the diluted sperm were added to a microscope slide. Each sample was air-dried and stained with 5µl DAPI and all sperm heads from three samples from each male were counted at 40x magnification. Sperm counts were then multiplied by the dilution factor (x 10,000). Sperm length was assessed by spreading 10µl of sperm solution in a microscope slide. Each sample was air dried and stained with 30µl of DAPI and photographs were taken at 20x magnification in a fluorescence microscope. Five sperm cells were measured from each male using the software ImageJ, and mean sperm length (from the tip of the head to the end of the tail) was calculated. After dissections for sperm analyses, males were fixed in alcohol and frozen, for later morphological measurements. Each male was placed between foam and a glass lid, allowing measurements under a stereomicroscope with a graduate ocular (Ribeiro et al. 2003). We used intertegular distance (the shortest distance between the bases of the tegulae) as an estimate of male size (Cane 1987).

Data analyses

Heritability was estimated using REMLF90 software, which relies on Restricted Maximum Likelihood (REML) (Misztal et al. 2002). This analysis allows the estimation of the unknown additive genetic variances and covariances, providing the phenotype and a relationship matrix (see parameter and input files in Supplementary Material). Even though this approach has already been used to evaluate colony traits in social insects (Zakour et al. 2012), this is its first application in stingless bees and individual male characteristics. In our case, phenotype was set as the response variable and colony was included as a fixed factor. The effect of time on the estimated parameters was also tested in a separate model, by including sampling month as fixed factor. Data was transformed to achieve normality and aid model convergence (sperm viability: logit + 10, sperm counts: log/100000). Maximum number of rounds was fixed at 100. Heritability estimates were calculated as the ratio between genetic variance and phenotypic variance (genetic + residual variance). Additionally, genetic correlations were obtained following the expression:

$$r_g = \frac{cov_{12}}{\sqrt{var_1 * var_2}}$$

,where cov_{12} is the genetic covariance between traits 1 and 2, and var_1 and var_2 are the genetic variances of traits 1 and 2, respectively. In order to test the robustness of the results, a sensitivity analysis was performed by removing each colony sequentially and comparing heritability and genetic correlations with the results obtained using the whole dataset. Phenotypic correlations were also computed, using Spearman correlation. Additive genetic coefficients of variance (CV_a) and residual coefficients of variance (CV_r) were calculated following Houle 1992.

To build the relationship matrix, we included the relatedness between all pairs of sampled males. In *Scaptotrigona* aff. *depilis*, a proportion of males can also be produced by unmated workers. Since we could not distinguish between queen and worker-produced males in each generation, we calculated weighted coefficients of relatedness, accounting for the probability of a male being produced by a queen or a worker. Three distinct relationship

scenarios were considered, following a study by Paxton et al. (2003), who studied male maternity in ten colonies of this species: (A) 13% of males produced by workers (mean proportion), (B) no worker reproduction (minimum observed), and (C) 51% of males produced by workers (maximum observed) (Table 1). In each generation, all possible pairs of males were considered, with the respective relatedness coefficients: both males being workers sons (assuming different workers, $r=0.375$), both males being queen's sons ($r=0.5$), and one worker son and one queen son or the opposite ($r=0.25$) (Fig. 1). Between generations, the same weighting was performed and possible male pairs were: both males being workers sons (assuming different workers, $r=0.1875$), both males being queen sons ($r=0.25$), one worker son and one queen son ($r=0.375$), and one queen son and one worker son ($r=0.375$) (Fig.1, Table 1).

Figure 1. Experimental design showing the two generations from which males were sampled. In each generation males could have been produced by queens or workers.

Table 1. Estimation of weighted coefficients of relatedness for males sampled in the same or in different generations in three distinct scenarios of worker reproduction (A: 13% of worker reproduction, B: no worker reproduction, C: 51% of worker reproduction). For each possible pair of males, the coefficient of relatedness was weighted according to the probability of this pair to occur (see Fig. 1 for male codes).

Relationship scenario	Generation	Pair of males	Probability	r	Weighted r ($\sum$ case probability*r)
A	same generation	both from queen	0.76	0.5	0.44
		one from queen, other from worker	0.23	0.25	
		both from workers (assuming different workers)	0.02	0.375	
	different generations	both from queen	0.76	0.25	0.25
		one from queen, other from worker (m1,m4)	0.11	0.125	
		one from queen, other from worker (m2,m3)	0.11	0.375	
		both from workers	0.02	0.1875	
B	same generation	both from queen	1	0.5	0.5
		one from queen, other from worker	0	0.25	
		both from workers (assuming different workers)	0	0.375	
	different generations	both from queen	1	0.25	0.25
		one from queen, other from worker (m1,m4)	0	0.125	
		one from queen, other from worker (m2,m3)	0	0.375	
		both from workers	0	0.1875	

Table 1 (continued)

Relationship scenario	Generation	Pair of males	Probability	r	Weighted r (Σ case probability*r)
C	same generation	both from queen	0.24	0.5	0.34
		one from queen, other from worker	0.50	0.25	
		both from workers (assuming different workers)	0.26	0.375	
	different generations	both from queen	0.24	0.25	0.23
		one from queen, other from worker (m1,m4)	0.25	0.125	
		one from queen, other from worker (m2,m3)	0.25	0.375	
		both from workers	0.026	0.1875	

Results

The assessed male traits in *Scaptotrigona* aff. *depilis* showed additive genetic variance. Heritability estimates were high, with the exception of sperm length which exhibited lower heritability (Table 2). Distinct scenarios of worker reproduction resulted in similar heritability estimates for all traits, and the inclusion of sampling month revealed no effect of collection time on heritability estimates (Supp. Table 1). Similarly, results remained unaltered when each colony was removed sequentially (Supp. Table 2). Sperm viability and sperm counts showed high additive genetics coefficients of variance (CVa), while sperm length and male size showed low CVa. However, residual coefficients of variance (CVr) for male size was lower than for sperm length, thus resulting in a higher heritability for male size and lower heritability for sperm length.

In general, genetic and phenotypic correlations were low. Similar to heritability estimates, genetic correlations were not influenced by the amount of worker reproduction, so

Table 2. Descriptive statistics (mean and standard deviation) and quantitative genetic parameters (heritability, additive genetic coefficient of variance, and residual coefficient of variance) for four male traits, in three distinct scenarios of worker reproduction (A: 13% of worker reproduction, B: no worker reproduction, C: 51% of worker reproduction).

