## ANA PAULA APRÍGIO ASSIS

## Seleção natural e mudanças climáticas na história evolutiva de esquilos (Sciuridae: *Tamias*)

Natural selection and climate change in chipmunks' evolutionary history (Sciuridae: *Tamias*)



São Paulo 2016

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Tese apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Doutora em Ciências, na área de Genética e Biologia Evolutiva.

Orientador: Prof. Dr. Gabriel Marroig

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Seleção natural e mudanças climáticas na história evolutiva de esquilos (Sciuridae: Tamias)

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Para os incríveis naturalistas que tornaram este trabalho possível:

Joseph Grinnell e Jim Patton

"In every walk with nature one receives far more

than he seeks."

— John Muir

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#### Resumo

O objetivo deste estudo foi compreender como seleção natural age sobre a variação fenotípica a fim de determinar como espécies respondem às mudanças ambientais. Para isso, usei esquilos do gênero Tamias (subgênero Neotamias, família Sciuridae) como um modelo em uma escala tanto macro quanto micro-evolutiva. Esse conjunto de 23 espécies de Neotamias é parte de uma radiação recente, ocupando uma ampla gama de hábitats com marcada partição de nicho entre as espécies. Um aspecto essencial que molda evolução fenotípica são características ambientais, tais como variações climáticas. Dessa forma, no primeiro capítulo eu examinei se as diferenças fenotípicas entre as espécies estão relacionadas às diferenças climáticas entre os hábitats que ocupam. Diversas características ambientais foram significativamente correlacionadas com atributos morfológicos, indicando que estas tiveram um papel importante como possíveis pressões seletivas conduzindo à divergência entre as espécies. Como consequência, é razoável supor que mudanças climáticas em tempo histórico (isto é, durante o Antropoceno) também afetam variação morfológica dentro de uma escala microevolutiva. No segundo capítulo, portanto, eu examinei esta expectativa usando espécimes de seis diferentes espécies, coletados com cerca de 100 gerações entre coletas (um século). Neste capítulo, não foi encontrada uma associação ente o grau de mudanças climáticas ao longo deste período e a magnitude de mudança morfológica ou de pressão seletiva. Contudo, as estimativas de força de seleção variaram substancialmente entre espécies: para a espécie Tamias alpinus observou-se uma alta estimativa de força de seleção, quase duas vezes maior do que para a espécie Tamias speciosus, a qual as menores forças de seleção foram observadas. Desta forma, a fim de avaliar o impacto de seleção direcional nos padrões de (co)variação fenotípica, no terceiro capítulo eu utilizei estas duas espécies, dado que representam extremos em termos de força de seleção dentre as populações analisadas. Estudos teóricos preveem que sob seleção direcional os padrões de (co)variação podem evoluir, realinhando-se com a paisagem adaptativa subjacente, aumentando a quantidade de variância genética na direção da seleção. Este padrão foi observado para T. alpinus, como esperado, dado que esta espécie sofreu a maior força de seleção. Além disso, para esta espécie foram observadas mudanças nos padrões de correlações entre os caracteres. Estes resultados apoiam expectativas obtidas a partir de modelos teóricos que consideram a evolução do mapa genótipo- fenótipo em resposta à seleção natural.

#### Abstract

The aim of this study was to understand how natural selection acts on phenotypic variation to determine species' response to environmental change. I used chipmunks of the genus Tamias (subgenus Neotamias; family Sciuridae) as a model at both a macro and microevolutionary scales. This set of 23 species is part of a recent radiation that occupy a wide range of habitats with marked niche partitioning among co-distributed members. As climate variation is an essential aspect believed to shape phenotypic evolution, in the first chapter I examined how phenotypic differences among these species were related to climatic differences among the habitats occupied. Several climatic variables were significantly correlated with morphological attributes differentiating taxa, suggesting a possible causal link between climate, through selection, and species divergence. As a consequence, it is reasonable to suggest that climate change within historic times (the Anthropocene) has also affected cranial morphological variation within species at a microevolutionary scale. In the second chapter, therefore, I examined this expectation using specimens from six different species, each collected about 100 generations apart (one century). Here, no relationship was found between the degree of climate change over this period and the magnitude of observed morphological change, or in a measure of selection strength. Nevertheless, the estimates of selection strength varied substantially among these species: those for the alpine chipmunk (Tamias alpinus) were the strongest and nearly twice that of the co-distributed lodgepole chipmunk (Tamias speciosus). As a result, to assess the impact of directional selection on the observed patterns of phenotypic (co)variation, in the third chapter I contrasted these two species, since they represent the extremes in the estimated strength of selection among all the species' populations I examined. Theory predicts that, under directional selection, patterns of phenotypic (co)variation might evolve in order to match the subjacent adaptive landscape. This prediction was upheld in the populations of alpine chipmunks, as perhaps expected since they exhibited the strong selective response. Equally importantly, I also observed changes in the overall correlation between traits for the alpine chipmunk in a pattern consistent with that expected under theoretical models that consider the evolution of the genotype-phenotype map in response to directional selection.

## Introdução geral

"... I wish to emphasize what I believe will ultimately prove to be the greatest value of our museum. This value will not, however, be realized until the lapse of many years, possible a century...

This is that the student of the future will have access to the original record of faunal conditions in California and the West, wherever we now work."

- Joseph Grinnell, The Uses and Methods of a Research Museum

#### Introdução geral

#### Genética quantitativa em populações naturais

Esta é uma tese sobre evolução em populações naturais e, como tal, apresenta os resultados de um estudo sobre mudanças em populações impulsionadas pelo processo de seleção natural. O estudo analisa possíveis fatores gerando a necessária força seletiva e também como a variação presente nas populações estudadas afetam e são afetadas por seleção natural.

A genética quantitativa é o estudo de características contínuas determinadas por múltiplos loci (Falconer & Mackay, 1996). Esta disciplina é uma ferramenta essencial para compreendermos como populações evoluem, uma vez que grande parte das características biológicas são contínuas. Mais do que isso, os organismos são formados por conjuntos de caracteres que interagem, por terem função ou desenvolvimento compartilhado e, portanto, não são livres para evoluírem independentemente (Gould & Lewontin, 1979; Olson & Miller, 1958). Dessa forma, para compreendermos o processo de evolução por seleção natural, temos que considerar como múltiplos caracteres afetam e são afetados pelo processo evolutivo.

Tais relações entre caracteres podem ser descritas através da equação multivariada de resposta à seleção (Lande, 1979):

#### $\Delta z = \boldsymbol{G}\boldsymbol{\beta}$

Na equação acima,  $\Delta z$  corresponde a um vetor de resposta evolutiva, isto é, a mudança na média de cada caráter de uma geração para a outra, sendo que a resposta evolutiva é resultado da interação entre  $\beta$  e G. O primeiro é um vetor de gradiente de seleção, ou seja, a força e direção da seleção em cada característica. Já o segundo representa a matriz de efeitos genéticos aditivos, e indica como os caracteres (co)variam geneticamente na população. A matriz G representa a porcentagem de variação na população que é herdada e, portanto, passível de ser selecionada (Lande, 1979; Lande & Arnold, 1983; Steppan et al., 2002). A ideia de covariação está relacionada a como os diferentes caracteres se correlacionam na população, sendo que uma correlação entre caracteres maior do que zero

surge principalmente através de pleiotropia e desequilíbrio de ligação entre loci (Lynch & Walsh, 1998).

A Figura I.1 auxilia na compreensão de como a distribuição da variação em uma população pode afetar a resposta evolutiva. Nessa Figura pode-se observar três diferentes populações que possuem médias iguais para os caracteres X e Y, diferindo no grau de associação entre estes caracteres. Se seleção estiver atuando de modo a favorecer um aumento do caráter Y ( $\beta$  - representado pela seta preta), a resposta evolutiva será em direções diferentes para cada uma das populações, dependendo de como estes caracteres estão correlacionados na população. A população vermelha responderá aumentando não só o caráter X como também Y, uma vez que eles estão positivamente correlacionados. Já para a população verde, a resposta evolutiva será de um aumento do caráter Y e diminuição do caráter X, dado que estes estão correlacionados negativamente na população. Por fim, para a população representada pela elipse azul, observa-se um aumento apenas do caráter Y, dado que não há correlação entre estes dois caracteres nesta população.



Figura I.1. Três populações cujas médias dos caracteres X e Y estão representadas pelo círculo preto e as elipses representam as dispersões dos dois caracteres em torno da média para cada uma das três populações representadas por cores diferentes. Na população vermelha, estes dois caracteres estão positivamente correlacionados, na população verde negativamente e na população azul não há correlação entre os caracteres. Desta forma, quando seleção ( $\beta$ ) atua na direção de aumento do caráter Y, a resposta evolutiva ( $\Delta z$ ) de cada uma destas populações será em direções diferentes.



**Figura I.2. a**: Representação esquemática de um loci cujos alelos determinam o grau de correlação entre dois caracteres X e Y. Indivíduos que carreguem o genótipo AA possuem uma correlação alta de 0,8 entre os dois caracteres; indivíduos com genótipo Aa possuem correlações moderadas entre os dois caracteres (0,5) e indivíduos *aa* não possuem correlações entre os dois caracteres. Note, no entanto, que este loci não afeta as médias das populações nestes dois caracteres (ponto preto no centro das elipses), afetando apenas o grau de correlação entre os caracteres (excentricidade das elipses). Em **b** e **c** tem-se os três fenótipos sobrepostos, sendo que em **b** a seleção (representada pela seta preta) está favorecendo um aumento em ambos caracteres X e Y; e em **c** a seleção está na favorecendo um aumento para o caráter Y e diminuição de X. Espera-se, portanto, que a população **b** evolua na direção de aumento da correlação positiva entre os dois caracteres, favorecendo indivíduos que carreguem o alelo A, aumentando assim sua frequência na população. Por outro lado, espera-se que a população em **c** evolua no sentido de diminuir a correlação entre os dois caracteres, levando a um aumento nas frequências do alelo *a*. Adaptado de (Wagner et al., 2007).

Por outro lado, a própria seleção pode afetar os padrões de covariação nas populações levando à mudanças destes, desde que haja variação herdável que afete o grau de covariação entre os caracteres (Pavlicev et al., 2008; Pavlicev et al., 2011; Pavlicev & Hansen, 2011; Wolf et al., 2005). Por exemplo, na Figura I.2 podemos observar um gene com dois alelos, sendo que cada genótipo determina um grau de associação diferente para dois caracteres (X e Y). Se seleção estiver atuando para aumentar ou diminuir ambos caracteres, o genótipo que determina uma forte correlação entre os caracteres será favorecido. Em contrapartida, se seleção favorecer o aumento de um caráter e diminuição do outro, o genótipo que determina uma dissociação entre os caracteres será favorecido.

Desta maneira, entender como seleção e variação interagem ao longo do processo evolutivo é um passo essencial, não só na compreensão da diversificação de grupos de espécies, como também para compreender se (e como) as espécies se adaptarão frente às mudanças ambientais (Gienapp & Brommer, 2014). Por conseguinte, na presente tese usei este arcabouço de genética quantitativa para compreender: 1) a diversificação fenotípica nas diferentes espécies de esquilos do grupo *Neotamias*; 2) mudanças morfológicas em escala microevolutiva, de cerca de 100 gerações, a fim de entender como seleção e variação morfológica interagiram na determinação da direção e magnitude da resposta evolutiva; e 3) como seleção direcional afetou os padrões de variação de duas espécies do grupo: *T. alpinus* e *T. speciosus.* 

#### Objetos de estudo: crânios de chipmunks

Os esquilos do gênero Tamias, conhecidos popularmente como chipmunks, possuem uma distribuição Holártica ocorrendo por toda Ásia e América do Norte. Atualmente são reconhecidas 25 espécies dentro do gênero (Thorington & Hoffmann, 2005), divididas em três subgêneros: o subgênero Eutamias, com apenas uma espécie que ocorre na Ásia, o subgênero Tamias, também com apenas uma espécie ocorrendo no leste da América do Norte e o subgênero Neotamias, grupo mais diverso com 23 espécies, ocorrendo no oeste da América do Norte, foco desta tese. Estudos recentes sugerem a elevação destes subgêneros à categoria de gênero com base em tempo de divergência (Patterson & Norris, 2015). No entanto, no presente trabalho decidi manter a nomenclatura adotada por (Thorington & Hoffmann, 2005). As diferentes espécies dentro do grupo Neotamias são definidas basicamente por variação molecular, características morfológicas cranianas e de anatomia genital (Johnson, 1943; Patterson, 1983; Reid et al., 2012; Sutton & Patterson, 2000). Importa notar que existem diversos casos relatados de hibridização entre espécies do grupo (Demboski & Sullivan, 2003; Good et al., 2008; Good et al., 2003; Reid et al., 2012; Rubidge et al., 2014; Sullivan et al., 2014). O grupo de esquilos *Neotamias* é diurno e onívoro com a dieta majoritariamente composta de sementes e frutas. Ao contrário do seu grupo irmão, os esquilos de chão (*ground squirrels*), *chipmunks* não estocam gordura durante o inverno. Durante os meses frios do ano, estes esquilos hibernam, acordando esporadicamente para comer sementes estocadas em suas tocas (Ingles, 1965). O ciclo de vida de *chipmunks* consiste de acasalamento no início da primavera, com os primeiros nascimentos da estação no começo de maio e junho. No final do outono, os jovens já adquirem tamanho adulto e estão completamente desenvolvidos antes do início do inverno (Grinnell & Storer, 1924).

O oeste da América do Norte é um ambiente extremamente diverso com inúmeros tipos de paisagens. Com base na composição vegetal, podemos dividir estas paisagens em zonas de vida (*life zones*) que correspondem à cinturões com espécies de plantas e animais características (Ingles, 1965; Schoenherr, 1992). As mudanças vegetais de uma zona de vida para outra ocorrem principalmente devido à diferenças latitudinais ou de altitude, sendo que diferentes espécies de *chipmunks* podem ser encontradas em cada uma destas áreas, havendo pouca sobreposição de áreas de vida entre as diferentes espécies distribuídas contiguamente (Grinnell & Storer, 1924; Heller, 1971; Heller & Gates, 1971; Heller & Poulson, 1972; Reid, 2006; Sullivan et al., 2014). Consequentemente, estas espécies ocorrem em hábitats extremamente variados, indo do nível do mar a cerca de 4000 metros de altitude; de áreas semi-desérticas a áreas úmidas e de regiões florestais à paredões rochosos (Grinnell & Storer, 1924; Heller, 1971; Ingles, 1965; Johnson, 1943). A diversificação das espécies deste subgênero é bastante recente, tendo ocorrido nos últimos 2,75 milhões de anos, possivelmente impulsionada por oportunidades ecológicas surgidas ao invadir novos hábitats (Sullivan et al., 2014).

Escolhi usar o crânio como modelo de estudo por se tratar de uma estrutura complexa, formada por diversos ossos que se interconectam desempenhando inúmeras tarefas essenciais, tais como proteção do cérebro e órgãos sensoriais, alimentação e regulação da perda de água e calor (Elbroch, 2006; Hanken & Hall, 1993; Schmidt-Nielsen et al., 1970). Também, inúmeros estudos mostraram correlações significativas entre morfologia craniana e variáveis ambientais em diferentes mamíferos (Burnett, 1983; Caumul & Polly, 2005; Eastman et al., 2012; Grieco & Rizk, 2010; Monteiro et al., 2003; Patton & Brylski, 1987). Além disso, já foi sugerido para *chipmunks* que diferenças cranianas entre espécies resultam de respostas às condições ambientais dos diferentes hábitats ocupados (Allen, 1890; Patterson, 1980, 1983; Sutton & Patterson, 2000). Sendo assim, o crânio em *chipmunks* pode ser considerado um excelente modelo para examinar processos evolutivos resultantes de mudanças ambientais nos hábitats destas espécies.

#### O Projeto Grinnell

Quando a filantropa Annie Alexander fundou o Museu de Zoologia de Vertebrados (MVZ, na sigla em inglês) da Universidade da Califórnia, em Berkeley, ela convidou o naturalista Joseph Grinnell para ser seu diretor. Grinnell atuou nessa posição de 1908 até o ano de sua morte em 1939. Durante este período, ele realizou extensivas coletas e expedições científicas por toda a costa oeste dos Estados Unidos, a fim de construir uma coleção representativa daquela fauna. Uma vez que grande parte de seu interesse científico estava ligado à distribuição de espécies e comunidades no espaço, ou aos fatores que determinam essa distribuição, em todas as suas coletas houve grande preocupação com o registro e caracterização do exato local em que cada espécime foi coletado. Esta visão, avançada para seu tempo, possibilitou registros e coletas de mais de 100.000 espécimes de mamíferos, aves, anfíbios e répteis de cerca de 700 localidades em múltiplos transectos. Além disso, seu trabalho foi registrado em 74.000 páginas de cadernos de campo e 10.000 fotos. Desta forma, temos hoje um registro bastante acurado das características das diversas comunidades de vertebrados terrestres na Califórnia no início do século XX (The Grinnell Resurvey Project, 2015). Tais registros possibilitaram o retorno aos exatos mesmos locais em que Grinnell e colaboradores coletaram, a fim de analisar como as comunidades e populações dos diferentes grupos de animais mudaram ao longo do último século. Este é o principal objetivo do "Projeto Grinnell".

As coletas de Grinnell foram realizadas antes dos efeitos das mudanças climáticas causadas por ações antrópicas (IPCC, 2014) o que torna estes registros ainda mais valiosos para cientistas que buscam compreender às respostas das espécies às mudanças climáticas e de uso da terra do último século. O Projeto Grinnell, portanto, está voltando aos mesmos locais a fim de analisar tais mudanças, além de criar outro ponto de referência para que estudos futuros possam se beneficiar de mais estas análises.

Utilizei nessa tese espécimes de *chipmunks* coletados por Grinnell e seus colaboradores no começo do século XX, bem como dados provenientes de coletas feitas por membros do Projeto Grinnell, amostras estas separadas por quase um século de diferença. Assim pude estudar, utilizando o arcabouço de genética quantitativa, mudanças evolutivas dentro das espécies analisadas. Esta amostra é ímpar não só pela época e riqueza de detalhes na coleta, mas também pela representatividade de espécimes e espécies. Estudos de genética quantitativa só podem ser feitos com um número amostral relativamente elevado (para que covariâncias consigam ser estimadas de forma confiável) e esta amostra me permitiu realizar isso. Além disso, uma vez que consegui uma representatividade de diferentes espécies, pude expandir a compreensão de como estas espécies foram afetadas por mudanças climáticas. Por fim, este trabalho faz parte de um esforço multidisciplinar, o que contribuiu em muito para uma melhor interpretação dos resultados obtidos (Eastman et al., 2012; Moritz et al., 2008; Rowe et al., 2015; Rubidge et al., 2012; Rubidge et al., 2011; Rubidge et al., 2014). Espero que os resultados obtidos nesta tese auxiliarão neste esforço, contribuindo para futuros estudos destas comunidades e melhores políticas e ações mitigadoras dos efeitos das mudanças climáticas na vida selvagem.

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## Chapter 1

# Evolutionary processes and its environmental correlates in the cranial morphology of western chipmunks (*Tamias*)

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"The study of natural history should develop the power of insightkeenness, not only in seeing what animals do, but in determining why those things are done. The interrelations existing between any animals and its environment are exceedingly manifold and vital."

-J. Grinnell and T. Storer; Animal Life in the Yosemite

#### Abstract

The importance of the environment in shaping phenotypic evolution lies at the core of evolutionary biology. The chipmunks of the genus Tamias (subgenus Neotamias) are part of a very recent radiation, occupying a wide range of ecological environments with marked niche partitioning among species. One open question is if and how those differences in environmental aspects affected the phenotypic evolution of this group. Herein we examined the relative importance of genetic drift versus natural selection in the origin of cranial diversity exhibited by clade members. We also explored the degree to which variation in potential selective agents (environmental variables) are correlated with the patterns of morphological variation presented. We found that natural selection has been the predominant evolutionary force at *Neotamias* diversification, although the strength of selection varied greatly among species. Moreover, selection promoted mainly divergence along the first axis of greatest phenotypic variation. This morphological diversification, in turn, was correlated with environmental conditions, with higher selection strength estimates correlated with a higher degree of climatic niche change, suggesting a possible causal relationship. These results underscore that extant Neotamias represent an adaptive radiation in which aspects of the environment have acted as the selective force driving species' divergence.

**Keywords**: Natural selection; morphometrics; quantitative genetics; climatic niche; phylogenetic comparative method; adaptive radiation

#### Introduction

For more than a century, understanding the role of evolutionary processes in driving phenotypic diversification and shaping the way species interact with their environment has been of central concern in biology. In a seminal paper, Lande (1979) demonstrated that even very complex structures could be generated, at least in theory, through a neutral process of genetic drift. Therefore, rather than simply assuming that species have diversified adaptively, the initial step in any study should be one that tests if a random evolutionary process could generate the observed phenotypic diversity. In this context, genetic drift is an appropriate null hypothesis. Moreover, to understand how species diversify and adapt to different environments is essential, especially in this world threatened by human induced changes. One major factor influencing species' phenotypic evolution is climate (Barnosky et al., 2003), which is also one of the major aspects affected by human actions (IPCC, 2014). Therefore, by studying a group of species that diversified recently occupying a wide range of climatic niches we might gain a better knowledge of how climate change might impact species under an evolutionary perspective.

The western North American chipmunks, genus Tamias, subgenus Neotamias, comprise 23 extant species that originated about ~2.75 million years ago in the early Pleistocene (Reid, 2006; Sullivan et al., 2014). This clade is one of the most speciose among North American mammals and exhibits the hallmarks of a recent, rapid radiation (Good et al., 2003; Reid et al., 2012; Sullivan et al., 2014). In striking contrast, the sister group to Neotamias includes two lineages, neither of which has apparently undergone any speciation event since their respective origins: Tamias sibiricus (subgenus Eutamias) is distributed through a large geographic region in temperate Asia, and Tamias striatus (subgenus Tamias) occurs throughout the eastern United States and adjacent Canada. Species of Neotamias are ubiquitous members of the diverse habitats found across western North America, which include alpine tundra, all types of conifer and western hardwood forests, sagebrush plains, brush covered montane slopes, and dense temperate rainforest (Nowak, 1999), and across an elevational gradient from sea level to 4,000 meters and an environmental gradient from coastal humid areas to the dry intermontane interior (Johnson, 1943; Reid, 2006). As many as seven species may be found along a single elevational transect, as in the central Sierra Nevada in California (Grinnell & Storer, 1924), with up four species co-occurring in a single area (Sullivan et al., 2014). Nevertheless, conspicuous niche partitioning is apparent in multi-species assemblages and sharp elevational zonation patterns are typical, resulting in limited true syntopy (Bergstrom, 1992; Grinnell & Storer, 1924; Heller, 1971; Heller & Gates, 1971; Heller & Poulson, 1972).

We chose to study the skull in these chipmunks since it is one of the most important structures determining how mammalian species perceive and interact with their environment. Therefore, studying the skulls in this group might enlighten us in how those species adapted and diversified to those strikingly different environments. Beyond the obvious role that the jaws and teeth play in food acquisition and initial processing, the interconnected bony elements of the skull serve to protect the brain and sensory organs (eye, inner ear, olfactory receptors; Elbroch, 2006) and serve in water balance and temperature regulation (counter-current water and heat exchange via the nasal passages and convoluted turbinal bones; Schmidt-Nielsen et al., 1970). Among mammals, rodents exhibit a great array of feeding specializations, with their characteristic single pair of gnawing incisors and highly specialized masticatory muscles hypothesized to underlie their extreme evolutionary success (Cox et al., 2012). Moreover, several studies have suggested that cranial trait differences among chipmunk species resulted from their response to environmental conditions associated with the differential habitats occupied (Allen, 1890; Patterson, 1980, 1983; Sutton & Patterson, 2000). Such striking features make this group a great model for examining underlying evolutionary processes.

Here, by combining phylogenetic comparative methods with the framework of the quantitative genetics theory, we examined the pattern of variation in quantitative attributes of the chipmunk skull. Our goal was to disentangle the relative roles of genetic drift and selection in their cranial phenotypic evolution and relate this to possible selective pressures. Our focal group is the radiation of those highly diverse species in western North America that collectively comprise the subgenus *Neotamias*, given their remarkable differences in abiotic niche occupancy in such short diversification time. We start by testing hypotheses of evolutionary diversification in *Neotamias*, to understand if the cranial diversity seen among

species could be explained solely by genetic drift, natural selection, or potentially by a combination of these two processes. We then investigate if climatic variables (potential selective agents) are associated with the evolution of morphological traits, which would be expected under natural selection. The phenotype-environmental correlation is thought to be an essential part of the adaptation process and one of the aspects that can demonstrate an adaptive radiation (Schluter, 2000; Wainwright & Reilly, 1994). Therefore, understanding how cranial traits are correlated with environmental variables might enlighten us on how ecological variation can promote divergence between species (Wainwright & Reilly, 1994). Finally, we reconstructed the selective regimen experienced along *Neotamias* evolutionary history and compared its selection magnitude with species' climatic niche shifts. The rationale behind this is our expectation that the invasion of new environments might lead to different selective pressures, which in turn could have triggered the speciation process in this clade (Benkman, 2003; Schluter, 2000).

#### Methods

#### Sample and measurement

We measured 2,238 skulls representing 20 of the 23 species of *Neotamias* and the single species in the subgenus *Tamias* (*T. striatus*) (taxa and sample sizes available in Table S1.1). All specimens are deposited in the Museum of Vertebrate Zoology (MVZ, Berkeley, CA) and National Museum of Natural History (NMNH, Washington, DC). We included only adult specimens, defined by full eruption of the permanent premolar 4 and a completely fused basisphenoid-basioccipital suture. The taxonomic arrangement used throughout this study follows Wilson and Reeder (2005), and the phylogeny presented is based on Sullivan et al. (2014). For polytypic species we included only a single representative subspecies. In a few cases (see Table S1.1) where specimen availability was limited, we included individuals assignable to two subspecies. We removed significant differences due to sex, age, locality, and subspecies prior to the estimation of the pooled within-species phenotypic variance/covariance (V/CV) matrices, using the residuals of a multivariate analysis of variance (MANOVA), and a pooled-by-subspecies mean was used for each trait (Table S1.1).
One of us (APAA) recorded three-dimensional coordinates for 27 landmarks on each skull (Figure 1.1; Table S1.2) using a Microscribe 3D MX digitizer (Microscribe, IL). Landmarks were positioned at the intersection of sutures or other discrete (and homologous) cranial features; each landmark was readily identifiable in all specimens. We chose this set of landmarks to reflect potentially important developmental and functional relationships among cranial structures while simultaneously representing the whole skull (Cheverud 1982; Marroig & Cheverud 2001). A set of 38 linear measurements was then calculated from the landmark coordinates (Figure 1.1); these typically encompass only a single bone of the skull and thus capture local developmental/functional processes. Bilaterally symmetrical measurements were averaged, and if the skull was damaged on one side, the other was used instead of the average. All specimens were measured twice, allowing the estimation of repeatability to account for measurement error (Lessels & Boag, 1987). The average of repeated measurements was used in all subsequent analyses.



**Figure 1.1.** Specimen of *T. alpinus'* skull displaying the landmarks and linear measurements used in the study. The scale bar represents 1 centimeter. A brief description of each landmark position is available on Table S1.2.

#### Genetic drift tests

We applied two different drift tests to evaluate which evolutionary process was responsible for the cranial diversity seen among *Neotamias* species. Both are based on quantitative genetics predictions for groups evolving through drift (Ackermann & Cheverud, 2002; Hohenlohe & Arnold, 2008; Lande, 1979). The first is referred as a regression test and the second as a principal components (PCs) correlation test. Both tests are based in the premise that the species patterns of covariance have remained relatively stable throughout their diversification. Therefore, in order to verify this premise we compared the phenotypic covariance matrices (**P**-matrices) among species using Random Skewers and Krzanowski methods (Blows et al., 2004; Cheverud & Marroig, 2007; Krzanowsky, 1979). We found considerable similarity in the covariance structure for all matrices using both methods (Supplementary material, Tables S1.3 and S1.4 and Figure S1.1). These results allowed us to continue with the investigation of the evolutionary processes responsible for the diversification of *Neotamias*.

*Regression test:*– Proposed by Ackermann and Cheverud (2002), the idea behind this test is that in populations evolving through genetic drift the amount of observed phenotypic divergence will be proportional to the amount of variation in the ancestral population (Ackermann & Cheverud 2002; Marroig & Cheverud 2004). This relationship can be expressed by the following equation:

$$\mathbf{B}_{\mathbf{t}} = \mathbf{G}(t/N_{e}) \tag{1}$$

Where  $B_t$  represents the V/CV matrix between groups at generation t; **G** is the additive genetic V/CV matrix of the founding population from which the group of species is derived, and  $N_e$  is the effective population size of the individual taxa (Lande, 1979, 1980; Lofsvold, 1988). For morphological traits, and particularly in mammalian skull studies, usually the phenotypic within-group V/CV matrix (**W**) is quite similar to **G** (Cheverud, 1988; Marroig & Cheverud, 2001; Oliveira et al., 2009; Roff, 1995), and can therefore be used as a substitute for **G** in the above equation. This assumption of **G** and **P**-matrices exchangeability is particularly robust on empirical cases where **P**-matrices are structurally similar among species under investigation, as is the case here (Supplementary material Tables S1.3, S1.4). **W** can be interpreted as an estimate of the ancestral population matrix. Given that t and N<sub>e</sub> are constant for a given comparison, the pattern of V/CV between groups must be proportional to the V/CV pattern within groups, in other words **B** should be proportional to **W** if the populations are evolving through genetic drift (Ackermann & Cheverud 2002, 2004; Marroig & Cheverud 2004). Alternatively, where **B** and **W** are not proportional, directional selection may have acted upon the evolution of the groups (Ackermann & Cheverud, 2004; Prôa et al., 2012). To simplify this relationship of within- to between-groups variation, we transformed **W** to its principal components (PCs). On a logarithmic scale, we can write the relationship between and within groups V/CV as a linear regression:

$$\ln(B) = \ln(t/N_e) + b \ln(W)$$
<sup>(2)</sup>

In this case b corresponds to the slope of the regression line. If the observed diversification was due to genetic drift, we expect the slope of regression not to deviate significantly from 1.0. A significant deviation from a slope of 1.0 indicates a pattern not likely to have been produced by genetic drift (Ackermann & Cheverud 2002; Marroig & Cheverud 2004). Regression slopes above 1.0 indicate that one or more of the first few PCs are more variable, relative to the other PCs, than expected under genetic drift. This could happen through diversifying selection for the highly variable PC or by stabilizing selection on the later PCs. Slopes significantly smaller than 1.0 occur when species are relatively highly divergent along minor PCs. This can occur through strong diversifying selection along these dimensions or stabilizing selection on the remaining PCs.

W-matrices for each node of the phylogeny were estimated in R environment (Melo et al., 2015; R Core Team, 2014), pooling by sample size and controlling for sources of variation, which were not of immediate interest (Table S1.1). Later,  $\mathbf{W}$  was reduced to their principal components and scores of the projection of each species means on each normalized

PC were calculated. The variance of the scores on each PC was then used as the estimate of the between-groups variance (**B**). Since genetic drift is rejected if the regression line between **B** and **W** deviates significantly from 1.0, the number of species involved in the analysis has an influence in the uncertainty of the confidence interval. The smaller the number of species used, the higher this uncertainty. Thus, in order to minimize the occurrence of type II error, this analysis was only applied to nodes of the phylogeny with more than four descent species. Type I error rates, on the other hand, for this regression test are acceptable when the diverging population/species satisfy the assumption of similarity in variance/covariance patterns (Prôa et al., 2012).

PCs correlation test:- By definition, principal components are uncorrelated with each other. Hence, when we apply a principal component transformation the result is a new set of uncorrelated variables. In a macroevolutionary context, therefore, a significant correlation between the average PC scores of each species in the PC space defined by the eigenvectors of **W**-matrix is an indication of co-selection between both traits (in this case PCs). The reason for this is that the **B**-matrix expected under diversifying directional selection is:

$$B = GCG \tag{3}$$

Where **C** is the V/CV matrix among selection gradients for the traits (Felsenstein, 1988; Zeng, 1988). Notice, therefore, that there are two potential sources of correlated evolution among traits: common inheritance (captured in **G**, or as discussed previously **W** in this case) and selective covariance (captured in **C**). Because PCs are, by definition uncorrelated, **G** is then a diagonal matrix in this case. Thus, any correlation in **B** must arise from **C** (selective covariance, Felsenstein, 1988). To test this, each species mean was projected onto **W**'s PCs (redefined at each node) and its scores were calculated. After this, we computed the Pearson correlation between those PC scores. As a general rule, the number of PCs used in the comparisons is equal to n-1, with n equal the number of species being compared. We rejected the null hypothesis of evolution through drift whenever significant correlations were found among at least one pair of PCs. Both regression and correlation tests can be viewed as complementary to investigate hypotheses of evolutionary diversification. While the regression test evaluates whether the variation within and between groups is proportional (deviations from proportionality indicate selection), the correlation test detects co-selection (uncorrelated traits being selected together).

### Directions of divergence and selection quantification

We also explored in a graphical way how the divergence observed among species was distributed along axes of high or low variation of the **W**-matrix (i.e., which morphospace direction divergence among species was concentrated). Therefore, we projected the divergence observed in **B** in the same space of **W**.

Moreover, we reconstructed the potential selection gradients responsible for the morphological changes of each species. In this way, we could access how directional selection was distributed on the phylogeny. It is a different question than simply estimating the total amount of morphological change, in the sense that by estimating the selection gradient, we are estimating the magnitude and direction of selection itself, irrespective of the possible effects of the amount of variation in the morphological change. The selection gradients were reconstructed based on Lande's (1979) multivariate equation;

$$\beta = W^{-1} \Delta z \tag{4}$$

Where  $\Delta z$  is the vector of evolutionary response, W<sup>-1</sup> is the inverse of the phenotypic pooled within- groups matrix and  $\beta$  is the selection gradient vector. Matrices are always estimated with some degree of error, whether due to sampling or measurement errors, and this error is amplified whenever a matrix inversion is required. So, in order to control for this noise, we calculated inverted **W**-matrices using an extension approach (described in Marroig et al., 2012). We reconstructed the ancestral states of the 38 traits using two different methods, a Brownian motion-based maximum likelihood estimator (Schluter et al., 1997) using function ace in ape package in R (Paradis et al., 2004; R Core Team, 2014) and the linear parsimony method using Mesquite version 3.02 (Maddison & Maddison 2006, 2015). Because linear parsimony and maximum likelihood reconstructions gave very similar results we will present only the maximum likelihood estimates for brevity. After reconstructing the ancestral states, we could then calculate the vector of response to selection ( $\Delta z$ ) as the difference vector between two nodes or between an extant species and its ancestor. We mean-standardized **W** and  $\Delta z$  estimates in order to obtain selection gradient values that were comparable among different nodes and species (Hansen & Houle, 2008; Hereford et al., 2004). The strength of selection was calculated as the norm of the mean standardized  $\beta$ -vector.