Trait	Mean	SD	Relationship scenario	h^2	CV_a	CV_r
sperm viability (%)	0.56	0.15	A	0.60	4.77	3.89
			B	0.60	4.87	4.01
			C	0.57	4.46	3.89
sperm counts	1,372,245	252,905	A	0.68	6.20	4.21
			B	0.71	6.55	4.21
			C	0.65	5.70	4.21
sperm length (μm)	87.35	3.76	A	0.22	1.94	3.64
			B	0.20	1.87	3.69
			C	0.25	2.06	3.55
male size (mm)	1.92	0.06	A	0.64	2.08	1.56
			B	0.67	2.20	1.56
			C	0.59	1.87	1.56

only the results for the scenario A are reported (Table 3). Sensitivity analyses revealed inconsistencies between some genetic correlations, with the exception of the correlations between sperm counts and male size, and sperm length and male size (Supp. Table 3). These were positive genetic correlations, indicating that larger males exhibit higher sperm counts and longer spermatozoa. We thus only considered these last correlations as meaningful, and tested if they were significantly greater than zero using a likelihood ratio test (Windig 1997), which revealed non-significant correlations. Phenotypic correlations were also not significant.

Table 3. Genetic and phenotypic correlations (above and below diagonal, respectively) between male traits. Genetic correlations are only reported for the scenario in which 13% of males are produced by workers (A), since similar results were found for the remaining scenarios. Only correlations between sperm counts and male size, and sperm length and male size were meaningful (showing consistency in a sensitivity analysis), but were not significant. Phenotypic correlations were also not significant ($p>0.05$).

	sperm viability	sperm counts	sperm length	male size
sperm viability	-	-	-	-
sperm counts	0.07	-	-	0.09
sperm length	-0.08	0.03	-	0.08
male size	-0.08	-0.01	0.13	-

Discussion

Our analyses revealed that, comparatively, sperm length in *Scaptotrigona* aff. *depilis* males exhibits low heritability, while sperm viability, sperm counts, and male size exhibit high heritability estimates. Additionally, we found positive albeit not significant genetic correlations between sperm counts and male size, and sperm length and male size.

High heritability estimates may be caused by a large additive genetic component, as was found to be the case for sperm viability and sperm counts. A previous study showed that persistence in a male aggregation selected stingless bee males of this species with higher sperm viability, suggesting that this trait was under sexual selection (Koffler et al. 2016). However, the high heritability found here indicates that sperm viability is not exposed to strong natural selection (Mousseau and Roff 1987, Postma 2014, Koffler et al. 2017). High additive genetic variance, however, may also be related to traits under directional selection (Houle 1992), so trade-offs with other related traits may also explain why sperm viability and sperm counts contain such high additive genetic variance. Our result contradicts

the low heritability found for sperm viability in *Atta colombica* ants (Stürup et al. 2011). However, in honeybees, ejaculate senescence was affected by colony, suggesting a possible heritable effect on long term viability (Sturup et al. 2013). On the other hand, sperm counts were not associated with male performance in male aggregations (Koffler et al. 2016). The high heritability of sperm counts found here also suggests this trait is weakly exposed to selection. Social context and colony condition were found to affect these male traits in other social bees (Pech-May et al. 2012, Stürup et al. 2013).

Even though high heritability was also found for male size, levels of additive genetic variance were low for this trait. Morphological traits, opposite to life-history traits, tend to exhibit high heritability estimates (Mousseau and Roff 1987, Postma 2014, Koffler et al. 2017). For instance, high heritability estimates for male body size were also found for other social insects and solitary bees (Koffler et al. 2017). We nevertheless expected that male size would show a low heritability in our study species, because body size was related to competitive ability in male aggregations (Koffler et al. 2016). Constraint in the evolution of male body size due to trade-offs with other traits may thus be driving this pattern.

Low levels of additive genetic variance and higher residual variance resulted in a low heritability estimate for sperm length, suggesting that selection is acting more strongly on sperm length than on the other measured traits. Sperm length thus seems to contribute to male fitness. Low additive genetic variance is related to stabilizing selection (Houle 1992), and previous results revealed that shorter sperm cells were found in more competitive males (Koffler et al. 2016). Low heritability for sperm length was also found in the monandrous social bee *Bombus terrestris*, when individual cells were analyzed (Baer et al. 2006), although higher heritability estimates were observed when mean sperm length was tested. High heritability for mean sperm length was also described for a polyandrous ant (Stürup et al. 2011) and other insects (Ward 2000, Morrow and Gage 2001, Dobler and Hosken 2010).

We found weak genetic correlations, suggesting independence in trait evolution. Phenotypic and genetic correlations were not equivalent, reinforcing the importance of this kind of quantitative genetic studies. Male size and sperm length were positively correlated, in agreement with the patterns found by Koffler et al. (2016), in which male aggregations selected smaller males and males with shorter sperm cells. Since sperm length was the only trait that we could relate to fitness, selection favoring males with shorter sperm cells also seems to be selecting smaller males. Male size was also positively correlated with sperm counts, and thus smaller males would also contain lower sperm counts. These possible interactions between sperm length, male size, and sperm counts may thus be contributing to avoid the depletion of additive genetic variance.

Although behavioral analyses suggested that male morphology and several sperm traits might be under selection, here we showed that only sperm length seems to be related to fitness. These results indicate that selection is favoring shorter sperm cells (Koffler et al. 2016), which may maximize the amount of sperm stored in queens' spermatheca, since shorter sperm cells occupy less volume than longer ones in a constrained volume (Boomsma et al. 2005). Importantly, the amount of stored sperm directly influences male fitness, which depends on the high investment in workers (for colony growth and establishment) prior to the production of virgin queens or males by unmated workers. Selection over sperm length is generally suggested for polyandrous species, favoring longer spermatozoa which confer competition advantages among ejaculates (Baer et al. 2003). However, our results indicate that selection may also occur in the absence of sperm competition (in a monandrous species), selecting sperm length in the opposite direction. As stingless bees are monandrous (Peters et al. 1999) and thus no sperm competition occurs after mating, traits that confer advantage in sperm competition as sperm viability and counts may be under a more relaxed selection than in polyandrous species (Fitzpatrick and Baer 2011). Low heritability for sperm length in *Bombus terrestris* males, which was attributed to rare multiple matings (Baer et al. 2006), might also be explained by selective pressures for long-term fertilizations.

Given the scarcity of quantitative genetic studies assessing heritability estimates of male traits in social insects, we cannot extrapolate our findings to the entire group. However, we expect selection for shorter sperm cells to be a general evolutionary force in groups where sperm storage is constrained. In addition, our findings suggest that future stingless bee breeding programs aiming to obtain long-lived colonies should invest in selecting colonies that produce males with shorter sperm cells, or instrumentally inseminate queens with these males to maximize fecundity.

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Data availability: We have included our dataset, parameter, and input files as Supplementary Material.

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Supplementary Material – Chapter 3

Supplementary Table 1. Male traits assessed in this study. Morphological traits were measured to identify predictors of male competitive ability and sperm quality traits were measured to test the relationship between male fertilization success and competitive ability.

traits	h^2	h^2 (with sampling month)
sperm viability	0.60	0.59
sperm count	0.68	0.72
sperm lenght	0.22	0.22

Supplementary Table 2. Sensitivity analyses testing the effect of removing each colony from the dataset on heritability estimates (only the scenario A was considered).

trait	removed colony	h^2	trait	removed colony	h^2
sperm viability	None	0.60	sperm length	None	0.22
	13	0.61		13	0.18
	15	0.62		15	0.19
	31	0.61		31	0.18
	42	0.60		42	0.19
	234	0.60		234	0.19
	247	0.60		247	0.18
sperm count	None	0.68	sperm length	None	0.64
	13	0.71		13	0.68
	15	0.72		15	0.68
	31	0.72		31	0.67
	42	0.70		42	0.68
	234	0.70		234	0.67
	247	0.71		247	0.68

Supplementary Table 3. Sensitivity analyses testing the effect of removing each colony from the dataset on genetic correlations (only the scenario A was considered). Bold coefficients indicate negative genetic correlation estimates. Since changes in the direction of the correlation indicate inconsistencies, only the correlations between sperm count and male size, and sperm length and male size were reported in the final results.

traits	genetic correlations (removing each colony from the dataset)						
	complete dataset	13	15	31	42	234	247
sperm viability - sperm count	0.01	0.00	0.01	-0.02	0.00	0.04	0.05
sperm viability - sperm length	-0.02	0.01	0.02	-0.03	0.01	-0.01	-0.02
sperm viability - male size	-0.01	-0.03	-0.02	0.00	-0.02	0.04	0.01
sperm count - sperm length	0.00	0.02	-0.01	0.02	-0.02	0.02	0.01
sperm count - male size	0.09	0.14	0.10	0.04	0.08	0.08	0.14
sperm length - male size	0.08	0.06	0.06	0.07	0.07	0.06	0.10

Photo by Paulo Menezes

Temporal variation in honey production by the stingless bee *Melipona subnitida* (Hymenoptera: Apidae): long term management reveals its potential as a commercial species in Northeastern Brazil

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Chapter 4.

Optimizing stingless bee breeding practices

Resumo

Embora a criação de abelhas sem ferrão apresente um grande potencial como ferramenta para o desenvolvimento sustentável, a atividade ainda é baseada principalmente em técnicas tradicionais e as práticas de manejo não possuem a sofisticação e a padronização encontradas na apicultura. Nesse estudo, contribuímos para o futuro desenvolvimento da meliponicultura investigando o impacto a longo prazo do manejo e do clima sobre a produção de mel e a sobrevivência em colônias da abelha sem ferrão *Melipona subnitida* Ducke (1910), popularmente conhecida como jandaíra. Analisamos uma série temporal de dez anos de 155 colônias de *M. subnitida* mantidas por um produtor de mel do nordeste brasileiro. Esse é o registro mais longo e mais preciso disponível para uma abelha sem ferrão. Modelamos a produção de mel em relação ao tempo, manejo (divisão de colônia e alimentação suplementar) e fatores climáticos (temperatura e precipitação) e, por meio de seleção de modelos, identificamos quais fatores melhor explicaram a produção de mel. Também modelamos a mortalidade das colônias em relação aos fatores climáticos. Embora a quantidade de mel produzido tenha diminuído ao longo do tempo, observamos que a probabilidade de produzir mel aumentou ao longo dos anos. A divisão de colônia afetou negativamente a produção de mel, mas não sua presença, enquanto a alimentação das colônias afetou positivamente a produção de mel. Em anos mais quentes, a probabilidade de produzir mel diminuiu e a produção de mel foi menor. Em anos com menor precipitação, menos colônias produziram mel. Já a mortalidade não foi afetada por fatores climáticos, e algumas colônias sobreviveram até nove anos, suportando condições extremas de clima. Nossos resultados fornecem diretrizes para o aperfeiçoamento do manejo e da produção de mel de abelhas sem ferrão.

Abstract

Even though stingless beekeeping has a great potential as a sustainable development tool, the activity remains essentially informal, technical knowledge is scarce, and management practices lack the sophistication and standardization found in apiculture. Here we contributed to the further development of stingless beekeeping by investigating the long-term impact of management and climate on honey production and colony survival in the stingless bee *Melipona subnitida* Ducke (1910). We analyzed a ten year record of 155 *M. subnitida* colonies kept by a commercial honey producer of Northeastern Brazil. This constitutes the longest and most accurate record available for a stingless bee. We modeled honey production in relation to time, age, management practices (colony division and food supplementation) and climatic factors (temperature and precipitation), and used a model selection approach to identify which factors best explained honey production. We also modeled colony mortality in relation to climatic factors. Although the amount of honey produced by each colony decreased over time, we found that the probability of producing honey increased over the years. Colony divisions decreased honey production, but did not affect honey presence; while supplementary feeding positively affected honey production. In warmer years, the probability of producing honey decreased and the amount of honey produced was lower. In years with lower precipitation fewer colonies produced honey. In contrast, colony mortality was not affected by climatic factors, and some colonies lived up to nine years, enduring extreme climatic conditions. Our findings provide useful guidelines to improve management and honey production in stingless bees.

Introduction

Although the commercial use of honeybees has become a major global business, the profitable use of stingless bees (designated meliponiculture after the bees from the Meliponini tribe) has received much less attention (Cortopassi-Laurino et al. 2006, Contrera et al. 2011). Currently, meliponiculture can be found in Central and South America, Asia, Africa and Australia (Quezada-Euán et al. 2001, Cortopassi-Laurino et al. 2006, Alves 2013, Halcroft et al. 2013). In developing nations, meliponiculture remains an essentially informal activity (Villanueva-G et al. 2005, Cortopassi-Laurino et al. 2006). Besides preserving the cultural heritage, stingless beekeeping has an important role in the conservation of native bees, which are increasingly threatened by habitat loss and fragmentation (Brown and Albrecht 2001, Kennedy et al. 2013). Furthermore, stingless beekeeping assures the provision of pollination services, as stingless bees are important pollinators for both native plants and commercial crops (Heard 1999, Cortopassi-Laurino et al. 2006, Giannini et al. 2014).

Important efforts have been directed to train beekeepers and standardize management practices (Nogueira-Neto 1997, González-Acereto et al. 2006, Venturieri 2008, Villas-Bôas 2012, Frazão 2013), quantify investment costs and profit perspectives (Lobato and Venturieri 2010), and assess honey properties, quality and commercialization routes (Vit et al. 2013). However, there is still a large knowledge gap in terms of the appropriate techniques needed to maintain healthy colonies and ensure the consistent production of honey over time (but see Jaffé et al. in press). Research devoted to this end could thus contribute toward transforming the activity into a powerful tool for sustainable rural development (Venturieri et al. 2003, Cortopassi-Laurino et al. 2006).

The main products commercialized by stingless beekeepers are colonies and honey. Stingless bee honey is quite distinct from *Apis mellifera* honey. It has higher water content (20 to 42% of water) and its flavor is sour and slightly sweet, varying among bee species and flowers visited by the bees (Nogueira-Neto 1997, Souza et al. 2006, Deliza and Vit 2013). Stingless bee honey consumption is deeply rooted in some communities, who believe it has medicinal properties (Zamora et al. 2013), and its price is much higher than the price of honeybee honey (Alves, 2013).