#### Climatic variables

We extracted climate data from each species locality georeference coordinates from the PRISM database (PRISM Climate Group, 2004). For four species (T. obscurus, T. cinereicollis, T. ruficaudus, and T. striatus), however, coordinate locality data from the morphological data were unavailable; for these, we estimated climate data from random points drawn from their mapped ranges (distribution maps available at the *IUCN*; IUCN, 2014). We used Worldclim climate data (Hijmans et al., 2005) for the Mexican species, T. durangae, since the PRISM dataset does not extend to that country. We used in our analyses extreme estimates of temperature and precipitation (minimum temperature in the coldest month; maximum temperature in the warmest month measured in Celsius degrees; precipitation of wettest and driest months measured in mm<sup>3</sup>) allied with mean annual temperature and precipitation indexes. We extracted those information from the climatic database using function *biovars* in package *dismo* for R (Hijmans et al., 2014). Subsequently, we estimated the impact of the climatic variables in the morphological variation through an evolutionary regression implemented in SLOUCH program (Hansen et al., 2008). The idea behind this analysis is to disentangle effects of phylogenetic inertia in the observed correlations between predictor (climatic variables) and dependent variables (morphological variation), from effects of adapting to an optimum that is influenced by the predictor variable. The model is built around an Ornstein-Uhlenbeck model (OU) of adaptive evolution for a single trait, while the predictor variable is modeled as a Brownian Motion (BM) process (Hansen et al., 2008). The method uses generalized least squares to estimate the regression parameters, i.e. the influence of the predictor variable on the primary

optimum. It also uses maximum likelihood to jointly estimate phylogenetic inertia (represented by a parameter called phylogenetic half-life,  $t_{1/2}$ ) and stochasticity ( $v_y$ ) effects. Those stochastic effects can be interpreted as unmeasured selective forces and/or drift effects (Voje & Hansen, 2013). In this way, this analysis, by disentangling phylogenetic effects due to phylogenetic inertia (slowness of adaptation) from phylogenetic effects generated due to closely related species adapting to similar environments, is a better choice when trying to estimate the impact of a certain variable in the evolution of a group (Hansen et al., 2008). The analysis, thus, returns an estimate of the regression coefficient of the linear regression, taking into account the phylogenetic history of a clade.

We used as predictor variables the above mentioned climatic variables, and as response variable scores of each species in the first two PCs of **W**. Measurement error, in both predictor and response variables, might impact the estimation of the evolutionary regression and to accommodate this uncertainty we included the variance for each individual parameter in the analyses (Hansen & Bartoszek, 2012). We compared the relative support for each model in relation to models estimated without the predictor variable (therefore, assuming a full Brownian motion process) using Akaike's Information Criterion correction for small sample sizes, AICc. A model was considered the best fit for the data if its AICc values was at least two units lower than the model without predictor (Hansen et al., 2008). High values of the coefficient of determination  $r^2$  indicate that a high amount of morphological variation is explained by the predictor variable. Because the estimation of phylogenetic inertia  $(t_{1/2})$  is rather inaccurate in small phylogenetic (<30 terminals), as pointed by Hansen et al. (2008), we decided to focus our comparisons in the outcome of the evolutionary regression and estimate the regression in two different scenarios as suggested by Hansen et al. (2008). The first, we allowed a small phylogenetic effect, with  $t^{1}/_{2}$  ranging from 0 to 0.1 (10%) of the total length of the tree). The second scenario we allowed high values of phylogenetic inertia, from 0 to 100% of the total length of the tree.

#### Selection gradient and magnitude of climatic change

Another approach to investigate if the climatic conditions are a candidate as the driver of the evolutionary change observed in *Neotamias* was through comparing the magnitude of selection gradients (strength of selection) and climatic change throughout the phylogeny. We therefore, transformed the climatic variables into z-scores and reconstructed the climatic states using a Brownian motion-based maximum likelihood estimator (Schluter et al., 1997). Then, we calculated the climatic changes as the vector of differences between two nodes or between an extant and its ancestor node. We compared the vector of climatic change with the selection strength vectors by a linear regression.

## Results

### Genetic drift tests

Both regression and correlation approaches indicate that morphological evolution in *Neotamias* cannot be explained by genetic drift alone and that natural selection was responsible for the majority of their cranial morphology diversification (Figure 1.2). In the regression test, of the 12 hierarchical levels analyzed, four have slopes significantly different from one: node 1 (all *Neotamias* + *T. striatus*), node 2, node 3, and node 10 (small-bodied *Neotamias*) (Figure 1.2, Table 1.1). Divergence within these groups is therefore unlikely to be due to genetic drift alone.

For the correlation test, significant correlations were found for nodes 1 (all *Neotamias* + T. striatus), 2, 3, 4, 5, 7 (quadrivittatus group), 8 and 11 (townsendii or large-bodied group), indicating deviations from the expectation under genetic drift in each case (Figure 1.2, Table 1.1). In most comparisons, PC1 was significantly correlated with the remaining PCs, except for node 11 (townsendii or large-bodied group), where the only correlation observed was between PC3 and PC5. Considering results of both drift tests together, genetic drift was rejected as an explanation for the three more inclusive nodes in phylogeny (node 1, 2, and 3; Figure 1.2, Table 1.1). As one moves further along the phylogeny, shallower branches show a less clear picture with genetic drift being rejected for at least one test for the majority of nodes. In only three, out of twelve nodes, each involving four species in the comparison, the phenotypic diversification was consistent with the null hypothesis of genetic drift (nodes 6, 9, and 12).

Table 1.1. Genetic drift test results, showing the slope (b) of the regression line calculated between W-matrix and B-matrix for the regression test with confidence interval. Regression coefficients significantly different from 1.0 are shown in bold. The node labels correspond to the node number displayed in Figure 1.2. For each node all species in the node were included in the drift tests. For the correlation test we present the number of PCs included (n-1 of the number of species with a maximum of 10) and the PCs were we found any correlation. The first number corresponds to a specific PC and the numbers in parentheses are the PCs to which a significant correlation was found.

	Regressi	on test			Correlation test
node		95 %	% CI	DCc included	
label	b	Lower	Upper	PCS Included	Correlated PCs
1	1.215	1.082	1.349	10	1-(2,4,8,9,10); 2-(8,9,10);4-(9); 8-(9)
2	1.204	1.071	1.337	10	1-(2,4,9,10);2-(4,9); 4-(5,9,10);5-(10);8-(9)
3	1.166	1.007	1.325	10	1-(2,4,8,10); 4-(8); 7-(8,9); 8-(10)
4	1.094	0.961	1.226	10	1-(7,8,9); 7(8,9); 8-(9)
5	1.072	0.936	1.209	9	1-(7,8,9); 7(8,9); 8-(9)
6	0.979	0.733	1.225	3	-
7	1.017	0.846	1.189	5	1-(3,5); 3-(5)
8	1.008	0.808	1.207	4	1- (3)
9	0.975	0.769	1.182	3	-
10	1.328	1.051	1.606	3	-
11	1.012	0.838	1.186	4	3-(4)
12	0.958	0.753	1.162	3	-

#### Directions of divergence and selection quantification

Most of the divergence observed in the Neotamias diversification was along the first principal component of  $\mathbf{W}$ . For some nodes, around 80% of the total divergence occurred along PC1 (nodes 1,2,3,6 and 10; Figure 1.3). PC1 is an allometric vector representing variation in cranial size and associated shape, with most loadings pointing in the same direction (Table S1.5). Therefore, most of the divergence in this group can be attributed to size related change. The only group that deviates from this pattern is the southern Rocky Mountains species (as defined by Reid, 2012, and including *T. umbrinus*, *T. rufus*, *T. quadrivittatus*, *T. cinereicollis*, *T. dorsalis*, *T. canipes*). In this group PCs 3 and 4 both had higher percentages of divergence than expected by drift, while PC1 divergence was in accordance with a drift scenario.



Figure 1.2. Phylogeny displaying the results of the genetic drift tests. The values close to the nodes represent the node number referred in the Table 1.1 and Figure S1.2. Whenever a node had 3 or less species, none of the tests was performed. The scale bar represents time in million years. Phylogeny based on(Sullivan et al., 2014).

The magnitude of selection greatly varied among branches. Smaller estimates are concentrated on more basal nodes, indicating that selection was stronger in the more recent branches (Figure 1.4). Also, the strength of selection was not correlated with a higher divergence along the first PC. For example, nodes 7, 8 and 9, which had patterns of divergence mainly in the direction of PCs 3 and 4, presented some of the stronger selection estimates. Lastly, the branch that lead to T. alpinus and T. minimus had the higher estimates of selection (76.77, Figure 1.4-a, Table S1.6), a value much larger than that of any other clade, indicating that in this particular clade selection was very strong. At the other extreme, the group represented by T. senex, T. siskiyou and T. townsendii had some of the weakest selection gradients in their diversification.



Figure 1.3. Percent variance explained by each principal component of the ancestral W-matrix (gray lines). The black lines represent the projection of the **B**-matrix in each of the 10 first PCs of W. For the majority of nodes most divergence occurred along PC1, in some cases it represented more than 70% of the total divergence among species (nodes 1,2,3,4,5,6 and 10).

## Phenotype-environment correlation

We found several high correlations between the first two PCs and climatic variables. The first two PCs of the pooled-within-species variance/covariance (V/CV) matrix are given in Table S1.5, with the 38 cranial traits classified according to functional/developmental groups. Together, these PCs account for 42.2% of the total within-species variation. PC1, as mentioned, is an allometric size vector, while PC2 is mainly a contrast between traits that affected the length of the face and the width of the neurocranium. In the context of the functional/developmental groups, PC2 contrasts larger oral/nasal group distances with

smaller zygomatic ones, suggesting a narrowing of the zygomatic arch. The second PC also represents a factor where an enlarged frontal bone contrasts with a smaller cranial vault (Table S1.5).

For the comparisons involving PC1, minimum temperature of the coldest month and mean annual temperature were the only climatic variables that had a better predictive power than the model without predictor (AICc values more than 2 units smaller). Minimum temperature of the coldest month explained from 39.9 to 48.7 % of the total variance in the scenario of strong and mild phylogenetic inertia, respectively (Table 1.2). For the annual mean temperature, the total amount of variance explained was smaller ranging from 24.5 to 34.2 % (Table 1.2, strong and mild phylogenetic inertia). Since PC1 is an allometric size vector, these results suggest that smaller animals live in relatively colder environments (Figure 1.5).



Figure 1.4. a) Phylogeny displaying the strength of selection estimates, with increasing values represented from light blue to dark blue/black colors. b) Linear regression between climatic change magnitude and selection strength. The color gradient follows the same as in  $\mathbf{a}$ , with increasing values going from light blue to dark blue/black colors. The regression line is also displayed. The estimated  $r^2$  is equal to 0.60 and p<0.001.

phylogenetic model with estimates ev	t inertia was allowed $(t^1/_2 \text{ values ranging from } 0-1)$ no predictor variable (Brownian motion). The amolutionary regression slope and standard error.	10% of total t nount of varia	tree length) ance explain	<ul> <li>Lower values of <i>i</i> ned by the predicto</li> </ul>	AICc indicate 1 r variable in e	nodels with be ach model is a	st fit compared to the lso shown $(r^2)$ and the
Response variable	Predictor variable	Model type	1: Strong ph	ylogenetic inertia	Model ty	<b>rpe 2:</b> Mild phylo	genetic inertia
		r² (%var)	AICc	slope ± se	r² (%var)	AICc	slope ± se
PC1	Annual Mean Temperature (°C)	24.59	101.57	0.047 0.018	34.24	110.75	$0.068 \pm 0.021$
PC1	Max Temperature of Warmest Month (°C)	0.89	106.64	$0.008 \pm 0.19$	7.43	117.70	$0.033 \pm 0.026$
PC1	Min Temperature of Coldest Month (°C)	39.94	97.69	$0.046 \pm 0.012$	48.97	105.60	$0.061 \pm 0.014$
PC1	Annual Precipitation (mm <sup>3</sup> )	16.07	103.72	0.003 ± 0.002	16.74	115.56	$0.004 \pm 0.002$
PC1	Precipitation of Wettest Month (mm $^3$ )	18.59	103.20	$0.018 \pm 0.008$	18.51	115.13	$0.021 \pm 0.010$
PC1	Precipitation of Driest Month ( ${ m mm}^3$ )	0.04	106.79	-0.006 ± 0.064	0.42	119.19	-0.025 ± 0.087
PC1		ı	103.63		ı	116.11	
PC2	Annual Mean Temperature (°C)	29.45	46.26	$0.013 \pm 0.005$	30.67	49.98	$0.014 \pm 0.005$
PC2	Max Temperature of Warmest Month (°C)	1.10	51.51	$0.002 \pm 0.005$	3.26	56.11	$0.005 \pm 0.006$
PC2	Min Temperature of Coldest Month (°C)	41.54	44.30	$0.012 \pm 0.003$	43.54	46.26	$0.012 \pm 0.003$
PC2	Annual Precipitation (mm <sup>3</sup> )	17.35	48.90	$0.001 \pm 0.000$	21.73	52.15	$0.001 \pm 0.000$
PC2	Precipitation of Wettest Month (mm $^3$ )	25.20	47.58	$0.005 \pm 0.002$	26.93	50.88	$0.005 \pm 0.002$
PC2	Precipitation of Driest Month ( ${ m mm}^3$ )	10.53	49.96	-0.023 ± 0.018	6.14	55.51	$-0.020 \pm 0.019$
PC2		I	101.95	I	ı	113.04	I

Table 1.2. Phylogenetic evolutionary regressions for effects of climatic variables on morphological PCs scores. The analyses were performed in two different scenarios: model type 1 where strong phylogenetic inertia was explored  $(t^1/_2 values ranging from 0-total length of the tree)$  and model type 2 where a mild  $ph_3$ mo est For PC2 all climatic variables analyzed had a better predictive power than the model without predictor (lower AICc values). However, maximum temperature of the warmest month and precipitation of the driest month explained very little of the morphological variation (1.10-3.12 % and 10.53-6.14% respectively). Annual mean temperature, minimum temperature of the coldest month, annual precipitation and precipitation of wettest month explained a high amount of the morphological variation ranging from 22 to 45% (Table 1.2). Since PC2 is a contrast between face length and neurocranium width, this result indicates that species with shorter faces in relation to a wider neurocranium (lower PC2 scores) tend to live in colder and dryer areas (Figure 1.5).



**Figure 1.5.** Schematic plots of the observed mean values for morphological (PC1, PC2 score) and climatic variables (the ones highlighted in bold in Table 1.2). The scale bars under each phylogeny indicate the observed values in the scores of the morphological PCs or the respective temperature or precipitation index.

### Strength of selection and magnitude of climatic change

The magnitudes of selection gradients for each node, represented by the norm of  $\beta$  (equation 4), and respective magnitude of the climatic change (represented by the norm of the differences vector between mean climatic variables) is presented in Table S1.6. The linear regression analysis found a significant correlation between those two vectors, with a coefficient of determination  $r^2 = 0.60$ , with p<0.001 (Figure 1.4-b).

## Discussion

Neotamias chipmunks represent one of the most speciose clades of North American mammals, exhibiting the hallmarks of a recent and rapid radiation, one that sharply contrast with its sister group that apparently has not undergone any speciation event (Good et al., 2003; Reid et al., 2012; Sullivan et al., 2014). Here we provide, for the first time, evidence supporting the long-lasting hypothesis that morphological diversification among clade members resulted from adaptive responses to changing climatic conditions (Allen, 1890; Patterson, 1980, 1983; Sutton & Patterson, 2000). To this end, we used an integrative framework that combines phylogenetic comparative methods with quantitative genetics to provide a comprehensive way to examine the association between evolutionary processes and potential selective agents during the species diversification.

Our analyses support a predominantly adaptive role in the evolutionary diversification of western chipmunks. The overall pattern for the 20 species in the *Neotamias* clade is one of too much variation between populations for divergence to have occurred solely by genetic drift. Nine of the 12 phylogenetic groups where tests could be applied rejected drift by one or both of the tests we applied; the three nodes that did not reject drift had only four descendent species. Since the power of both tests is dependent upon sample size in each comparison, that power diminishes substantially when too few species are included (Harmon & Gibson, 2006; Marroig & Cheverud, 2004), it is possible that the sample size in these three tests influenced the results. That natural selection has shaped the phenotypic evolution of these species is not surprising, since most biologists agree that natural selection is important at the morphological level. On the other hand, the mere existence of a speciose lineage does not necessarily imply that member taxa have diversified solely or mainly by adaptive means (Schluter, 2000).

Most of the divergence observed among species was along the first principal component, which at first glance might suggest that genetic constraints dominated the diversification in this group. In this multivariate context, genetic constraints can be understood as the impact of the axis of greatest variation (PC1) on the evolutionary change. In other words, simply because there is more variation in the direction of PC1, the response to selection could be biased along PC1 even if selection was acting in another direction. The idea that patterns of variation might constrain and bias evolutionary change was first proposed by Schluter (1996), who emphasized that this bias should be more marked during rapid radiations, as is *Neotamias* (Sullivan et al., 2014). Alternatively, divergence along PC1 could be the result of selection in PC1 direction. A future contribution will deal with disentangling the relative contributions of genetic constraints and selection in those observed patterns of divergence.