Generally, larger stingless bee species produce more honey and hence are more likely to be exploited for honey production (Alves 2013). One such species is *Melipona subnitida* Ducke (1910), which is among the main cultivated species across Northeastern Brazil. Colonies produce relatively large amounts of honey compared to other stingless bee species, can be artificially reared in wooden boxes, and support management practices such as honey extraction and colony divisions (Cortopassi-Laurino et al. 2006). A central figure in the commercial use and conservation of *M. subnitida* was Father Huberto Bruening (1914-1995). A priest in the city of Mossoró, Rio Grande do Norte, he improved informal beekeeping practices, established links with national and international researchers, and motivated the local population to engage in stingless beekeeping (Bruening 2006). Mr. Paulo Menezes, one of his disciples, inherited Bruening's colonies and has continued his work as a teacher and promoter of meliponiculture. Currently, Mr. Menezes is a major stingless bee honey producer and well known beekeeper across Northeastern Brazil, having received the first license for stingless bee honey commercialization in the country. Mr. Menezes (co-author of this study) meticulously recorded honey production and management practices in 155 of his colonies over a period of 10 years. Here we analyze his dataset, which to our knowledge is the longest and most accurate yet available for any stingless bee. By assessing the long-term impact of management and climate on honey production and survival in *Melipona subnitida*, we provide an important contribution to the development of commercial stingless beekeeping.

Fig. 1. Colonies of *Melipona subnitida* held in the “Meliponário Monsenhor Huberto Bruening”, Mossoró (Rio Grande do Norte, Northeastern Brazil). Photo by Paulo Menezes.

Materials and Methods

The data set

We analyzed a temporal record of 155 *Melipona subnitida* colonies, closely monitored for a period of ten years (Supp. Tables S1 and S2). The colonies were kept at the “Meliponário Monsenhor Huberto Bruening”, in Mossoró, Rio Grande do Norte, Brazil (Fig. 1). This state is dominated by the Caatinga biome, a semi-arid region with extreme climate (high temperatures and low and irregular precipitation) and defined seasonality (Prado 2003). Between 1999 and 2008, Mr. Menezes followed his colonies (identifying each one with a different code), and recorded the total amount of honey produced by each colony each year, the management provided to each colony, and whether the colony died (the variables recorded are described in Supp. Table S3). Whenever a colony was reported dead, a new colony was established in the same wooden box, and a new identification code was given to it.

In order to replace dead colonies and increase the size of the beekeeping operation, colonies were periodically divided. To this end, 50 to 70% of the brood discs containing bee pupae near emergence were removed from selected colonies (those with more brood and workers) and placed in an empty wooden box in order to create a new daughter colony. In the *Melipona* genus, many queens are produced per brood comb (14-20%, Wenseleers and Ratnieks (2004)) and hence a queen will normally hatch from the transplanted combs. Upon emergence, the new queen will mate (presumably with a single male), return to the colony and begin laying eggs. Colony divisions were performed between January and July, overlapping with the rainy season (which comprehends November to April) (Prado 2003), when more floral resources are available (Zanella and Martins 2003) (Supp. Table S4). The colonies were divided once per year, except in one case in 2004 when a colony was divided twice in the same year. Honey was harvested from the divided colonies, but only 1 to 5 months after the procedure. When new colonies were purchased from other beekeepers (either inside tree branches or in boxes), the entire colony was transferred to a new wooden box and assigned a new identification code.

Honey harvesting was performed between April and September, after the rainy season, when the honey storages are full (Supp. Table S4). Honey was normally collected once per year, but in atypical years two honey batches were harvested from each colony. In these cases (12% of all honey producing colonies), honey production was still quantified as the total volume of honey produced per colony per year. In stingless bee colonies, honey and pollen are stored in egg-shaped pots, apart from the brood area (Nogueira-Neto 1997, Fig. S1). During honey collection, the pots were opened and honey was either harvested by flipping the boxes and leaving the honey to drain into a collection container, or in the later years, by suction with a plastic hose attached to an electric pump. Although the total honey reserves were harvested, some honey always remained in honey-pots and was left for the colonies. The volume of honey harvested was recorded and honey production below 100 ml was considered essentially nil, and was registered as zero production (Supp. Table S3). This happened when virtually no or very little honey was found in the colonies, either because they were too weak (and thus consumed more than what they could store) or when floral resources were scarce. In 2008, colonies received supplementary feeding consisting of sugar syrup (1:1 sugar, water; 250 milliliters) every fortnight. Syrup was provided in feeders placed inside the colonies and the total amount was consumed by the bees in 2-5 days. Supplementary feeding was only provided during the dry season months, between May and October, and it ceased at the onset of the rainy season.

Climate data

Climate data was obtained for the nearest meteorological station with available historic data, which is located in the city of Apodi, 73km from Mossoró (INMET 2014). We gathered all available climatic data for the years 1999-2008, including: (a) number of days with precipitation, (b) total precipitation (mm), (c) mean maximum temperature (°C), (d) mean weighted temperature (°C), (e) mean minimum temperature (°C) and (f) mean relative humidity (%) (Supp. Table S5).

Statistical analyses

We modeled honey production across time using generalized linear mixed models (R package glmmADMB, Skaug et al. 2013). Because some colonies did not produce any honey in many years (all honey produced was consumed by the bees or the production was below 100 ml and considered nil), we jointly modeled the amount of honey that the colony produces and the probability that a given colony will produce honey in a given year. To do so we employed a hurdle model, which consists of two submodels: (A) a zero-truncated count model (henceforth count model) for which the response variable is honey production (using only those data for which honey production is greater than zero) and follows a negative binomial distribution, and (B) a logistic regression for which the response is binary (honey presence, using all data) and follows a Bernoulli distribution (henceforth zero model). The hurdle model implicitly assumes that the zero-generating process is distinct from the count-generating process: In the context of our study, we consider that an overall greater honey production per colony does not necessarily imply more frequent honey production, and that different causative factors may mediate these two processes.

Both the count- and zero- processes reflect the response of individual colonies over time. To account for these dependencies, we included a random intercept for each colony (e.g. the mean honey production or probability of producing honey varies randomly with colony), and a random slope (e.g. the change in honey production across time varies randomly with colony). In the zero model (honey presence), the interaction between colony and time was not included as year showed zero variance in a random slope model. For both sub-models, we fitted a full model with time (year), colony division (brood comb removal for new colony foundation), supplementary feeding, and climate variables as fixed effects. Yearly mean minimum temperature (henceforth referred to as temperature) and yearly total precipitation (henceforth referred to as precipitation) were chosen as the climatic predictors since they were found to minimize multicollinearity. Supplementary feeding was not included in the zero model for honey presence, since there was a complete separation

(all fed colonies produced honey) and thus feeding was not an informative predictor (Supp. Table S6). All continuous predictor variables were centered and scaled to aid model convergence. For each submodel, we used marginal likelihood ratio tests to choose the best model and assess the explanatory power of predictors. We used likelihood ratio test to compare reduced models without each predictor variable, with full models containing them. Because our aim was to assess how management practices, time and climatic variations influenced honey production, we decided to analyze only established colonies that had enough time to respond to such predictors. Thus, for this analysis we excluded shortly established colonies (recently bought or created), and colonies that died right after being created (see Table 1 for final sample sizes).

To assess the sensitivity of these results to the inclusion of data from 2008 (the only year supplementary feeding was provided), we fitted an additional model without the data from this year. The effect of supplementary feeding in 2008 was also assessed in a separate analysis, comparing honey production in 2008 against all previous years. We modeled honey production using a negative truncated binomial ANOVA, with a custom set of orthogonal contrasts, in which a given year was compared to all previous years. As in the previous models, a random intercept was included for each colony (the production of honey between years varies randomly with colony). The years 1999 and 2001 were not included, because 1999 had only one observation on honey production, and in 2001 no colony produced honey.

In order to discriminate between the effect of time from the effect of colony age on honey production and honey presence, we fitted similar Hurdle models to a subset of the data containing only colonies with known age (born from divided colonies, $n=47$). Colony age was included as an additional fixed factor in the best zero and count models previously found using the whole data set. As described above, marginal likelihood ratio tests were used for model selection and assess the explanatory power of predictors.

Table 1. Ten year record for 155 *Melipona subnitida* colonies. The table shows the number of colonies (used in the mortality and honey production analyses, respectively), the number (proportion) of lost colonies, the number of colony divisions, honey production per colony (for all colonies and only for colonies that produced honey), minimum and maximum honey production and total honey production per year. Honey production is reported in milliliters.

Year	Number of colonies (mortality analysis / honey production analysis)	Number (proportion) of lost colonies	Number of colony divisions	Honey production per colony (mean $\pm$ SD) (milliliters)	Honey production per colony: colonies producing honey (mean $\pm$ SD) (milliliters)	Min-Max honey production per colony (milliliters)	Honey production per year (milliliters)
1999	9/1	1 (0.11)	0	800	800	800	800
2000	17/10	6 (0.35)	3	160 $\pm$ 267	400 $\pm$ 294	0 – 800	1,600
2001	50/25	22 (0.44)	1	0	-	0 – 0	0
2002	63/42	5 (0.08)	0	486 $\pm$ 464	817 $\pm$ 293	0 – 1,380	20,430
2003	65/58	5 (0.08)	16	726 $\pm$ 605	1002 $\pm$ 474	0 – 1,800	42,100
2004	84/63	12 (0.14)	32	522 $\pm$ 372	710 $\pm$ 254	0 – 1,250	34,800
2005	90/75	8 (0.09)	0	51 $\pm$ 188	633 $\pm$ 280	0 – 1,000	3,800
2006	108/82	17 (0.16)	2	501 $\pm$ 309	586 $\pm$ 247	0 – 1,300	41,050
2007	92/92	0 (0)	0	276 $\pm$ 261	454 $\pm$ 175	0 – 900	25,400
2008	92/92	0 (0)	0	662 $\pm$ 135	662 $\pm$ 135	400 – 1,000	60,900
Mean $\pm$ SD	67 $\pm$ 34	7.6 $\pm$ 7.38 (0.16 $\pm$ 0.14)	5.4 $\pm$ 10.5	421 $\pm$ 284	674 $\pm$ 186	120 $\pm$ 270- 1,023 $\pm$ 474	23,088 $\pm$ 21,414

Table 2. Parameter estimates and hypothesis tests for the best models describing honey production (honey amount and honey presence models).

Model	Data sets	N. obs / colony number	Fixed effects Predictor	Estimate	SE	χ^2	Degrees of freedom	P-value
Honey amount (count model)	Established colonies 1999-2008	345 / 109	Year	-0.1143	0.0155	46.54	1	< 0.0001
			Colony division	-0.2073	0.0702	8.44	1	0.0037
			Supplementary feeding	0.3142	0.0675	20.70	1	< 0.0001
			Temperature	-0.4670	0.0964	20.78	1	< 0.0001
Honey amount (count model)	Established colonies 1999-2007	253 / 97	Year	-0.1138	0.0176	31.34	1	< 0.0001
			Colony division	-0.2142	0.0799	6.94	1	0.0084
			Temperature	-0.4733	0.1120	16.64	1	< 0.0001
Honey amount (count model)	Established colonies 1999-2008	123 / 42	Year	-0.1414	0.0221	34.12	1	< 0.0001
			Supplementary feeding	0.4501	0.0965	19.62	1	< 0.0001
Honey presence (zero model)	Established colonies 1999-2007	540 / 124	Year	0.2307	0.0519	20.69	1	< 0.0001
			Temperature	-0.8169	0.3193	73.70	1	0.0095
			Precipitation	0.0048	0.0006	6.73	1	< 0.0001
Honey presence (zero model)	Established colonies of known age 1999-2007	326 / 65	Colony age	0.6033	0.0982	52.05	1	< 0.0001
			Temperature	-1.7832	0.7109	9.67	1	0.0019

N.obs is the number of observations of each model and colony number refers to the total number of colonies included. For each predictor, estimates and standard errors from the model are given. The p-values and degrees of freedom refer to the marginal likelihood ratio tests (using a χ^2 test statistic), in which the full model was compared to a reduced model without each of the predictor variables. For more details on the different data sets employed see Table S6.

In order to assess the influence of climate on colony mortality, we analyzed the number of lost colonies per year with a generalized linear model with an overdispersed binomial distribution. We treated the number of lost colonies (out of a total number of colonies) as the response variable, and included temperature and precipitation as predictor variables (Supp. Table S6). Marginal likelihood ratio tests were also employed to assess the explanatory power of predictor variables. We excluded data for the year 2008 in this analysis, since supplementary feeding is known to affect colony performance (Nogueira-Neto 1997). In addition, we excluded mortality cases related to queen removal (n=3), colony transportation or ant attacks (n=3).

Results

The total number of colonies monitored each year varied from 9 to 108 (Table 1). Honey production per colony varied from zero to 1.8 liters, with a mean ($\pm$ SD) yearly production of 0.43 ($\pm$ 0.4) liters. Considering only colonies that did produce some honey, mean yearly production was 0.67 ($\pm$ 0.3) liters. Total joint honey production ranged from zero to 60.9 liters per year, with a mean joint production of 23.1 ($\pm$ 21.4) liters per year. Overall, we analyzed data from 155 different colonies through the ten years.

The best count model (honey production) included all predictors except annual precipitation. The best zero-model (honey presence) included time and climatic factors (temperature and precipitation) (Table 2). The quantity of honey harvested decreased over the years (Fig. 2). This reduction was not related to colony aging, as in a complementary analysis age was not included in the best model, while honey production still showed a significant decrease with time (Table 2). Colony division decreased the amount of honey produced in a given year (reduction of 19% on average) (Fig. 4), while food supplementation increased honey production (increment of 37% on average) (Fig. 5). Years with higher temperatures were associated with decreased honey production (Table 2). The

results remained unaltered when we excluded data for 2008 (the only year supplementary feeding was provided, Table 2). In addition, the mean difference between honey production in 2008 and all previous years was positive and significant (0.1165 ± 0.0255 , p-value < 0.001), revealing that honey production was significantly higher in the year when supplementary feeding was provided.