The correlations observed between morphological traits and several climatic variables (PC1 x minimum temperature of coldest month and annual mean temperature; PC2 x minimum temperature of coldest month, annual mean temperature, annual precipitation and precipitation of wettest month) suggest that some morphological differences among species likely reflect the climatic differences among the habitats they occupy. Even though we were unable to access the relative role of phylogenetic inertia (because of the small number of species in the phylogeny), the amount of variance explained by the predictor variables remained similar with small and high degrees of phylogenetic inertia, indicating that the regression coefficient estimates were robust. Considering the temperature variables, minimum temperature of coldest month had both the higher regression coefficients for both PC1 and PC2 and a better model fit (smaller AICc-Table 1.2, Figure 1.5). This indicates that minimum temperatures had a greater impact on the expressed morphological variation than higher temperatures or mean annual temperature. These correlations also suggest that species with higher scores on these two PC axes inhabit places with higher temperatures and species with lower scores occur in colder habitats (Figure 1.5). Considering that the first morphological PC is an allometric size component, this is exactly the opposite of what would be expected according to Bergmann's Rule, the ecogeographic prediction that organisms living in colder climate should have larger body sizes and, alternatively, that warm-climate denizens should have smaller body sizes (Bergmann, 1847; Mayr, 1970). This positive correlation is not surprising since the smaller chipmunks are those that inhabit the highest elevations (T. minimus scrutator and T. alpinus, for example, both of which extend into the arctic-alpine zone above 10,000 ft) and, therefore, endure the lowest temperatures. Interestingly, the branch leading to these two species was also the one where we observed the strongest selection strength, again supporting the hypothesis that minimum temperature (or some other environmental aspect correlated with minimum temperature) has been an important selective agent. On the other extreme, species of the townsendii group (such as T. townsendii, T. sonomae) are the largest chipmunks and occupy mostly coastal areas at lower elevations and with seasonally more moderate climate. Estimates of selection strength are lowest in this clade. One possible explanation for this trend is that species living in warmer climates experience longer growing seasons and shorter hibernation periods, attributes that may lead to greater growth potential and thus to larger body size, which has already been demonstrated for other hibernating mammals (Eastman et al., 2012; Ozgul et al., 2010).

In a similar fashion, species with higher loadings on the second PC axis, those with longer faces and narrower neurocrania, inhabit hotter climates while those with lower scores (wider neurocrania and shorter faces) occur in colder environments. This pattern conforms to what we would expect according to Allen's rule, which predicts that animals living in colder environment should have relatively shorter and stouter appendages (such as the snout) in order to reduce heat loss (Allen, 1877; Yom-Tov & Nix, 1986). One of the species with small loadings in both PC1 and PC2, the least chipmunk (T. minimus), occupies the widest distribution of all western chipmunks (Reid, 2006), and therefore occurs in places with very different temperature indices. Our analyses, however, are exclusive to the subspecies T.minimus scrutator, which is confined to sagebrush steppe in the Great Basin and eastern slope of the Sierra Nevada, extending above treeline in some parts of that high range (Johnson, 1943; Reid, 2006). Thus, a more broad-based geographic sampling of T. minimus would represent an opportunity to assess if the inter-specific patterns of morphological and climatic relationship observed here have correspondence at the intra-specific level. If so, this would reinforce the important role of environmental variables in determining morphological variation.

Precipitation has been hypothesized to be equally important to temperature as the mechanistic basis for body size trends observed in mammals (Burnett, 1983; Millien et al., 2006). The rationality behind this hypothesis is that wetter habitats will have higher primary productivity, and consequently greater food availability, which could lead to bigger animals (Burnett, 1983). However, in our analyses precipitation variables explained a low amount of the morphological PC1 (allometric size; Table 1.2), and the associated AICc values were similar to the model without a predictor. Those results contravene Burnett's hypothesis of precipitation being an evolutionary driver of body size diversification. On the other hand, the evolutionary regressions between PC2 and both precipitation of the wettest month as well as annual precipitation had smaller AICc value compared to the model without predictor, explaining about 20% of the morphological variation in PC2 (Table 1.2). These relations mean that species with narrower faces and wider neurocrania inhabit dryer habitats and those with longer faces and narrower neurocrania occur in wetter places (Figure 1.5). Species of the townsendii group (T. townsendii, T. senex, T. siskiyou, T. quadrimaculatus, T. sonomae) once again are on one extreme of this trend, being the species with higher loadings in PC2 living in the wettest environments, and species with smaller PC2 loadings, are on the other extreme of this trend living in dry-habitats (Figure 1.5).

Felsenstein (1988) defines "selective covariance is the covariance in the distribution of traits, owing to covariance of the changes in these traits brought about by a correlation of their selection pressures". Therefore, a more interesting picture of the evolution in these chipmunks skulls' can be gained when we considered together all analyses. The selective covariance recovered between PC1 and PC2 (Table 1.1), the results from the evolutionary regression and the estimates of selection strength, points to a scenario where minimum temperature of the coldest month has been the most important agent in this group skulls' morphological diversification and a potential source for the selective covariance. Thus, the lower the temperature the higher the selective force on morphological attributes. Possibly, the cold environment might have resulted in selection for smaller animals because of the smaller growth period available and the stouter appendages to prevent heat loss, which might have led to the positive correlation observed between PC1 and PC2 between species, as discussed above.

The distribution of some of these chipmunk species has changed in the last century due to climate change (Moritz et al., 2008). Some species have shifted their elevational distribution (e.g., T. alpinus), others their latitudinal distribution (e.g., T. senex, (Moritz et al., 2008). In the Sierra Nevada of California, which encompasses the range of nine chipmunk species, including both T. alpinus and T. senex, minimum temperature of the coldest month has increased over the past century while maximum temperature of the warmest month has remained constant (Rowe et al., 2015). We showed that minimum temperature is one of the most important climatic variables associated with morphological attributes of chipmunks. Hence, we might expect that climatic change would affect not only their distribution but also, their morphology. Consequently, we might predict that species living in colder areas will respond by becoming morphologically more similar to their warm climate cousins, increasing their size and narrowing their neurocranium in relation to more elongated snouts (PC1 and PC2 described above).

Although we cannot pinpoint the mechanistic factors that led to the correlations observed, the association between climatic variables and cranial morphology suggests that abiotic environmental conditions are remarkably important in determining skull morphology in *Tamias*, either directly through influences on the growth period or indirectly via food availability (Patterson, 1980, 1983; Sutton & Patterson, 2000). Moreover, the high concordance between the strength of selection (represented by the magnitude of the selection gradient) and climatic changes supports the notion that morphological differences among species represent adaptations to the habitats they occupy. We only tested few a priori hypotheses of the abiotic niche dimensions of these species thought to be important for the group (Allen, 1890; Patterson, 1980, 1983; Sutton & Patterson, 2000). Thus, it is likely that unmeasured ecological variables are equally important determinants of morphological variation as are those climatic variables we examined. A study measuring exactly the availability of food in the habitats of each species would be an interesting follow up to our work and a very informative way to specifically test the importance of both temperature and precipitation variables. Another important venue of future research would be to investigate the biophysical functional significance of the traits we examined structurally.

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# Supplementary material: Chapter 1

Species	Subspecies	MANOVA model	Sample size
T. amoenus	amoenus	Age, sex and era	188
T. canipes	-	-	39
T. cinereicollis	cinereicollis	Age and sex	47
T. dorsalis	grinnelli	-	59
T. durangae	durangae, solivagus	-	21
T. merriami	merriami	Age, sex and era	60
T. minimus	scrutator	Age, sex and era	212
T. obscurus	obscurus, davisi	-	28
T. panamintinus	acrus, panamintinus	Age and sex	55
T. quadrimaculatus	-	Age, sex and era	100
T. quadrivitattus	quadrivitattus	Age and sex	46
T. ruficaudus	simulans	Age and sex	50
T. rufus	-	Age and sex	49
T. senex	senex	Age, sex and era	313
T. siskiyou	-	Age and sex	50
T. sonomae	sonomae	Age and sex	49
T. townsendii	townsendii	Age and sex	50
T. umbrinus	inyoensis	Age, sex and era	57
T. alpinus	-	transect, sex and era	251
T. speciosus	frater, sequoiensis	subspecie, age, sex and era	514

**Table S1.1.** Sample size (n) by species indicating the subspecies measured when appropriate and the factors controlled for prior to the analysis in the MANOVA model.

Table S1.2. I           Landmark	Landmarks recorded from crania by using a 3 Descirption	D digitizer with description and anatomical reference Anatomical reference
SI	Intradentale superior	Interincisive suture, right below the gnatic process
PM	Premaxillari-maxillari suture at the alveolus	Maxilloincisive suture
NSL	Nasale	Internasal suture
NA	Nasion	Internasal and frontonasal suture
BR	Bregma	Coronal and sagittal sutures
PT	Pterion	Point where parietal, frontal and temporal bones meet
ZS	Zygomaxillare superior	Most dorsal point of suture that delimits the zygomatic process between maxilla and jugal bones
ZI	Zygomaxillare inferior	Most ventral point of suture dividing maxilla and jugal bones
MT	Maxillary tuberosity	Palatomaxillary suture
PNS	Posterior nasal spine	Interpalatine suture
APET	Anterior petrous temporal	Spheno-occipital suture
BA	Basion	Midsagittal point on anterior margin of foramen magnum
OPI	Opisthion	Midsagittal point on posterior margin of foramen magnum
EAM	External auditory meatus	Point at the anterior external auditory meatus
ZYGO	Inferior zygo-temporal suture	Temporozygomatic suture
TSP	Temporo-spheno-parietal junction	Temporo-spheno-parietal junction
TS	Temporo-spenoidal junction at petrous	Posterior extremity point in the suture between squamosal and alisphenoid
JP	Jugular process	Ventral extremity point in the suture between basioccipital and squamosal at the jugular foramen
LD	Lambda	Sutura lambdoidea (occipitointerparietal suture)
AS	Asterion	Occipitoparietal suture
FIV	Ventral portion of infraorbital foramen	Ventral portion of infraorbital foramen
EZ	Zygomatic plate antero-dorsal point	Antero-dorsal point at zygomatic plate
PMZ	Dorsal portion of infraorbital foramen	Dorsal portion of infraorbital foramen
M1	First molar	Anterior margin at the alveolus of 1st molar.

#### Matrices comparisons

#### Similarity between phenotypic covariance matrices

In the present work we used the phenotypic covariance matrix, **P**, as a surrogate for its genetic counterpart the genetic covariance matrix,  $\mathbf{G}$ . In this sense, one potential approach to investigate the similarity of  $\mathbf{G}$  among lineages is to analyze and to compare their phenotypic counterparts in a broad phylogenetic context. Given that phenotypic patterns are the result of genetic and environmental influences, the eventual constancy of Pmatrices in several related taxa constitutes strong evidence that G also remained constant. There is also considerable evidence supporting that  $\mathbf{G}$  could be substituted for its phenotypic correspondent, at least for morphological characters (Cheverud, 1988, 1996; Marroig & Cheverud, 2001; Oliveira et al., 2009; Porto et al., 2009; Roff, 1995). Therefore, before analyzing the processes underlying the diversification in *Neotamias*, we tested the null hypothesis of no similarity among **P**-matrices using Krzanowski (Blows et al., 2004; Krzanowsky, 1979) and Random Skewers (Cheverud, 1996; Cheverud & Marroig, 2007) methods. The Random Skewers method is based in the multivariate response to selection equation (Cheverud, 1996; Cheverud & Marroig, 2007; Lande, 1979). This method consists of multiplying each matrix by random selection vectors and comparing the response to these random vectors in the two matrices being compared (Cheverud & Marroig, 2007). Because any selection vector applied is the same in both matrices, any difference in the response vectors is due to differences between the two matrices. To quantify these differences we calculate the correlation between each pair of response vectors. The average correlation across 10,000 response vectors pairs gave us the similarity value between any two matrices. Sampling error associated with the estimation of the V/CV matrices might affect the results, limiting the maximum possible correlation between any two matrices (Cheverud, 1996; Cheverud & Marroig, 2007; Porto et al., 2009). To circumvent this problem we estimated a matrix repeatability value through a self-correlation procedure as described in Oliveira et al. (2009) and adjusted the matrix RS similarity values observed by these repeatability values. Raw and adjusted vector correlations, along with the respective matrix repeatabilities for

each species, are presented in (Table S1.3). The Krzanowski method (Blows et al., 2004; Krzanowsky, 1979) allows the comparison of any two matrices (or k-dimensional subspaces) by calculating the angles between the best-matched pairs of orthogonal axes (Principal Components, PC). Here, the following relationship was used to find a projection matrix, S, based on a subspace of the first 16 PCs of the 35 PCs in the full dimensionality space: S =  $A^{T}BB^{T}A$ , where A, corresponds to the first 16 PCs column arranged of the first V/CV matrix being compared, B stands for the first 16 PCs of the second matrix, and  $^{T}$  for the The sum of the eigenvalues of the S matrix divided by the k dimensions transpose. represents the similarity of the two subspaces, expressed between zero and one, where zero indicates that the two subspaces are dissimilar and one indicates strictly similarity. Table S1.3 provides the results of Krzanowski comparison method. In general, there was considerable similarity in the covariance structure for all matrices compared using Random Skewers and Krzanowski methods (Blows et al., 2004; Cheverud & Marroig, 2007), suggesting that the overall pattern of cranial morphological elements has remained similar during Neotamias morphological diversification. Lower correlation values were observed in comparisons between species with lower sample sizes (Table S1.1, S1.3, S1.4).

#### Rarefaction Analysis

In order to evaluate the impact of sample size error between matrices, we performed a rarefaction analysis using *Tamias quadrimaculatus*, represented by 100 sampled individuals. The analysis is based on the correlation of random re-samplings from an original sample (100 specimens) to the smallest possible sample sizes. For each sample size we obtained correlation values, which enables to establish the relationship of these two variables. Graphical results for Rarefaction analysis for *T. quadrimaculatus* are presented in Figure S1.1, considering all possible sample sizes for covariance matrices estimated by Random Skewers and Krzanowski. The average of correlation values for 35 individuals was very similar to the correlation values obtained considering 100 individuals, both for comparisons made by Krzanowski and for Random Skewers. The rarefaction result reinforces the existence of structural similarity among phenotypic matrices of *Neotamias* (Figure S1.1).



Figure S1.1. Rarefaction analyses plots showing the distribution of self-correlation between matrices (y-axis; left compared using Krzanowski and right using Random Skewers) estimated from the same population (*T. quadrimaculatus*) with different sample sizes (x-axis). The lower values observed for matrices estimated with fewer than 25 individuals shows that sample size impacts the similarity between matrices. For sample sizes higher than 35 individuals the mean correlation observed is higher than 0.8 for both methods showing those matrices are a good representation of the V/CV matrix.

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		4	2	з	4	ъ	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
4	T. amoenus	0.94	0.85	0.90	0.93	0.79	0.88	1.00	0.88	0.90	0.96	0.92	0.88	0.85	1.00	0.92	0.88	0.89	0.93	0.92	0.95	1.02
2	T. canipes	0.79	0.93	0.84	0.85	0.71	0.82	0.85	0.80	0.79	0.84	0.80	0.81	0.81	0.85	0.80	0.72	0.84	0.79	0.85	0.86	0.91
ω	T. cinereicollis	0.85	0.79	0.94	0.87	0.72	0.84	0.93	0.88	0.86	0.90	0.87	0.90	0.86	0.94	0.91	0.85	0.83	0.86	0.87	0.90	0.94
4	T. dorsalis	0.87	0.80	0.82	0.94	0.82	0.91	0.90	0.85	0.88	0.88	0.87	0.88	0.81	0.95	0.86	0.79	0.90	0.85	0.83	0.88	0.95
ы	T. durangae	0.73	0.66	0.67	0.76	0.92	0.79	0.75	0.70	0.76	0.74	0.71	0.72	0.69	0.83	0.75	0.64	0.77	0.72	0.67	0.73	0.82
6	T. merriami	0.83	0.77	0.79	0.86	0.74	0.95	0.86	0.81	0.86	0.83	0.85	0.83	0.78	0.92	0.85	0.73	0.89	0.82	0.82	0.86	0.91
7	T. minimus	0.93	0.79	0.87	0.84	0.69	0.81	0.93	0.87	0.92	0.98	0.93	0.89	0.90	1.00	0.93	0.90	0.88	0.94	0.92	0.98	1.01
8	T. obscurus	0.83	0.75	0.83	0.80	0.66	0.76	0.82	0.95	0.79	0.85	0.83	0.85	0.81	0.90	0.84	0.79	0.80	0.83	0.84	0.85	0.90
9	T. panamintinus	0.84	0.74	0.81	0.83	0.71	0.81	0.86	0.74	0.94	0.89	0.84	0.84	0.84	0.95	0.90	0.81	0.84	0.83	0.81	0.86	0.92
10	T. quadrimaculatus	0.89	0.78	0.84	0.82	0.68	0.77	0.91	0.80	0.82	0.92	0.91	0.89	0.86	0.97	0.90	0.88	0.86	0.92	0.90	0.96	0.99
11	T. quadrivitattus	0.87	0.75	0.82	0.82	0.66	0.81	0.87	0.78	0.79	0.85	0.94	0.85	0.79	0.93	0.86	0.81	0.85	0.88	0.88	0.89	0.93
12	T. ruficaudus	0.82	0.76	0.85	0.83	0.67	0.79	0.83	0.80	0.79	0.83	0.80	0.94	0.85	0.93	0.89	0.82	0.83	0.82	0.84	0.88	0.93
13	T. rufus	0.79	0.76	0.81	0.76	0.63	0.73	0.83	0.76	0.79	0.80	0.73	0.79	0.93	0.89	0.89	0.81	0.80	0.80	0.76	0.86	0.90
14	T. senex	0.93	0.79	0.88	0.89	0.76	0.86	0.93	0.84	0.89	0.90	0.87	0.87	0.83	0.93	0.96	0.89	0.91	0.94	0.90	0.96	1.02
15	T. siskiyou	0.86	0.75	0.86	0.81	0.69	0.80	0.87	0.79	0.84	0.84	0.81	0.83	0.83	0.89	0.94	0.85	0.86	0.85	0.83	0.91	0.95
16	T. sonomae	0.83	0.68	0.80	0.74	0.59	0.68	0.84	0.74	0.76	0.82	0.76	0.77	0.76	0.83	0.80	0.94	0.74	0.85	0.76	0.86	0.90
17	T. striatus	0.84	0.79	0.78	0.84	0.72	0.84	0.82	0.76	0.79	0.80	0.80	0.78	0.75	0.85	0.81	0.69	0.94	0.84	0.83	0.90	0.94
18	T. townsendii	0.87	0.74	0.81	0.80	0.67	0.77	0.88	0.78	0.78	0.86	0.83	0.77	0.75	0.88	0.79	0.80	0.80	0.94	0.85	0.88	0.95
19	T. umbrinus	0.86	0.79	0.82	0.78	0.62	0.77	0.86	0.79	0.76	0.84	0.83	0.79	0.71	0.84	0.77	0.72	0.78	0.80	0.94	0.90	0.92
20	T. alpinus	0.89	0.80	0.84	0.82	0.67	0.80	0.90	0.79	0.80	0.89	0.83	0.82	0.80	0.89	0.85	0.80	0.84	0.82	0.84	0.92	1.00
21	T. speciosus	0.95	0.84	0.88	0.89	0.75	0.85	0.94	0.84	0.85	0.92	0.87	0.86	0.83	0.94	0.88	0.84	0.88	0.89	0.86	0.92	0.92

Raw average vector correls	tions ar	e belov	v the d	iagonal	, and ac	ljusted	vector	correls	ations a	tre abo	ve the	liagona	l.				·			- -	
	1	2	3	4	5	9	7	8	6	10	11	12	13	14	15	16	17	18	19	20	21
<b>1</b> T. amoenus	0.85	0.89	0.91	0.93	0.84	0.91	1.01	0.85	0.89	0.97	0.86	0.86	0.89	1.02	0.89	0.89	0.88	0.91	0.88	1.02	1.02
<b>2</b> T. canipes	0.78	0.91	0.79	0.86	0.81	0.82	0.88	0.76	0.80	0.84	0.80	0.76	0.80	0.86	0.81	0.81	0.79	0.81	0.79	06.0	0.89
3 T. cinereicollis	0.79	0.72	0.91	0.84	0.77	0.83	0.92	0.77	0.82	0.86	0.77	0.80	0.77	0.91	0.82	0.83	0.80	0.80	0.79	0.88	0.91
4 T. dorsalis	0.80	0.77	0.75	0.87	0.81	0.86	0.91	0.78	0.87	0.89	0.83	0.81	0.84	0.94	0.84	0.84	0.84	0.84	0.83	06.0	0.98
5 T. durangae	0.69	0.69	0.66	0.68	0.81	0.80	0.85	0.77	0.77	0.82	0.78	0.79	0.79	0.85	0.78	0.77	0.79	0.76	0.76	0.84	0.84
6 T. merriami	0.79	0.74	0.74	0.76	0.68	0.89	06.0	0.79	0.82	0.88	0.82	0.79	0.80	0.92	0.83	0.81	0.81	0.81	0.80	0.89	0.90
7 T. minimus	0.86	0.77	0.80	0.78	0.70	0.78	0.84	0.83	0.93	0.97	0.84	0.87	0.91	1.03	06.0	0.89	0.90	0.88	0.86	0.99	1.03
8 T. obscurus	0.73	0.68	0.69	0.69	0.65	0.70	0.72	0.89	0.80	0.81	0.74	0.75	0.78	0.83	0.79	0.77	0.77	0.76	0.75	0.82	0.83
9 T. panamintinus	0.78	0.73	0.75	0.78	0.66	0.74	0.82	0.72	0.91	0.87	0.80	0.80	0.82	0.91	0.81	0.83	0.81	0.82	0.81	06.0	0.92
<b>10</b> T. quadrimaculatus	0.84	0.75	0.77	0.78	0.69	0.78	0.84	0.71	0.78	0.87	0.85	0.83	0.85	0.95	0.85	0.85	0.86	0.86	0.85	0.98	0.99
<b>11</b> T. quadrivitattus	0.75	0.73	0.70	0.74	0.67	0.73	0.73	0.67	0.73	0.76	0.91	0.77	0.80	0.84	0.79	0.79	0.80	0.81	0.76	0.89	0.86
<b>12</b> T. ruficaudus	0.75	0.69	0.72	0.72	0.67	0.71	0.75	0.67	0.72	0.73	0.69	0.89	0.80	0.89	0.81	0.78	0.81	0.81	0.77	0.83	0.88
<b>13</b> T. rufus	0.77	0.72	0.69	0.74	0.67	0.71	0.79	0.69	0.74	0.75	0.72	0.71	0.89	0.87	0.80	0.79	0.82	0.83	0.79	0.87	0.89
<b>14</b> T. senex	0.87	0.76	0.80	0.82	0.71	0.81	0.88	0.72	0.81	0.83	0.74	0.78	0.76	0.86	0.91	0.88	0.90	0.88	0.87	0.99	1.02
15 T. siskiyou	0.77	0.73	0.74	0.74	0.66	0.74	0.78	0.70	0.73	0.75	0.71	0.72	0.71	0.80	0.89	0.81	0.82	0.81	0.82	0.89	0.93
<b>16</b> <i>T. sonomae</i>	0.78	0.74	0.76	0.75	0.65	0.73	0.77	0.69	0.76	0.75	0.72	0.70	0.71	0.78	0.73	0.90	0.80	0.81	0.80	0.89	0.90
<b>17</b> T. striatus	0.76	0.71	0.72	0.74	0.67	0.72	0.78	0.68	0.72	0.76	0.72	0.72	0.72	0.78	0.72	0.72	0.88	0.80	0.79	0.88	0.88
<b>18</b> T. townsendii	0.80	0.73	0.72	0.75	0.65	0.72	0.77	0.68	0.75	0.77	0.73	0.73	0.74	0.78	0.72	0.73	0.72	0.91	0.80	0.91	0.91
<b>19</b> T. umbrinus	0.76	0.71	0.71	0.73	0.64	0.71	0.74	0.66	0.73	0.74	0.68	0.69	0.70	0.76	0.73	0.72	0.70	0.72	0.89	0.89	0.88
20 T. alpinus	0.87	0.80	0.77	0.77	0.70	0.78	0.84	0.72	0.79	0.84	0.78	0.72	0.76	0.85	0.78	0.79	0.76	0.80	0.77	0.85	1.00
21 T. speciosus	0.85	0.78	0.79	0.83	0.69	0.77	0.86	0.71	0.80	0.84	0.74	0.76	0.76	0.86	0.80	0.78	0.76	0.79	0.76	0.84	0.83

**Table S1.4.** Structural similarity for covariance matrix based on Krzanowski comparison method. The bolded diagonal contains the matrix repeatability for each species.

Trait	PC1	PC2	Cranial region	Functional/developmental group
EAM-ZYGO	0.051	0.011	face	Zygomatic
EZ-M1	0.146	0.089	face	Zygomatic/Oral
IS-NSL	0.079	0.047	face	Nasal
IS-PM	0.086	0.057	face	Oral
IS-PNS	0.279	0.222	face	Oral, Nasal
MT-M1	0.067	0.045	face	Oral
MT-PNS	0.039	0.031	face	Oral
NA-PNS	0.210	0.231	face	Nasal
NFI-FIV	0.031	0.027	face	Oral
NSL-NA	0.207	0.030	face	Nasal
NSL-ZI	0.316	0.243	face	Oral, Nasal
NSL-ZS	0.258	0.216	face	Nasal
PM-MT	0.178	0.135	face	Oral
PM-ZI	0.192	0.141	face	Oral
PM-ZS	0.154	0.106	face	Oral
PT-ZYGO	0.220	-0.297	face	Zygomatic
ZI-MT	0.085	0.048	face	Oral
ZI-TSP	0.071	0.031	face	Zygomatic
ZI-ZYGO	0.121	0.042	face	Zygomatic
ZS-ZI	0.100	0.062	face	Oral
ZYGO-TSP	0.144	-0.109	face	Zygomatic
APET-BA	0.079	0.033	neurocranium	Cranial base
APET-TS	0.057	0.020	neurocranium	Cranial base
BA-EAM	0.102	0.055	neurocranium	Cranial base
BA-OPI	-0.013	-0.021	neurocranium	Cranial base
BR-APET	0.109	0.020	neurocranium	Cranial vault
BR-LD	0.135	-0.071	neurocranium	Cranial vault
BR-PT	-0.019	0.088	neurocranium	Cranial vault
JP-AS	0.083	0.025	neurocranium	Cranial base
LD-AS	-0.010	0.035	neurocranium	Cranial vault
NA-BR	0.189	0.344	neurocranium	Cranial vault
OPI-LD	0.107	0.056	neurocranium	Cranial vault
PNS-APET	0.090	0.029	neurocranium	Cranial base
PT-APET	0.199	-0.150	neurocranium	Cranial vault
PT-AS	0.329	-0.450	neurocranium	Cranial vault
PT-BA	0.293	-0.225	neurocranium	Cranial vault
PT-EAM	0.280	-0.364	neurocranium	Cranial vault
PT-TSP	-0.041	0.253	neurocranium	Cranial vault, zygomatic
% variance				
explained	31.10	11.10		

Table S1.5. Eigenvectors for the first two principal components extracted from the pooled-withingroups morphological matrix (W-matrix). The traits represent linear distances between the landmarks displayed in Figure 1.1. The classification of the 38 distances in functional/developmental groups is also presented. Boldface identifies the more extreme loading values.



Figure S1.2. Regression test plots for each node in the phylogeny. The blue line indicates the estimated regression line with respective confidence interval. The estimated slope (b) is displayed in the left upper corner for each test. The labels inside the plots correspond to the respective principal component. The genetic drift test has been rejected for nodes 1, 2, 3, and 10 as indicated in Figure 1.2.

Phylogeny branch	selection strength	Magnitude of climatic change
T. amoenus_34	42.80	1.16
T. canipes_30	34.55	1.53
T. cinereicollis_31	43.47	1.38
T. dorsalis_29	43.20	1.29
T. durangae_23	57.32	2.61
T. merriami_27	30.27	0.89
T. minimus_35	36.99	1.46
T. obscurus_27	27.39	1.17
T. panamintinus_26	34.68	1.66
T. quadrimaculatus_36	36.54	2.13
T. quadrivittatus_32	23.66	0.94
T. ruficaudus_33	49.17	2.63
T. rufus_32	37.95	0.69
T. senex_39	16.26	1.02
T. siskiyou_39	14.53	0.87
T. sonomae_37	44.66	2.83
T. townsendii_38	20.71	1.54
T. umbrinus_28	42.29	2.41
T. alpinus_35	28.72	1.63
T. speciosus_26	43.66	1.79
39_38	14.54	0.97
38_37	29.88	1.00
37_36	13.41	0.52
36_21	35.67	1.14
35_34	76.77	2.32
34_33	25.60	0.57
33_22	21.18	0.44
32_31	8.83	0.35
31_30	8.34	0.31
30_29	19.48	0.46
29_28	12.82	0.94
28_24	20.19	0.64
27_25	37.42	1.57
26_25	14.74	0.53
25_24	14.15	0.49
24_23	11.44	0.47
23_22	19.97	1.12
22_21	30.30	1.14

**Table S1.6.** Estimates of the strength of selection and magnitude of climatic change for each branch in the phylogeny. The branch numbers corresponds to the ones displayed in Figure S1.3.



Figure S1.3. Neotamias phylogeny displaying branch numbers relative to Table S1.6.

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# Chapter 2

# Adapting to climate change: phenotypic responses of a Sierra Nevada chipmunk community to a century of environmental change

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"At every moment natural selection is operating to change the genetic composition of populations in response to the momentary environment, but as that composition changes it forces a concomitant change in the environment itself. Thus organisms and environments are both causes and effects in a coevolutionary process."

-R. Lewontin, The Triple Helix: Gene, organisms, and environment

# Abstract

Human activities can expose wild populations to alterations in their environment that can be translated into phenotypic adaptive change. We asked here if the degree of climate change observed in specific habitats correctly predicts changes in phenotypes of six species of chipmunks from nine populations inhabiting the Sierra Nevada, California (USA). Moreover, we estimated the strength of selection and related it to changes in climate conditions. The question we ask is timely, given that we expect an increasing impact of climate change on wild populations, degrading their habitats leading those populations with adaptation as their only option for persistence over evolutionary time. Therefore, comprehending how adaptation occurs is relevant to any conservation action. We found that chipmunk populations skulls responded in strikingly different directions and magnitudes, although we were not able to find any correlation between the degree of climate change and morphological change or selection strength. Nonetheless, we found that populations from T. *alpinus* experienced the highest selective pressures, suggesting that this species may be under a higher extinction risk when compared to other Sierra Nevada chipmunks, at least in regard to the fitness covariance with skull morphology.

Keywords: Quantitative genetics; Chipmunks, climate change, natural selection, morphometrics

# Introduction

Human activities have greatly altered the natural habitat for most species in the world. This, in turn, requires both measuring and understanding species' responses, be these behavioral, morphological, and/or genetic as an essential activity in a world where changes are increasing at a rapid pace. When a species is confronted with changes in its environment, it has three response options: it can move, tracking its preferred habitat; it can adapt to the new conditions in its existing range; or it can fail at either and go extinct. Dispersal to new, unaffected habitats is not always possible, due either to unavailability of the preferred habitat or because of an inability to disperse (Gienapp & Brommer, 2014). And, even if a species can track its preferred habitat, it remains likely that the conditions of the altered range will be different, thus also requiring an adaptive response (Berteaux et al., 2004). Since more and more habitats will be affected by continuing climate change, we expect an increasing importance of adaptive evolution for the persistence of wild populations (Etterson & Shaw, 2001). Consequently, to understand how species adapt to new conditions is essential in order to predict, and mitigate, possible impacts of climate and environmental changes.

The Sierra Nevada of California, US, is a topographically diverse mountain range averaging 80 km in width and extending north to south for about 640 kilometers (Schoenherr, 1992). Its western flank slopes gently from the floor of the San Joaquin Valley (about 150 m) to the crest at more than 4300 m (Mt. Whitney, at 4421 m, is the highest point in the continental US) and then descends precipitously on the east to the floor of the Owens Valley (at about 1100 m). This remarkable ecological gradient results in elevationally distinct climate and habitat zones (from low-elevation grasslands successively through chaparral, deciduous woodlands, conifer forests, and arctic-alpine above treeline), different climate and especially precipitation gradients between the west-slope (wet) and east-slope (dry), and thus in patterns of species distributions.

The complex relationship among climate and species distribution seen in the Sierra Nevada had a fundamental role in the development of the ecological niche theory by Joseph Grinnell (1917). In the early part of the 20<sup>th</sup> century, Grinnell and his colleagues devoted considerable effort to detail the distributional limits of one particular group of species in the Sierra Nevada: the western chipmunks species of the genus *Tamias* (subgenus *Neotamias*). This group of 23 species distributed throughout western North America radiated relatively recently, within the last 2.75 million years (Sullivan et al., 2014). Nine of these species co-inhabit the Sierra Nevada (Grinnell & Storer, 1924; Johnson, 1943), occupying vegetation communities from the Upper Sonoran zone to the Alpine Zone (Grinnell & Storer, 1924; Ingles, 1965; Reid, 2006). Individual species, however, exhibit strong elevational zonation patterns (Figure 2.1), which limits the degree of sympatry. Nonetheless, seven species may be found on a single west-to-east transect and up to three species may occur in syntopy (Bergstrom, 1992; Grinnell & Storer, 1924; Heller, 1971; Heller & Gates, 1971; Heller & Poulson, 1972).

Climate and land use changes have affected the distribution of Sierra Nevada chipmunks in different ways during the past century (Moritz et al., 2008). These changes have also been heterogeneous along the length of the range (Rowe et al., 2015). Distributional shifts include near extinction in one case (T. umbrinus), northward latitudinal retraction (T. senex, now nearly extinct in the central Sierra Nevada but remaining common further to the north), and upward elevational range retraction [T. alpinus, the lower limits of which have retracted by ~600 meters (Moritz et al., 2008; Rowe et al., 2015; Rubidge et al., 2011)]. In contrast, the other six other Sierran chipmunks have not changed their distribution appreciably (T. amoenus, T. merriami, T. minimus, T. panamintinus, T. quadrimaculatus, and T. speciosus).

How climate has changed across each species' specific habitat range and if and how these species have adapted to cope with change remain open questions. Rubidge et al. (2011), for example, found that the minimum temperature in the coldest quarter had the greatest explanatory effect on the elevational retraction of the alpine chipmunk (T. alpinus) on the Yosemite transect (Central distribution). And Rowe et al. (2015), combining data from three distinct transects spanning the length of the Sierra Nevada, found that no climate model performed better than a random one in explaining low elevation species shifts while, in contrast, four temperature variables (minimum annual temperature, overall warming, local mean annual temperature, and local maximum annual temperature) were significantly better than random for high elevation species. On a macroevolutionary scale, climate conditions are associated with morphological variation in chipmunks (Chapter 1). Hence, how climate change affects morphological variation at the microevolutionary scale is both an important question and venue of research as we search for predictions of the impacts of climate change in different species.