The probability of a given colony producing any quantity of honey (honey presence) increased over time (Table 2, Fig. 3a). However, this temporal pattern was best predicted by age, since including colony age in place of time resulted in a substantial increase in the model's likelihood (Table 2). Hence, older colonies had a higher probability of producing honey. Higher temperatures were associated with the absence of honey, and higher levels of precipitation were associated with the presence of honey (Fig. 3b-c). Colony mortality was variable over the years, ranging from 0 to 44% (22 cases). Temperature and precipitation did not influence the number of colonies lost in a year (Table 3). Even though many colonies died after few years of observation, some colonies survived up to nine years.

Discussion

By meticulously following 155 colonies over a period of ten years, Mr. Paulo Menezes provided the longest and most accurate record of honey production and mortality available for a stingless bee. Analyses of these records reveal that honey production by *Melipona subnitida* is influenced by time, colony age, management techniques (colony division and supplementary feeding) and climatic factors.

Fig. 2. Honey production (ml) over ten years in *Melipona subtinida* colonies. While each point represents a record, the line represents the fitted curve and the grey area shows the 95% confidence interval. The figure reveals a decrease in the amount of honey produced over time.

Fig. 3. Proportion of *M. subnitida* colonies producing honey in relation to (a) time, (b) temperature and (c) precipitation. The line represents the fitted curve and the grey area shows the 95% confidence interval of the model. For each plot the other covariates were held constant at their mean and the size of the points indicates the number of colonies in each year.

Fig. 4. Honey production per colony (ml) in years in which colony divisions were performed, showing that colonies that underwent division produced less honey than colonies that were not divided. Only colonies that produced honey are shown ($n = 161$; all colonies are shown in Fig. S2).

Table 3. Parameter estimates and hypothesis tests for the model describing colony mortality.

Response	N	Predictor	Estimate	SE	χ^2	Degrees of freedom	P-value
Colony mortality	76	Temperature	0.8718	0.5448	2.21	1	0.14
		Precipitation	-0.0007381	0.001122	0.44	1	0.51

N is the number of observations of the model. For each predictor, estimates and standard errors from the model are given. The p-values and degrees of freedom refer to the marginal likelihood ratio tests (using a χ^2 test statistic), in which the full model was compared to a reduced model without each of the predictor variables.

Along the years, the amount of honey produced by each colony decreased and this reduction was not related to colony age. The increase in colony number during the ten years of record could have resulted in competition for floral resources, leading to a lower honey production per colony. However, our results do not suggest an effect of colony density on honey production since the total number of colonies was not found to have an effect on honey production (results not shown). We nevertheless caution that our study could not fully quantify the effect of the total number of colonies on honey production per colony, because there were more colonies located in the vicinity of the study colonies which were not accounted for. In addition, *Apis mellifera* colonies are commercially reared in the same region, probably competing with native bees for limited food resources (Bruening 2006). Another possible cause for the observed decrease in honey production over time is that larger colonies were usually selected for division. By so doing, colonies were not allowed to grow indefinitely, thus constraining honey production (which is usually proportional to colony size (Chinh & Sommeijer, 2005)). Furthermore, colony division itself has a negative impact on honey production (see below). Although this effect was probably stronger in 2003 and 2004, when more colonies were divided, this practice could have had a long-term impact on honey production. In addition to colony division, the extraction of

Fig. 5. Honey production per colony (ml) for the year 2007, when no supplementary food was provided, and for 2008, when colonies were fed with sugar syrup every fortnight. Each line represents a colony and only colonies that produced honey are shown ($n = 56$; all colonies are shown in Fig. S3).

honey itself could also have influenced long-term honey production. However, our dataset did not allow assessing the effect of honey extraction on honey production, because the amount of honey produced by each colony was only quantified after being extracted. The different techniques employed to collect honey do not seem to have affected the amount of honey harvested, since the method used in later years (syringe coupled with a pump) causes less harm to colonies, but we still observe a decrease in honey production. Future studies aiming to quantify the effect of honey extraction could implement less invasive methods to measure honey production (perhaps weighting colonies).

Despite the decrease on honey amount produced per colony, the proportion of colonies producing honey increased over the course of the study, potentially due to colony aging. In the beginning of the colony life cycle, most resources (nectar and pollen) are expected to be invested in colony growth (Oster and Wilson 1978), and they only begin to be stored after colony establishment. This behavioral pattern would result in an increase in the probability of honey presence over the years, even though not related to an increase in honey amount.

Management techniques also affected colony performance. Colony division decreased the amount of honey produced, which could be related to the decline in the worker population of the colony by the removal of some brood combs. Since brood combs are constructed sequentially (Nogueira-Neto 1997), removing combs results in a gap of bees of a determined age cohort, which can affect the entire dynamic of the colony and temporarily reduce the number of foragers. However, the probability of honey production was not affected by colony division, revealing that even with a dramatic decrease in colony population, colonies would not cease producing honey. These results indicate that colony division has a short-term negative impact for the beekeeper – an immediate decrease in the amount of honey produced – which may be offset by a long-term increase in the number of honey-producing colonies.

Supplementary feeding had a positive effect on the amount of honey produced. In the year when colonies were fed with sugar syrup, we observed a sharp increase in posterior honey

production. Providing food resources helps the maintenance of the nest, especially in the dry season when floral resources are scarce and the colonies become weaker (Nogueira-Neto 1997, Contrera et al. 2011). During this season, the bees consume the sugar syrup provided and no honey is normally stored. Honey production starts at the onset of the rainy season, with the first blooms. At this point, supplementary feeding is ceased and honey is harvested a few months later. An additional strategy to avoid the harvesting of sugar syrup-based honey is discarding any stored honey before the rainy season, a known practice amongst beekeepers. Thus, since only nectar-based honey was harvested, the observed increase in honey production was not due to the transformation of sugar syrup-based into honey. This result suggests that honey production was constrained, and that it can be boosted through supplementary feeding. Supplementary feeding thus proved a particularly important practice to help strengthen colonies during periods when floral resources are scarce, and later maximize honey production when resources are available. Another strategy for stingless beekeepers to enhance honey production is to place their colonies near areas with abundant floral resources, and thus minimizing the need for supplementary feeding.

We found that temperature had a more drastic effect on honey production than precipitation, as both the probability of a colony producing honey and the amount of honey produced were significantly influenced by annual mean temperature. In warmer years, fewer colonies produced honey, and those that did produce yielded a lower amount. This is a concerning situation as global warming predictions point to an increase in temperature (IPCC 2014) that could compromise honey production. In contrast, annual precipitation only affected the presence of honey in the colonies. In the Brazilian Caatinga, the relationship between climate and plant phenology is well documented, with two distinct seasons: A rainy season during which the majority of plant species flower, and a dry season with less abundant floral resources (Zanella and Martins 2003). Seasonal variations in floral resources are known to affect bee foraging intensity and honey production (Schneider and Blyther 1988). For instance, father Huberto Bruening described the bee's ability to predict climate, noticing that *M. subnitida* colonies "intensify egg-laying activity 45 days before the

first rain" (Bruening 2006). Water availability is also important for colony thermoregulation through evaporative cooling (Nogueira-Neto 1997, Jones and Oldroyd 2006).