Figure 2.1. Schematic representation of elevational distribution of Sierra Nevada chipmunks. It is important to keep in mind that most species are not found in all the latitudinal distribution of the Sierras. Especially, T. senex which was a common species in Central Sierras, has been extinct from its southern distribution, only occurring in upper latitudes nowadays. Moreover, T. umbrinus (\*) appears to be extinct, or nearly so, in the Sierra Nevada (based on unpublished resurveys at each historical site where the species had been recorded; see Johnson 1943). Dashed lines represent transition zones between different life zones as defined by Grinnell & Storer, 1924.

Here we use a quantitative genetics framework to investigate phenotypic changes in the skulls of Sierra Nevada chipmunks. Quantitative genetics is a suitable framework to examine evolutionary responses, especially given that we can estimate selective pressures in heritable quantitative phenotypic traits. We believe that a full appreciation of how those species have accommodated to the environmental changes they have encountered over the past century will enable us to understand better why phylogenetically close species have respond to change in such idiosyncratic ways (Moritz et al., 2008; Rowe et al., 2015; Rubidge et al., 2012; Walsh et al.,accepted). One prediction we might pose is that the degree of morphological change will scale with the degree of environmental change that a species has encountered. And, as a corollary, species that have experienced greater environmental perturbation are under higher selective pressure.

In order to analyze the relationship between climate and morphological changes in Sierra Nevada chipmunks, we compared climate conditions that occurred over the past century with morphological changes in the skull. We used in our analysis specimens derived from a unique sample of chipmunk specimens from six species collected almost a century apart. Joseph Grinnell and his colleagues collected the historical sample at the beginning of the 20<sup>th</sup> century; members of the Grinnell Resurvey Project (GRP), which was designed to resample the same set of localities, collected the modern specimens nearly a century later (see Moritz et al., 2008; Rowe et al., 2015). The species used here span a broad distribution of Sierra Nevada, from north to south and on both slopes of the Sierra Nevada, and thus encompass a substantial elevational range and comprehensive array of vegetation zones and local habitats.

#### Methods

#### Samples and measurements

We determined the degree of morphological change over the past century by comparing skulls from historical samples, collected by J. Grinnell and collaborators between 1911 and 1925, with those obtained by the GRP between 2003 and 2013 (Table 2.1). All specimens used in this study are deposited in the Museum of Vertebrate Zoology (Berkeley, CA). We included as many species of Sierra Nevada as sample size allowed, both to cover phylogenetic diversity as well as the maximal range in latitudinal and elevational distribution among species (Figure 2.2). We were able to measure specimens from six out of nine species that occur in the Sierra Nevada: *T. alpinus*, *T. speciosus*, *T. minimus scrutator*, *T. quadrimaculatus*, *T. senex* and *T. amoenus* (Grinnell & Storer, 1924; Heller & Gates, 1971; Reid, 2006). We did not include *T. umbrinus* since it has become effectively extirpated from its historical sites along the crest of the Sierra Nevada (Moritz et al., 2008), nor were we able to include *T. merriami* (a west-slope species) or *T. panamintinus* (an east-slope species) due to a paucity of available specimens. Samples from three species (*T. alpinus*, *T. speciosus* and *T. quadrimaculatus*) presented a high discontinuity of sampling, because of that we separated each of those species in two different populations and analyzed them separately.



**Figure 2.2.** Geographical distribution of the samples used in the study. Blue crosses represent historical samples and black circles represent modern samples. Species T. alpinus, T. speciosus and T. quadrimaculatus were separated into two different transects (represented by rectangles), based on the discontinuity of the sampling. The scale represents elevation.

We registered 21 landmarks on each skull, each of which were placed at intersections of sutures or other discrete cranial features, using a Microscribe 3DMX digitizer (Microscribe, IL). Landmarks were chosen to reflect homologous, functional and developmental processes (Cheverud, 1982; Marroig & Cheverud, 2001). We then transformed the landmarks into 35 linear distances, which we used in the subsequent analyses (Figure S2.1). Bilaterally symmetrical measurements were averaged, and if the skull was damaged on one side, the other was used instead of the average. Each skull was measured twice to evaluate measurement error through a repeatability analysis (Lessels & Boag, 1987), with the averages of both measurements used in subsequent analysis. Only adult specimens were used, defined by full eruption of the permanent premolar 4 and a completely fused basisphenoid-basisoccipital suture.

				Modern
	Ν	Ν	Historical median	median year
	historical	modern	year of collection	of collection
T.alpinus-Central	51	38	1915	2006
<i>T. alpinus</i> – South	75	33	1912	2010
T. speciosus- Central	77	221	1915	2004
T. speciosus- South	83	100	1911	2009
T. quadrimaculatus- North	21	8	1913	2008
T. quadrimaculatus- Central	32	28	1915	2003
T. minimus	39	137	1917	2007
T. senex	38	112	1923	2006
T. amoenus	54	77	1923	2006

Table 2.1. Sample sizes for historical and modern samples and the median of collection years.

Morphological changes and selection gradient estimates: direction and magnitude

Using the 35 linear distances, we calculated the mean change for each trait as:

$$\Delta z = \overrightarrow{z_m} - \overrightarrow{z_h}$$

where  $\overrightarrow{z_m}$  represents a vector of modern mean for each trait and  $\overrightarrow{z_h}$  represents the mean vector for historical samples. These morphological change vectors  $(\Delta z)$  can be decomposed in two its magnitude and its direction. We estimated the magnitude of morphological change in two different ways: 1) by calculating the norm of the  $\Delta z$  vector divided by the historical skull's geometric mean, which accounts for potential scale differences among species; and 2) by estimating the Mahalanobis distance from historical to modern samples. The Mahalanobis distance can be understood as a multivariate way to express distance in standard deviation and was calculated using:

$$D^{2} = (x - \mu)^{T} \Sigma^{-1} (x - \mu)$$

where  $\mu$  represents each trait mean and  $\Sigma$  is the covariance matrix. In order to have a grasp in the amount of morphological change per generation between ages, we divided the observed Mahalanobis distance by the number of years between historical to modern samples [assuming one year generation time(Ingles, 1965)].

To understand the direction in which morphological change occurred, we correlated the normalized  $\Delta z$  vectors with the four first principal components from the covariance matrices, as well as a hypothetical isometric vector (same loadings for all 35 traits). The covariance matrices were estimated after removing differences due to sex, age, locality and historical period by using the residuals from a multivariate analysis of variance (MANOVA).

We reconstructed the selective gradient responsible for the morphological changes observed by calculating:

$$\beta = \mathbf{G}^{-1} \Delta z$$

where  $\beta$  represents the selection gradient,  $\Delta z$  is the vector of morphological change and  $G^{-1}$ is the inverse of the genetic covariance matrix (Lande, 1979). We used the phenotypic matrix **P**, as a substitute for its genetic counterpart, since there is considerable evidence suggesting they are similar, at least for morphological traits, and particularly in mammals (Cheverud, 1988; Garcia et al., 2014; Marroig & Cheverud, 2001; Porto et al., 2009). Due to the fact that inverted matrices are dominated by small eigenvalues usually estimated with a lot of noise, we controlled our **P**-matrices for noise using an eigenvalue extension method (Marroig et al., 2012). We then estimated the strength of directional selection by calculating the norm of  $\beta$  divided by the skulls' geometric mean. We also correlated the normalized selection gradient vector with the first four principal components derived from the **P**matrices, a hypothetical isometric vector and the morphological change vector, in order to understand the direction in which selection was acting. Finally, we compared morphological change vectors and selective regimens were similar between species.

#### Climate changes

The climate conditions over the distributional range of each species have changed remarkably in the last century (Rowe et al. 2014). Thus, to determine how climate has changed across the range of each pooled species-geographic sample (here after 'population') and which aspects of those changes are different among the separate populations, we downloaded climate data from PRISM database (PRISM Climate Group, 2004) for the decade spanning both the historical (1910-1019) and modern (2000-2009) sample periods. We then extracted climate data from each georeferenced specimen locality for both periods. We used three biologically relevant temperature variables: mean annual temperature, minimum temperature of coldest month and maximum temperature of warmest month. We also explored changes in three precipitation variables: annual precipitation, precipitation of wettest quarter and precipitation of driest quarter.

#### Comparison of climate to morphological changes

To determine if both the magnitude of morphological change and selection strength were correlated with the degree of changes observed in climate variables. We defined the degree of climate change as the norm of the vector composed by the changes observed in each climate variables. We then fit a linear regression using the degree of climate change (for temperature and precipitation separately) as independent variable, and the selection strength/magnitude of morphological change as the dependent variables. We also estimated a total climate change magnitude, as the norm of the z-scored climate variable changes.

### Results

#### Morphological changes and selection gradient estimates: direction and magnitude

**Table 2.2.** Magnitude of morphological change ( $\Delta z$  - Norm), Mahalanobis distance ( $D^2$ ), Mahalanobis distance divided by generations ( $D^2$ /generations) and selection strength estimates ( $\beta$ - Norm) for each population.  $\Delta z$  - Norm and  $\beta$ - Norm were standardized by the geometric skull's mean from the historical samples in each population.

	$D^2$	D <sup>2</sup> /generatio	Magnitude of morphological	Strength of selection-
		115	change- $  \Delta z  $	ß
T. quadrimaculatus -North	13.808	0.145	0.13	0.37
T. quadrimaculatus - Central	7.407	0.084	0.09	0.32
T. minimus	7.591	0.084	0.08	0.42
T. alpinus - Central	33.014	0.363	0.15	0.66
<i>T. alpinus</i> - South	9.930	0.101	0.09	0.50
T. sepeciosus - Central	5.094	0.057	0.07	0.21
T. speciosus - South	5.147	0.053	0.06	0.24
T. senex	4.015	0.048	0.08	0.19
T. amoenus	2.951	0.036	0.07	0.35

The magnitude of morphological change ( $\Delta z$  – Norm) varied greatly, with the populations of *T. alpinus*-Central and *T. quadrimaculatus*-North exhibiting the greatest

magnitude of morphological change, 60% or more than other species population groups. For *T. alpinus*–Central, the magnitude observed was almost three times higher than the lowest magnitude estimate (*T. speciosu*-South; Table 2.2). The Mahalanobis distance estimates presented a similar picture, again with *T. alpinus*-Central and *T. quadrimaculatus*-North with the highest distances between their historical to modern samples than all the other populations (Table 2.2). Selection strength estimates also varied greatly, with both samples of *T. alpinus* exhibiting the strongest selection and *T. senex* with the lowest, more than three times weaker than *T. alpinus*-Central (Table 2.2).

**Table 2.3.** Directions of morphological change ( $\Delta z$ ) as expressed by the correlations between  $\Delta z$  -vector with the four first principal components from the covariance matrices (**P**-matrices), the selection gradient ( $\beta$ -vector) and a hypothetical isometric vector. Values higher than 0.40 are significant based in a 95% confidence interval from correlations for 35-elements random vectors drawn from a normal distribution. Significant values (bold) suggest that the correlated vectors are more similar than expected by chance, indicating that the morphological change occurred in that particular direction. Since principal components directions are arbitrary, the correlations for  $\Delta z$  –vectors and the four PCs are shown as absolute values.

	ß	PC1	PC2	PC3	PC4	Isometric vector
T. minimus	0.97	0.22	0.46	0.00	0.08	-0.34
T. quadrimaculatus - North	0.90	0.48	-0.37	0.42	0.19	-0.61
T. quadrimaculatus – Central	0.89	0.55	0.24	0.50	0.18	-0.66
T. senex	0.81	0.78	0.31	0.07	0.05	0.67
T. amoenus	0.86	0.55	0.24	0.24	0.18	0.51
T. speciosus - South	0.94	0.36	0.18	0.13	0.18	0.10
T. speciosus - Central	0.89	0.52	0.27	0.15	0.47	0.45
T. alpinus - South	0.83	0.66	0.13	0.17	0.17	-0.58
T. alpinus - Central	0.85	0.56	0.05	0.48	0.43	0.80

Morphological changes in all populations were strongly correlated with the selection gradient, indicating that the response to selection was not very different from the direction in which selection was acting (Table 2.3). In addition, morphological change for most populations was in the direction of the first principal component of the covariance matrices, which in all populations represents an allometric size component (loadings for all traits pointing to the same direction; Table S2.3). It is, therefore, not surprising that for those populations where a significant correlation with PC 1 was found there was also a significant correlation with an isometric vector (Table 2.3). Moreover, two trends are apparent in the correlations with the isometric vector: population samples either increased (positive correlations with isometric vectors) or decreased in size (negative correlations, Table 2.3, Table S2.1). This pattern can also easily be observed in the similarity matrix of morphological changes, where two clusters are readily visible (Figure 2.3-a). There were only two populations where correlations with either PC1 or the isometric vector were not significant: T. minimus and T. speciosus-South. This indicates that morphological changes in those populations have proceeded in a different direction when compared to the other seven. In four population samples, morphological change also occurred along PC axes other than the first: T. alpinus-Central, in which morphological change was also correlated with the PC3 and PC4; which indicates higher loadings in nasal bones (PC3) and a contrast between nasal traits and zygomatic traits (PC4) respectively; T. speciosus-Central correlated with PC4, which is a contrast between traits related to the rostrum with traits related to the length of the zygomatic arch; T. quadrimaculatus-Central with PC3, which indicates higher loadings in the dorsal traits; and T. minimus with PC2, which is closely similar to T. alpinus-Central PC4 (correlation 0.68).

Table 2.4. Directions of selection gradient estimate ( $\beta$ - vector) as expressed by the correlations between  $\beta$ - vectors with the four first principal components from the covariance matrices (**P**-matrices) and a hypothetical isometric vector. Values higher than 0.40 are significant based on a 95% confidence interval from correlations for 35-elements random vectors drawn from a normal distribution. Significant values (bold) suggests that the correlated vectors are more similar than expected by chance, indicating that selection was orientated in that particular direction. Since principal components directions are arbitrary, the correlations for  $\beta$ - vectors and the four PCs are shown as absolute values.

	PC1	PC2	PC3	PC4	Isometric vector
T. minimus	0.04	0.30	0.00	0.08	-0.15
T. quadrimaculatus- North	0.17	0.20	0.30	0.21	-0.35
T. quadrimaculatus- Central	0.14	0.19	0.45	0.21	-0.37
T. senex	0.29	0.27	0.08	0.08	0.30
T. amoenus	0.10	0.11	0.15	0.14	0.23
T. speciosus- South	0.05	0.07	0.07	0.13	0.12
T. speciosus- Central	0.10	0.17	0.13	0.49	0.09
T. alpinus- South	0.15	0.06	0.15	0.16	0.19
T. alpinus- Central	0.19	0.02	0.29	0.38	0.52

The selection gradients, on the other hand, were not significantly correlated with either the isometric vector or PC-1 in most populations, except for T. alpinus-Central, where a significant correlation was found with the isometric vector (0.52- Table 2.4). The populations T. quadrimaculatus-Central and T. speciosus-Central also had the selection gradients correlated with PC3 and PC4 respectively (Table 2.4). The selection gradient estimates did not show substantial similarity among species, with most populations exhibiting an almost zero correlation between selection vectors (Figure 2.3- c). Moreover, both T. alpinus-Central and T. quadrimaculatus-Central showed rather dissimilar (i.e., in opposite directions) selection vectors with the remaining species samples (Figure 2.3- c).



Figure 2.3. Degree of similarity (correlation) between: **a**. the morphological change vector ( $\Delta z$ -vectors similarity), **b**. climate change vector similarity and **c**. selection gradient estimate ( $\beta$ -vectors similarity) for all nine populations. The scale panel to the left of each graph shows correlation values and their respective color code: pinkish tones indicate negative correlations and bluish ones indicate positive correlations. The order of populations was arranged to maximize similarity clustering in the morphological change graph. Both  $\beta$ -vector similarity and climatic change similarity graphs were, therefore, organized in the same order.

#### Climate changes

As expected, changes observed in climate conditions have been very idiosyncratic between population habitats (Figure 2.3- b). Even those of the same species, which are expected to occupy a similar climate niche, exhibit very different relationships to the climate variables (Figure 2.4). A striking example is T. *alpinus* where the climatic conditions of the central and southern Sierra Nevada populations have changed in opposite directions for each climatic variable except mean annual temperature (Figure 2.4).



**Figure 2.4.** Climate changes observed across the ranges of the nine species-populations analyzed. Blue bars represent a decrease in temperature (degrees Celsius - upper panels) or rainfall (millimeters - lower panel), while red bars represent an increase in temperature or precipitation.

#### Comparison of climate to morphological changes

Linear regressions between both population morphological changes and selection strength estimates and climate changes were not significant for any of the comparisons (Figure 2.5, all p-values>0.05).



Figure 2.5. Relationship between magnitude of morphological change, strength of selection and climate change. Upper panels represent linear regressions with magnitude of morphological change and lower panels present regressions with strength of selection as the dependent variable. None of the regressions were significant.  $\Delta$  Temp+ Prec= Norms of the vectors calculated from the six z-scored climatic variables;  $\Delta$ Temperature = Norms of the vectors from untransformed temperature variables (°C);  $\Delta$ Precipitation = Norms of the vectors from untransformed precipitation variables (mm<sup>3</sup>).

# Discussion

Global climate change has the potential to cause sustained and consistent selective pressures on wild populations of every species (Gienapp & Brommer, 2014). Hence, conservation protocols that ignore the potential of a species to evolve in response to directional selection are flawed from the onset (Stockwell et al., 2003). The different species of chipmunks studied here presented strikingly different responses to a century of observed climate change across their respective ranges. By combining quantitative genetics with estimates of climate change we showed that the amount of climate change did not effectively predict changes in morphological traits, a result that differs from our initial expectation. Moreover, since the selection imposed on each species population differed greatly both in direction and magnitude, a simplistic expectation that a common set of climate change parameters will elicit a common response is clearly in error, even for phylogenetically closely related species.

Even though the expectation of climate change worldwide is to a mean increase of temperature, it will most certainly not be homogenously distributed throughout the planet (IPCC, 2014). Climate change in the habitat of the chipmunks' populations we analyzed corroborate this expectation. Although we observed an increase in mean annual temperature for eight of our species samples, a decrease was observed in one (T. speciosus-South; Figure 2.3). In a simplified way, we might expect that an increase in temperature would lead to larger body size, either due to direct physiological acclimation to a warmer environment (following Bergmann's Rule) or to a plastic response to an increase in growing season length, as has been already observed for other hibernating mammal species (Berteaux et al., 2004; Eastman et al., 2012; Ozgul et al., 2010). In fact, a body-size trend has been observed on a macroevolutionary scale for chipmunks, as larger-bodied species inhabit warmer climates (Chapter 1). Contrary to this simple expectation, however, and even though morphological change did occur along the first principal component, which is an allometric size component (Table 2.3), there were two distinct outcome trajectories of size change. Size did increase over the past century in four of the species-population comparisons but became smaller in the other five (Figure 2.3). It is noteworthy that no readily recognizable pattern of climate change conditions is apparent in either group, or that might explain the different size trajectory responses.

One of the most relevant questions to ask is how climate change can a population tolerate to persist through evolutionary time. Theoretical models predict that a population can only sustain a given long-term selective pressure, such as global warming, if it has sufficient genetic variation to respond to selection (Blows & Hoffmann, 2005). If the rate of environmental change is sufficiently slow to enable a population to track it, it will adapt and avoid extinction. Alternatively, if the rate of environmental change is so rapid that a population cannot keep pace (by adding genetic variation through new mutations), eventual extinction is inevitable (Burger & Lynch, 1995). This model predicts a greater extinction risk over a given change in phenotypic variation, which can be expressed in standard deviations per generation: changes higher than 0.1 standard deviation per generation would lead to a greater extinction risk (Burger & Lynch, 1995). At least three Sierra Nevada chipmunk populations had phenotypic changes higher than this threshold, including both regional samples of T. alpinus and the North sample of T. quadrimaculatus (Mahalanobis distances/ generation - Table 2.2). We can conclude, therefore, that these three are under a higher extinction risk than the other species we examined. This result, taken in conjunction with observed local near-extirpations for some Sierra Nevada chipmunks (T. senex from the Yosemite transect and T. umbrinus from along the southern Sierran crest), highlights a need for conservation action. Of course, our assessment is simplistic, since it does not account for other factors that may impact the ability of these populations to avoid extinction. One potential factor that could diminish the extinction risk is phenotypic plasticity (Chevin et al., 2012), therefore, future work that assess the degree to which those traits are plastic could provide us with further knowledge regarding the specific extinction risk for which population. Another aspect that could ameliorate this extinction perspective would be through a reorganization of the genetic architecture in response to selection itself (Chapter 3; Jones et al., 2014; Pavlicev et al., 2010).

It is noteworthy that the selection pressures we measured were both very dissimilar among the different populations and uncorrelated with the axis of greatest variation (allometric size, PC-1), and only correlated with the isometric size vector in a single case, that of *T. alpinus*-Central (Table 2.4). This implies that selection was not actually aligned with a size axis, even though the morphological change resulting from these selection gradients was biased in that direction. The impact of the axis of greatest variation biasing evolutionary trajectory is widely documented in biological systems (eg. Marroig & Cheverud, 2005, 2010; Schluter, 1996). Moreover, the very different directions in which selection has acted indicates that climate change cannot be understood as a singular predictor of selective pressures in chipmunks, or other hibernating mammals more generally. The pressures a population experiences due to climate are complex, probably involving multiple direct effects, as well as indirect ones through competition, predation, resource availability and parasitism. We thus need a broader understanding of how both abiotic and biotic components of a species niche interplay to result in the selective pressure imposed on a population over time. Although the selection gradients we estimated are very different, one trait did exhibit a uniformly strong selection for increase in all nine populations samples. This trait (IS-NSL; Table S2.2) measures the height of the nasal aperture and provides a rough estimate of the size, or volume, of the nasal cavity itself (Figure S2.1) – the larger the aperture, the larger the nasal cavity. Importantly, the nasal cavity houses the turbinate complex of bony elements that house a membrane system involved in the regulation of both heat and water balance in mammals (Schmidt-Nielsen et al., 1970). Thus, one plausible explanation for the observed uniformly high selection on IS-NSL across all chipmunk populations has been to compensate for the potential elevation in water loss and heat gain in an increasingly warming environment. A more explicit quantification of changes in the area and volume of the turbinal membranes over the past century would be an important step in verifying this direct cause-and-effect hypothesis (Van Valkenburgh et al., 2011; Van Valkenburgh et al., 2004).

We observed both the greatest morphological change and selective pressures in the alpine chipmunk (T. alpinus). In contrast, however, this species has not experienced the largest degree of climate change among the nine species-populations we examined. Nonetheless, it is important to note that T. alpinus does live in the most extreme habitat in terms of temperature, enduring the lowest temperatures of all the populations analyzed (Figure S2.2). Moreover, numerous studies have demonstrated that high elevation species are more prone to suffer changes in their distribution and/or morphology due to climate change (Eastman et al., 2012; Moritz et al., 2008; Ozgul et al., 2010; Rowe et al., 2015). Deutsch et al. (2008) suggested that more important than the degree of climate change is the ability of a given population to cope with change per se. Tamias alpinus is a habitat specialist (Rubidge et al., 2011) that has changed several aspects of its biology in the last century, including genetic diversity and population genetic structure (Rubidge et al., 2012), diet coupled with facial morphology (Walsh et al., accepted) and elevational range (Moritz et al., 2008). Why specifically this species has experienced a larger response to environmental changes it has encountered than other chipmunks remains a question for further research.

Allen's Chipmunk (T. senex) has become nearly extinct in the central Sierra Nevada during the last century (Moritz et al., 2008). The samples available to us are all from the northern part of the species range (Figure 2.1; for range map see Johnson, 1943). This population had the weakest selective pressure on its skull than any other chipmunk sample we examined. So, while nearly gone from the southern terminus of its historical range, Allen's Chipmunk is apparently under no, or only minimal, extinction risk across the remaining part of its range in the Sierra Nevada, at least in regard to the fitness covariance with skull morphology. We remain puzzled by what factors might have contributed to loss of southern populations but concomitant maintenance of those to the north. Nevertheless, our results reinforce the likelihood that multiple aspects of each chipmunk population in the Sierra Nevada must to be taken into account to assess the actual reality of extinction risk.

We studied phenotypic changes, which are the result of the interaction between environmental and genetic variation. For evolutionary purposes, only the genetic variation is relevant, given that this is the hereditary part (Falconer & Mackay, 1996). We believe this does not hinder our conclusions, given that hereditability is thought to be high for morphological traits (Mousseau & Roff, 1987), which indicates that most of the effect we observed has a genetic basis. Another important point is that we are using a net selection gradient, given that we only have access to two time periods (one historical and one modern). Therefore, we cannot tell the degree to which there have been fluctuations in selective pressures in each population over time. This could have a substantial impact in the amount of total morphological change observed (Grant & Grant, 2002). Periodic resurveys of these chipmunks would help in determining if selection has proceeded along a linear temporal trajectory or has been fluctuation episodically over time.

Our study reinforces the conclusion that understanding the climate change impacts on different species is necessarily complex. We showed that even closely related species living in similar and sometimes overlapping habitats could experience very different climate and selective pressures on their phenotypes. A nice follow up to our work would be to assess other aspects of environmental change relatable to the differences in the selective regimen we observed. For example, studies that incorporate information about other community features just as diet niche, competition, parasitism or landscape changes in the Sierras. More importantly, our study emphasizes the substantial need for long-term interdisciplinary studies that deal with climate change impacts on wildlife populations taking into account the evolutionary change potential in each population.

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Figure S2.1. Specimen of T. alpinus' skull displaying the 21 landmarks and 35 linear measurements used in the study. The scale bar represents 1 centimeter.

Table S2.1. Mo	orphlogical	change	vectors	$(\Delta z)$	estimates	for	each	popul	ation
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	T. minimus	T. quadrimaculatus- North	T. quadrimaculatus- Central	T. senex	T. amoenus	T. speciosus-South	T. speciosus- Central	T. alpinus-South	T. alpinus- Central
APET.BA	0.001	-0.044	-0.057	0.067	0.004	0.014	0.088	-0.095	0.092
APET.TS	-0.018	-0.196	-0.030	0.053	0.070	-0.027	-0.023	-0.105	0.053
BA.EAM	-0.063	-0.031	-0.058	0.088	-0.009	0.026	0.091	-0.063	0.111
BA.OPI	-0.051	-0.041	-0.033	-0.098	0.023	-0.147	-0.098	-0.067	0.062
BR.APET	-0.010	-0.289	-0.092	0.082	0.075	-0.116	0.016	0.013	0.037
BR.LD	-0.068	0.094	-0.342	0.229	0.111	-0.052	0.121	0.048	0.413
BR.PT	-0.024	-0.074	-0.071	-0.033	0.063	-0.048	0.019	0.102	0.135
EAM.ZYGO	0.048	0.015	0.017	-0.054	0.076	-0.013	-0.006	-0.058	0.139
IS.NSL	0.182	0.336	0.073	0.169	0.087	0.133	0.124	0.061	0.250
IS.PM	-0.014	-0.134	-0.116	0.085	-0.040	-0.051	-0.003	0.008	0.156
IS.PNS	-0.294	-0.242	-0.159	0.180	-0.121	-0.071	0.095	-0.164	0.381
JP.AS	-0.220	-0.214	-0.235	-0.038	0.080	-0.122	-0.081	-0.264	-0.034
LD.AS	0.109	-0.093	-0.026	-0.012	0.021	-0.057	-0.017	-0.026	0.182
MT.PNS	-0.023	-0.047	-0.033	0.060	0.011	-0.026	0.063	0.017	0.069
NA.BR	-0.058	-0.352	-0.156	0.061	0.217	0.153	0.013	0.042	0.101
NA.PNS	-0.076	-0.281	-0.077	0.154	0.072	-0.005	0.086	-0.068	0.223
NSL.NA	-0.010	-0.031	-0.058	0.290	0.100	0.166	0.280	-0.051	0.217
NSL.ZI	0.012	-0.102	-0.230	0.151	0.028	0.071	0.127	-0.130	0.385
NSL.ZS	0.013	0.105	0.013	0.238	0.103	0.143	0.188	-0.183	0.254
OPI.LD	0.011	-0.215	-0.032	0.115	0.062	0.112	0.064	0.020	0.068
PM.MT	-0.251	-0.093	-0.033	0.060	-0.085	0.003	-0.013	-0.208	0.175
PM.ZI	-0.117	-0.270	-0.255	-0.036	-0.085	-0.059	-0.046	-0.202	0.126
PM.ZS	-0.017	0.089	-0.064	0.101	0.028	0.084	0.052	-0.219	0.178
PNS.APET	0.041	-0.055	0.014	0.011	-0.044	-0.094	-0.029	-0.150	-0.017
PT.APET	0.024	-0.226	-0.048	0.133	0.105	-0.013	-0.023	-0.053	0.089
PT.AS	0.065	0.032	-0.048	0.209	0.191	0.156	0.092	-0.093	0.082
PT.BA	0.003	-0.234	-0.131	0.124	0.084	0.014	0.029	-0.127	0.133
PT.EAM	0.019	-0.244	-0.092	0.047	0.110	0.022	-0.045	-0.026	-0.014
PT.TSP	-0.084	-0.014	-0.156	0.058	-0.052	0.025	0.000	0.088	0.168
PT.ZYGO	0.021	-0.270	-0.061	0.121	0.069	0.037	-0.006	-0.025	0.023
ZI.MT	-0.020	-0.045	-0.008	-0.015	-0.023	0.051	-0.007	-0.077	0.090
ZI.TSP	-0.054	-0.098	0.089	0.041	0.095	-0.009	0.085	-0.064	0.141
ZI.ZYGO	-0.144	-0.094	-0.038	0.124	-0.004	0.010	0.105	-0.011	0.080
ZS.ZI	-0.053	-0.298	-0.291	-0.057	-0.018	-0.001	-0.045	-0.064	0.178
ZYGO.TSP	-0.011	-0.353	-0.112	0.126	0.122	-0.034	0.077	-0.022	0.201

Table S2.2. Selection gradient	(ß	) estimates	for	each	popul	ation
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	T. minimus	T. quadrimaculatus- North	T. quadrimaculatus- Central	T. senex	T. amoenus	T. speciosus-South	<i>T. speciosus</i> - Central	T. alpinus-South	<i>T. alpinus</i> - Central
APET.BA	0.061	0.093	-0.173	0.153	0.000	0.010	0.256	-0.502	0.418
APET.TS	0.005	-0.600	0.036	0.075	0.384	-0.108	-0.139	-0.636	-0.131
BA.EAM	-0.249	0.153	-0.087	0.184	-0.183	0.096	0.250	-0.239	0.312
BA.OPI	-0.295	-0.350	-0.115	-0.319	0.070	-0.591	-0.355	-0.458	0.207
BR.APET	0.058	-0.895	-0.262	0.108	0.305	-0.512	-0.042	0.166	-0.081
BR.LD	-0.263	0.396	-1.115	0.797	0.384	-0.102	0.323	0.670	2.611
BR.PT	-0.085	-0.293	-0.339	-0.076	0.371	-0.091	0.091	0.707	0.776
EAM.ZYGO	0.356	0.121	0.256	-0.292	0.449	-0.090	-0.028	-0.375	0.630
IS.NSL	1.130	1.223	0.480	0.530	0.525	0.538	0.410	0.747	1.287
IS.PM	0.037	-0.114	-0.426	0.085	-0.286	-0.318	-0.078	0.495	0.331
IS.PNS	-1.340	-0.277	-0.228	0.071	-1.105	-0.524	0.091	-0.287	1.281
JP.AS	-1.156	-0.764	-0.974	-0.246	0.434	-0.592	-0.422	-1.914	-1.086
LD.AS	0.628	-0.248	-0.038	-0.023	0.189	-0.194	-0.090	-0.149	1.062
MT.PNS	-0.075	-0.169	-0.030	0.103	-0.044	-0.093	0.217	0.204	0.199
NA.BR	-0.031	-0.750	-0.520	0.019	1.064	0.683	-0.073	0.759	-0.079
NA.PNS	-0.145	-0.681	0.077	0.247	0.067	-0.065	0.174	0.086	0.690
NSL.NA	0.074	-0.034	0.067	0.644	0.605	0.501	0.854	0.356	0.288
NSL.ZI	0.406	0.058	-0.451	0.079	-0.075	0.045	0.155	0.150	0.834
NSL.ZS	0.329	0.662	0.526	0.410	0.429	0.347	0.449	-0.441	0.649
OPI.LD	0.141	-0.513	0.012	0.109	0.456	0.369	0.148	0.073	-0.021
PM.MT	-1.263	-0.026	0.202	-0.036	-0.769	-0.147	-0.268	-1.090	0.700
PM.ZI	-0.451	-0.644	-0.785	-0.426	-0.727	-0.448	-0.387	-0.814	-0.095
PM.ZS	0.048	0.534	-0.093	0.052	0.015	0.193	-0.001	-1.077	0.572
PNS.APET	0.279	0.038	0.092	-0.088	-0.262	-0.516	-0.231	-0.784	-0.403
PT.APET	0.236	-0.394	0.092	0.185	0.262	-0.144	-0.239	-0.079	-0.099
PT.AS	0.347	0.531	0.301	0.316	0.381	0.623	0.209	-0.247	0.145
PT.BA	0.111	-0.238	-0.176	0.027	-0.032	-0.041	-0.136	-0.458	0.044
PT.EAM	0.114	-0.437	-0.094	-0.262	0.083	-0.084	-0.369	0.223	-0.775
PT.TSP	-0.291	-0.332	-0.835	0.340	0.008	0.110	0.051	0.604	0.897
PT.ZYGO	0.099	-0.663	-0.126	0.176	-0.059	0.020	-0.192	0.157	-0.225
ZI.MT	-0.013	0.074	0.071	-0.227	-0.191	0.125	-0.099	-0.200	0.367
ZI.TSP	-0.316	0.090	0.556	-0.003	0.507	-0.075	0.200	-0.024	1.059
ZI.ZYGO	-0.777	0.027	-0.170	0.155	-0.117	-0.019	0.261	0.330	0.309
ZS.ZI	-0.203	-0.973	-1.174	-0.270	-0.224	-0.015	-0.311	-0.148	0.429
ZYGO.TSP	-0.073	-1.049	-0.485	0.280	0.493	-0.209	0.137	0.277	0.786



Figure S2.2. Historical to modern changes in temperature. The colors indicate the different populations studied with the first boxplot referring to the historical 10 years temperature mean estimates (1910-1919) and the second to the 2000-2009 temperatures estimates. The box encompasses the first, median and third quartiles ( $25^{\text{th}}$ ,  $50^{\text{th}}$  and  $75^{\text{th}}$  percentiles) and whiskers represent 95% distribution. The y-axis is in degree Celsius for mean annual temperature; maximum temperature of warmest month and minimum temperature of coldest month respectively.