In contrast to honey production, colony mortality was not affected by climatic factors. This indicates the resilience of *M. subnitida* colonies to long intervals of drought and warm weather. *M. subnitida* occurs in regions with the highest temperatures and lowest precipitation recorded in Brazil, suggesting that the species is highly adapted to arid regions (Silveira et al. 2002, Giannini et al. 2012). Colony failure thus seems to be related to factors other than climate, such as resource availability, management, parasite attacks or disease (Nogueira-Neto 1997, Maia-Silva et al. 2013, Jaffé et al. 2015).

According to our results, two different strategies can be outlined for stingless beekeepers: To maximize honey production in a given year, beekeepers should avoid excessive colony divisions, and only divide colonies to maintain an optimal number of honey-producing colonies. Likewise, by providing colonies with sugar syrup when floral resources are scarce, beekeepers could boost posterior honey production. On the other hand, to maximize the production of new colonies, beekeepers should divide their colonies frequently, at a rate that does not compromise colony survival. Not harvesting honey and providing the colonies with sugar syrup could help increase survival and maximize colony division rate. For instance, many stingless beekeepers already specialize in colony sells (Jaffé et al. in press). Both strategies are expected to yield economic returns, since the mean local prices for *M. subnitida* honey and colonies are \$25/liter and \$50/colony respectively (Maia, 2013). Future efforts are needed to determine the optimal division rate which maximizes total joint honey production.

Overall, our results show that both environmental factors and management can influence honey production in stingless bees. Likewise, our work highlights the benefits of *M. subnitida* as a commercial species for honey production in Northeastern Brazil, given that it proved resilient to management and extreme weather conditions. Our study emphasizes the importance of keeping detailed long-term records on honey production and management practices, and illustrates the importance of establishing partnerships between scientists and beekeepers to further develop stingless beekeeping.

Acknowledgements

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Supplementary Material – Chapter 4

Table S1. Honey production data set for *Melipona subnitida* colonies, from 1999 to 2008 (see csv file online).

Table S2. Mortality data set for *Melipona subnitida* colonies, from 1999 to 2008 (see csv file online).

<https://academic.oup.com/jee/article-abstract/108/3/858/2379798/Temporal-Variation-in-Honey-Production-by-the?redirectedFrom=fulltext>

Table S3. Description of the variables assessed in this study.

Variable	Definition	Type
Honey production	volume of honey in milliliters produced per colony	continuous
Honey presence	presence or absence of honey in a colony	binary
Time	year	categorical
Age	colony age in years	categorical
Colony mortality	colony dead or alive	binary
Colony division	colony divided or not	binary
Supplementary feeding	feeding or not the colony with sugar syrup	binary
Temperature	mean minimum annual temperature (°C)	continuous
Precipitation	total precipitation in the year (mm)	continuous

Table S4. Monthly distribution of colony divisions and honey extraction for *Melipona subnitida*, in relation to the rainy season. Colony divisions were performed between January and July, while honey harvesting was normally done between April and September. Both processes are synchronized with the rainy season (which occurs between November and April), when the floral resources are most abundant. Hence, colony divisions are usually performed at the onset and during the rainy season, ensuring that the divided colonies have sufficient food resources available. Likewise, honey harvesting is done after the rainy season, when honey storage peaks.

Table S5. Climate data for the city of Apodi, 73km from Mossoró (Rio Grande do Norte, Brazil) for the years 1999-2008. The mean weighted temperature represents the daily variation in air temperature and is calculated by $[\text{maximum temperature} + \text{minimum temperature} + \text{temperature at 12 UTC} + 2 \times \text{temperature at 24 UTC}]/5$.

Year	Number of days with precipitation	Total precipitation (mm)	Mean maximum temperature (°C)	Mean weighted temperature (°C)	Mean minimum temperature (°C)	Mean relative humidity (%)
1999	61	462.9	33.7	28.6	25.2	58.0
2000	107	1059.3	31.7	27.7	25.0	79.1
2001	15	112.5	36.0	28.9	23.9	64.0
2002	77	595.4	33.9	27.3	23.0	73.6
2003	82	758.9	34.0	27.4	23.0	77.2
2004	107	1158.6	33.1	27.0	23.0	80.8
2005	74	602.1	34.3	27.8	23.3	75.5
2006	75	721.4	33.8	27.5	23.4	76.9
2007	76	610.2	34.0	27.5	23.3	77.8
2008	95	980.1	33.4	27.2	23.1	84.8

Table S6. Summary of the datasets included in the different models.

Model	Response	Predictors	Datasets ^a
Honey production	Honey amount (count model)	time, colony division, supplementary feeding, temperature, precipitation	Established colonies 1999-2008, established colonies 1999-2007
		time, age, colony division, supplementary feeding, temperature	Established colonies of known age 1999-2008
	Honey presence (zero model)	time, colony division, temperature, precipitation	Established colonies 1999-2007
		time, age, temperature, precipitation	Established colonies of known age 1999-2007
Colony mortality	Proportion of dead colonies	temperature and precipitation	All colonies 1999-2007

^aEstablished colonies: Considered only established colonies that survived at least one year, excluding shortly established colonies (recently bought or created), and colonies that died right after being created. Established colonies of known age: Considered a subset of the previous data, containing only colonies with known age. All colonies: Considered all colonies, including shortly established colonies, and colonies that died right after being created. See methods for more details.

Fig. S1. Interior of the nest of *Melipona subnitida*: A) brood combs and B) honey pots. Photo by Ulysses Madureira Maia.

Fig. S2. Honey production per colony (ml) in years in which colony divisions were performed, showing that colonies that underwent division produced less honey than colonies that were not divided. All colonies are shown.

Fig. S3. Honey production per colony (ml) for the year 2007, when no supplementary feeding was provided, and for 2008, when colonies were fed with sugar syrup every fortnight. Each line represents a colony and all colonies are show.

Concluding Remarks

In this thesis we contributed to stingless beekeeping improvement by:

- (1) Performing the first systematic meta-analysis of heritability and genetic correlations across the social Hymenoptera.
- (2) Identifying which male traits underpin stingless bee male reproductive success and integrating pre and post-copulatory sexual selection episodes.
- (3) Using novel statistical tools to quantify heritability and genetic correlations in stingless bee male traits, and thereby understand how selection has shaped these traits.
- (4) Providing novel insights into stingless bee breeding optimization by assessing the impact of the environment and long-term management on stingless bee colony productivity and survival.

We believe these findings may help developing large scale meliponiculture, providing high quality colonies for honey extraction and eventual use in crop pollination.

Results from chapter 1 revealed that quantitative genetic parameters essential for breeding are still scarce for stingless bees, as we only found two studies reporting heritability estimates in two *Melipona* species (Barros 2006; Oliveira et al. 2015). Only productivity traits were investigated, indicating that the main aim was honey production. However, future quantitative genetic studies in stingless bees may explore results already obtained for honeybees, which showed the majority of estimates in our meta-analysis. In general, our results confirmed that morphological traits exhibit higher heritability estimates than

fitness-related traits (in this case, defense, fertility, metabolism, and colony productivity traits) (Mousseau and Roff 1987; Postma 2014). Even though showing lower heritability than morphological traits, traits that affect beekeeping as colony productivity and defense traits showed moderate levels of heritability, indicating potential for breeding. A quite explored feature in honeybees was pathogen defense, since colony losses or reduced productivity due to diseases is a major problem in apiculture. At present, few reports are available for pests and pathogen impact in stingless bees (Maia-Silva et al. 2013; Ueira-Vieira et al. 2015; Nunes-Silva et al. 2016; Shanks et al. 2017). We highlight however that this is an important subject for future studies, because colony overcrowding in large scale meliponiculture will likely lead to disease emergence and spread (MacIvor and Packer 2015). Fertility traits were less explored and most heritability estimates were made for ants and wasps. Only 34 heritability estimates were found for males, indicating a knowledge gap in male mating biology. Even though most breeding programs focus on queen selection (Büchler et al. 2013), males are also important in maintaining selected traits in the population.

In chapter 2, we help to fill the knowledge gap in mating biology of social insects by investigating how sexual selection acts on stingless bee males. In stingless bees, reproductive events implicate in the formation of male aggregations, however, the proximate and ultimate causes of this behavior have not been elucidated yet (Engels and Engels 1984; Mueller et al. 2012). Mating occurs in the air and queens mate with only one male (Peters et al. 1999). Single mating and the high number of males in aggregations suggest high male-male competition, while long term sperm storage and fertilizations imply selection for high quality sperm (Boomsma et al. 2005). In the studied species, *Scaptotrigona* aff. *depilis*, competitive males were found to exhibit higher quality sperm. Male persistence in aggregations selected smaller males and males with higher sperm viability, shorter sperm cells, and high sperm length variation. Our findings revealed thus the importance of male aggregations selecting males with longer potential for sperm storage and fertilizations. In addition, the selected traits may be suggested for breeding

programs to improve colony fertility and survival. Male persistence in aggregations may be an indirect form of selection of males (Wiley and Poston 1996), assuming that opportunity for mate choice in mating flights is low. Since instrumental insemination has not yet been developed for stingless bees, male aggregations may thus work as a natural form of selecting high quality males to inseminate queens. Selection of colonies that produce high quality males may help in this process.

Despite the selection of specific male phenotypes in male aggregations, the heritable basis of these traits still needed confirmation, which was the focus of chapter 3. We used a mixed model with Restricted Maximum Likelihood (Misztal et al. 2002; Thompson 2008) to estimate quantitative genetic parameters for the first time in a social insect male. This approach has the advantage of combining individuals with distinct relatedness degrees in a single analysis, allowing parameter estimation with more precision. The successful application of this methodology can generate protocols for estimation of quantitative genetic parameters for other traits, as colony productivity or pollination related traits. Low heritability was only found in sperm length, suggesting that selection acts stronger on this trait. Since male aggregations selected males with shorter sperm cells, selection for sperm length in order to reduce its length may be interpreted as selection for long-term sperm storage and fertilizations. These findings provide interesting insights in relation to the evolution of social insect male traits, suggesting that ejaculate may be under selection even in singly mated species and thus absence of sperm competition. In addition, this result may be applied to breeding initiatives. For instance, to improve colony fertility, artificial selection must focus on colonies that produce males with shorter sperm, and these males may also be chosen for future artificial insemination of queens.

Finally, to stimulate and optimize stingless bee breeding programs, in chapter 4 we investigated management and environmental effects on colony productivity in the commercially managed stingless bee *Melipona subnitida* (Jandaíra). Specifically, we assessed the effect of colony multiplications, which is central to breeding and also a constraint

to large-scale meliponiculture. The presence of honey in colony storage was affected by colony age. Colony multiplication reduced the amount of honey produced (19%), while supplementary feeding increased honey production (37%). Climatic factors did not influence colony mortality, but in warmer years, the amount of honey produced was lower. These results help planning beekeeping activities (investing and estimating profit, scheduling colony multiplications and honey harvesting) and strategy decision by beekeepers (investing in colony trade or honey production, for instance). A trade-off was observed between honey harvesting and colony multiplication. Thus, to maximize honey production in a given year, the beekeeper must invest in supplementary feeding and avoid excessive colony multiplications, while to increase colony number, avoiding honey harvesting and supplementary feeding may help strengthen colonies and allow a higher rate of colony divisions. Climatic influence warns for the effects of climate warming, which is predicted to induce distribution shifts in this species, also affecting beekeeping practice (Giannini et al. 2017). Although colony multiplication was performed according to colony strength criteria, outlining a breeding scheme, relatedness among colonies was available for few colonies, restraining possibilities to assess genetic variance of colony productivity traits. However, these results allow the identification of traits that may be explored for breeding in future studies, as honey production, balance between honey and pollen stores (which can affect pollination efficacy), capacity of recovering after colony divisions, and resistance to warmer climates. Breeding in *Melipona* species may also account for queen parasitism, since queens from different colonies may invade colonies temporarily without queens (Wenseleers et al. 2011), resulting in the loss of the artificial selection accomplished. This issue may be circumvented by following queen supersedure events through marking and genotyping queens, and controlling queen origin by artificially inseminating queens. Finally, this study revealed the importance of keeping records in beekeeping and maintaining partnerships between researchers and beekeepers to the future development of meliponiculture.

This thesis shows that breeding in stingless bees is feasible, considering past progress with honeybees and bumblebees. However, some precautions must be considered, especially

regarding the depletion of genetic variance, which may threaten bee populations (discussed in chapter 1). Artificial selection of colonies according to specific traits may lead to elevated inbreeding and to a severe reduction in additive genetic variance for important traits and local adaptations (Meixner et al. 2010; López-Urbe et al. 2017). This situation is especially negative for Hymenoptera species (Zayed and Packer 2005), since sex determination in this group depends on the *cds* locus (complementary sex determination). Inbreeding lowers allele diversity in the sex locus, resulting in more homozygous individuals, which will develop into diploid males. These males are usually infertile or unviable, causing a great cost to colonies. Indeed, diploid male production in stingless bees was shown to result in queen death and substitution (Vollet-Neto et al. 2017). Stingless beekeeping and breeding success may thus require the maintenance of genetic diversity, through the conservation of different wild populations, which may serve as genetic repositories in the future. This will be especially important given the probability of infectious disease emergence. For instance, feral honeybees exhibited higher genetic variance, which was related to higher immunocompetence (López-Urbe et al. 2017). We thus advise combining beekeeping and conservation efforts in order to achieve a sustainable use of stingless bees as pollinators and for honey production.

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