Figure S2.3. Historical to modern changes in precipitation. The colors indicate the different populations studied with the first boxplot referring to the historical 10 years temperature mean estimates (1910 - 1919) and the second to the 2000 - 2009 temperatures estimates. The box encompasses the first, median and third quartiles ( $25^{\text{th}}$ ,  $50^{\text{th}}$  and  $75^{\text{th}}$  percentiles) and whiskers represent 95% distribution. The y-axis is in mm<sup>3</sup> of precipitation for total annual precipitation; precipitation of wettest quarter and precipitation of driest quarter, respectively.

	PC4	0.061	0.019	0.014	-0.078	0.198	0.160	0.069	0.088	0.070	-0.046	0.077	0.052	0.026	0.061	0.038	0.146	-0.335	0.317	-0.063	0.055	0.043	0.406	0.071	0.087	-0.014	-0.197	0.023	-0.159	0.169	-0.114	-0.017	-0.267	-0.450	0.302	-0.001
	PC3	-0.051	-0.076	-0.028	-0.005	-0.040	0.003	-0.218	-0.076	-0.017	0.034	-0.016	-0.076	-0.041	-0.013	-0.664	-0.244	0.395	0.195	-0.035	-0.063	-0.088	0.203	0.000	0.097	0.064	0.123	0.060	0.136	-0.092	0.186	0.016	-0.147	-0.194	0.148	0.053
South	PC2	0.040	-0.034	0.055	-0.060	-0.129	-0.095	0.024	-0.037	-0.056	0.083	0.261	-0.059	-0.021	-0.004	-0.155	0.001	0.350	0.144	0.239	0.005	0.141	0.049	0.108	0.014	-0.213	-0.388	-0.257	-0.411	0.137	-0.341	0.068	0.124	0.171	-0.017	-0.085
T. alpinus-	PC1	0.072	0.069	0.082	0.035	0.030	0.114	-0.007	0.027	0.111	0.150	0.305	0.037	0.024	0.026	0.263	0.237	0.211	0.344	0.323	-0.027	0.161	0.189	0.189	0.106	0.147	0.237	0.215	0.221	-0.048	0.173	0.131	0.196	0.195	0.080	0.173
	PC4	-0.043	-0.133	-0.139	-0.015	-0.145	-0.098	-0.092	-0.072	0.002	-0.180	-0.237	-0.164	-0.108	-0.008	-0.101	-0.203	-0.243	0.035	-0.268	-0.148	-0.036	0.166	-0.183	-0.108	-0.059	0.339	0.041	0.157	-0.275	0.199	-0.139	-0.155	-0.394	0.210	0.085
	PC3	0.009	-0.100	-0.012	-0.033	-0.105	-0.188	-0.124	-0.050	-0.026	-0.063	-0.182	-0.119	-0.002	-0.081	-0.560	-0.346	0.375	-0.309	-0.023	-0.033	-0.091	-0.232	-0.029	0.027	-0.091	-0.038	-0.094	-0.031	-0.019	0.019	0.051	0.049	0.133	-0.284	-0.087
Central	C2	0.027	-0.033	-0.029	-0.125	-0.090	-0.154	0.048	-0.012	-0.010	0.004	-0.025	-0.037	-0.037	-0.030	-0.146	-0.078	0.109	0.299	0.011	0.005	-0.008	0.266	-0.010	0.051	-0.230	-0.424	-0.254	-0.347	0.144	-0.269	0.002	-0.152	-0.302	0.287	-0.182
r. alpinus- (	C1	-0.062	-0.020	-0.072	-0.032	0.042	0.077	0.066	-0.043	-0.138	-0.111	-0.201	-0.089	-0.023	-0.026	0.207	0.065	-0.542	-0.403	-0.217	-0.062	-0.088	-0.231	-0.109	-0.057	-0.114	-0.230	-0.206	-0.225	0.033	-0.200	-0.057	0.059	0.027	-0.173	-0.163
	C4 F	0.085	0.021	0.009	0.022	-0.059	-0.119	-0.084	0.039	0.029	0.025	-0.015	0.038	-0.080	-0.041	-0.206	-0.149	0.314	-0.335	0.179	-0.060	0.072	-0.339	0.128	0.061	0.015	0.040	0.072	0.131	-0.025	0.091	0.123	0.166	0.494	-0.426	-0.039
	G	-0.070	-0.021	-0.084	-0.096	-0.162	-0.526	-0.235	0.011	-0.007	-0.004	-0.096	-0.095	-0.065	-0.025	-0.411	-0.247	0.045	0.036	-0.084	0.141	-0.104	0.073	-0.022	-0.063	0.044	0.139	0.020	0.245	-0.253	0.224	-0.069	-0.017	-0.318	0.100	0.074
- South	G	0.007	-0.040	-0.090	-0.141	-0.114	-0.384	-0.108	-0.006	0.040	0.082	0.158	-0.013	-0.081	-0.051	0.044	-0.027	0.195	0.205	0.263	0.154	0.127	0.146	0.169	0.043	-0.163	-0.479	-0.317	-0.257	0.202	-0.197	0.066	0.034	0.020	0.002	-0.059
speciosus	C1	0.075	-0.002	0.079	-0.037	0.075	0.085	-0.080	0.040	0.073	0.095	0.268	0.078	-0.033	0.000	0.121	0.159	0.224	0.391	0.328	0.125	0.203	0.273	0.195	0.125	0.176	0.262	0.259	0.289	-0.017	0.231	0.124	0.009	0.053	0.078	0.094
1	C4 P	0.051	0.035	0.043	0.069	-0.004	-0.023	-0.075	0.141	0.119	-0.060	0.011	0.086	0.034	0.034	-0.424	-0.206	0.695	-0.066	0.229	-0.067	0.072	-0.190	0.100	0.021	0.036	-0.186	0.053	-0.006	0.009	-0.020	-0.028	0.163	0.183	-0.133	-0.010
	C E	0.057	-0.116	-0.064	0.001	-0.264	-0.348	-0.202	0.229	0.122	-0.042	-0.031	-0.083	-0.202	-0.063	-0.118	-0.128	0.132	0.232	0.009	-0.176	0.069	0.237	-0.067	-0.014	-0.091	0.249	-0.032	0.134	0.045	-0.038	0.067	-0.231	-0.378	0.124	-0.345
. Central	C	-0.069	0.015	-0.086	0.001	0.036	-0.047	-0.080	0.052	-0.089	-0.014	-0.230	-0.075	-0.004	-0.036	-0.230	-0.121	-0.101	-0.374	-0.187	-0.045	-0.217	-0.308	-0.108	-0.037	0.188	0.407	0.195	0.348	-0.152	0.283	-0.055	-0.057	-0.012	-0.163	0.031
speciosus	C1	060.0	0.029	0.092	-0.072	0.100	0.144	-0.045	0.00	0.070	0.073	0.242	0.037	-0.008	0.023	0.147	0.190	0.152	0.316	0.241	0.105	0.152	0.180	0.167	0.100	0.193	0.380	0.318	0.317	-0.098	0.269	0.069	0.084	0.101	0.123	0.168
7.	C4 P	0.023	0.067	0.044	0.014	0.397	0.647	0.168	0.054	0.038	0.121	0.107	0.098	0.211	0.010	0.128	0.009	0.010	0.206	0.127	0.295	0.008	0.117	0.001	0.037	0.137	0.000	0.123	0.027	0.159	0.020	0.028	0.194	0.161	0.001	0.032
	E3	0.025	0.023	0.021	0.032	0.024	0.056	0.009	0.008	0.020	0.059	0.074	0.045	0.015	0.004	0.567	0.249	0.733	0.118	0.157	0.015	0.026	0.004	0.015	0.044	0.020	0.077	0.026	0.044	0.075	0.023	0.031	0.028	0.037	0.021	0.015
	22 P(	0.028 -	0.110 -	060.0	0.002	0.112	0.049	0.106 -	0.066	0.064	0.082	0.270	0.076	0.012	0.041	0.292 -	0.252 -	0.065	0.248	0.195	0.066	0.135	0.161	0.113	0.010	0.058	0.424 -	0.159 -	0.306 -	0.376	0.269 -	0.094	0.017 -	0.049	0.057 -	0.111
minimus	C1 P(	0.061 -	0.059 -	- 060.0	0.006	0.052 -	0.118 -	0.031 -	0.070	0.092	0.115 -	0.275 -	0.071 -	0.005	0.049	0.193 -	0.214 -	0.155 -	0.320 -	0.231 -	0.045 -	0.130 -	0.192 -	0.125 -	0.060	0.217	0.409	0.316	0.332	- 060.0	0.250	0.066 -	0.008	0.006	0.103 -	0.097
Τ.	PC	APET.BA -	APET.TS -	BA.EAM	BA.OPI	BR.APET -	BR.LD	BR.PT	- EAM.ZYGO	- ISNSL	- Marsi	- IS.PNS	- JP.AS	LD.AS	- MT.PNS	NA.BR	- NA.PNS	- NSL.NA	- IZ'ISN	- NSL.ZS	- OPI.LD	PM.MT	- IZIMA	- SZ.MG	PNS.APET -	PT.APET -	- PT.AS	PT.BA -	PT.EAM -	PT.TSP	PT.ZYGO	ZI.MT	ZI.TSP	- ZI.ZYGO	- IZ.SZ	ZYGO.TSP -

**Table S2.3.** Loadings in the first 4 principal components to all populations.

		C				T	1		/							
	T. amoenu	S			T. senex				T. quadrim	<i>aculatus</i> - Ce	ntral		T. quadrimo	aculatus- No	rth	
	PC1	PC2	РСЗ	PC4	PC1	PC2	РСЗ	PC4	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
APET.BA	-0.050	-0.077	0.022	0.018	-0.067	0.013	0.012	0.017	0.038	0.010	0.065	0.008	-0.122	0.087	0.020	-0.064
APET.TS	-0.052	-0.016	0.027	-0.046	-0.069	-0.033	-0.019	0.009	0.077	0.030	0.047	-0.151	-0.069	0.110	0.051	0.061
BA.EAM	-0.109	-0.089	0.008	-0.001	-0.086	-0.019	-0.057	0.010	0.094	0.040	0.053	0.070	-0.048	0.141	-0.071	-0.018
BA.OPI	-0.006	0.058	0.018	-0.178	0.055	-0.079	-0.021	-0.013	0.039	-0.042	-0.012	-0.083	0.040	-0.108	0.039	-0.048
BR.APET	-0.112	-0.079	-0.010	-0.194	-0.094	-0.126	0.012	0.035	0.033	0.067	0.141	-0.214	-0.054	-0.056	-0.163	-0.289
BR.LD	-0.133	0.039	0.227	-0.807	-0.022	-0.133	0.113	-0.499	0.098	-0.065	0.610	-0.514	-0.106	-0.170	0.014	-0.313
BR.PT	-0.013	-0.104	-0.113	-0.115	0.023	0.049	-0.088	-0.036	-0.035	0.014	0.088	-0.085	0.050	-0.027	-0.098	-0.053
EAM.ZYGO	-0.035	-0.043	-0.002	-0.047	-0.048	-0.009	-0.013	0.014	0.066	0.059	0.046	-0.077	-0.072	0.029	0.100	-0.090
IS.NSL	-0.061	-0.067	-0.017	0.078	-0.075	0.004	0.020	0.069	0.086	-0.017	-0.033	-0.019	-0.108	-0.099	0.121	0.210
IS.PM	-0.049	-0.041	0.083	0.009	-0.104	-0.144	-0.144	-0.026	0.066	-0.032	0.096	0.068	-0.092	0.185	-0.067	-0.149
IS.PNS	-0.241	-0.215	-0.032	-0.104	-0.302	-0.233	-0.280	-0.221	0.214	0.179	0.235	0.136	-0.174	0.327	-0.081	-0.033
JP.AS	-0.060	-0.040	0.016	-0.100	-0.056	-0.016	0.030	0.060	0.104	0.021	0.002	-0.090	-0.044	-0.060	-0.108	0.254
LD.AS	0.008	-0.100	0.012	-0.068	0.002	0.054	-0.029	0.052	0.047	-0.087	0.073	0.004	0.126	0.080	-0.162	-0.504
MT.PNS	-0.054	-0.010	-0.042	-0.077	-0.063	-0.052	-0.051	-0.100	0.038	0.050	0.082	0.014	-0.012	-0.013	-0.028	0.104
NA.BR	-0.060	-0.090	-0.723	-0.077	-0.286	0.708	-0.351	0.069	0.156	-0.420	0.320	0.467	0.076	0.561	-0.211	0.037
NA.PNS	-0.169	-0.109	-0.353	-0.208	-0.242	0.181	-0.205	-0.213	0.160	-0.098	0.375	0.104	-0.051	0.296	-0.060	0.085
NSL.NA	-0.277	-0.305	0.478	0.140	-0.155	-0.512	-0.127	0.118	0.222	0.344	-0.207	0.109	-0.317	-0.093	0.315	0.182
NSL.ZI	-0.263	-0.328	-0.079	0.092	-0.266	-0.106	-0.191	-0.275	0.311	0.318	0.095	0.071	-0.331	0.243	0.343	-0.066
NSL.ZS	-0.252	-0.261	0.021	0.040	-0.265	-0.123	-0.229	0.075	0.262	0.025	0.075	0.294	-0.174	0.187	0.191	0.128
OPI.LD	-0.062	-0.184	-0.022	0.239	-0.190	-0.042	-0.046	0.376	0.109	0.101	-0.038	0.227	-0.049	0.015	-0.260	-0.005
PM.MT	-0.177	-0.188	-0.042	-0.035	-0.149	-0.034	-0.046	-0.062	0.167	0.161	0.061	0.136	-0.126	0.176	0.030	0.020
PM.ZI	-0.167	-0.206	-0.085	0.086	-0.163	-0.017	-0.069	-0.220	0.179	0.373	0.064	0.007	-0.225	0.187	0.176	-0.177
PM.ZS	-0.145	-0.107	-0.013	0.056	-0.181	-0.072	-0.070	0.060	0.104	0.120	0.040	0.168	-0.100	0.084	0.038	0.061
PNS.APET	-0.049	-0.121	0.065	0.065	-0.073	-0.020	-0.018	0.189	0.075	-0.011	-0.131	0.135	-0.059	0.117	-0.024	-0.153
PT.APET	-0.235	0.110	-0.013	0.039	-0.200	-0.015	0.138	0.086	0.231	-0.038	-0.088	-0.144	-0.199	-0.010	-0.194	-0.105
PT.AS	-0.394	0.437	-0.029	-0.012	-0.342	0.119	0.416	-0.159	0.421	-0.138	-0.213	-0.224	-0.367	-0.203	-0.061	0.011
PT.BA	-0.335	0.121	0.016	0.034	-0.287	0.022	0.240	0.067	0.336	-0.088	-0.104	-0.138	-0.297	-0.041	-0.263	-0.137
PT.EAM	-0.325	0.305	0.020	0.122	-0.293	0.041	0.362	0.075	0.316	-0.177	-0.177	-0.166	-0.341	-0.195	-0.174	-0.156
PT.TSP	0.145	-0.205	0.097	-0.123	0.087	-0.011	-0.318	0.104	-0.120	0.113	0.161	0.075	0.100	0.073	0.260	-0.170
PT.ZYGO	-0.248	0.296	0.005	0.142	-0.196	0.035	0.292	0.094	0.225	-0.235	-0.186	-0.078	-0.274	-0.175	-0.155	0.011
ZI.MT	-0.060	-0.053	0.028	0.052	-0.086	-0.058	-0.058	0.146	0.075	-0.031	-0.003	0.005	-0.053	0.121	-0.050	-0.040
ZI.TSP	-0.060	-0.031	-0.037	-0.080	-0.074	-0.088	-0.012	0.160	0.057	-0.133	0.117	0.046	-0.091	0.125	-0.314	0.387
ZI.ZYGO	-0.085	-0.069	0.082	-0.093	-0.163	-0.118	-0.089	0.312	0.069	-0.240	0.016	0.155	-0.056	0.061	-0.301	0.093
ZS.ZI	-0.075	-0.084	-0.090	0.028	-0.030	-0.001	0.009	-0.301	0.059	0.301	0.081	-0.085	-0.180	0.055	0.196	-0.123
ZYGO.TSP	-0.147	0.122	-0.011	-0.032	-0.120	-0.037	0.090	0.056	0.126	-0.220	-0.082	-0.002	-0.212	-0.092	-0.122	0.146

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# Chapter 3

# Directional selection effects on patterns of phenotypic (co)variation in wild populations

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"How fast, as a matter of fact, do animals evolve in nature?"

-G.G. Simpson, Tempo and Mode in Evolution

# Abstract

It is well known and documented that covariation between traits affect a population's evolutionary trajectory. On the other hand, the impact of natural selection on the patterns and magnitudes of covariation between traits is less explored and mainly through a theoretical and experimental perspective. In a quantitative genetics framework, the ability to respond to selection is dependent upon the patterns and amount (co)variation that exist in a given population. Recent theoretical models predict that under directional selection the (co)variation will respond by realigning itself with the subjacent adaptive landscape and by increasing the amount of genetic variance in the direction of selection. Whether either occurs in natural populations is an open question and thus an important gap in evolutionary theory. Here we present empirical evidence of the impact of natural selection in the organization and magnitude of correlations in population's phenotypes. We documented changes in the phenotypic (co)variation structure in 2 separate natural populations in each of two mammalian species, the chipmunks Tamias alpinus and T. speciosus, undergoing directional selection. In populations where selection was strongest (those of T. alpinus) we observed an increase in the phenotypic variance in the direction of the selection gradient, a result that contradicts the traditional view that selection might deplete genetic variation. Moreover, we also observed changes in the overall phenotypic integration for those populations. Our results give further support to recent theoretical advances that take into account the complexities of the genetic architecture.

**keywords**: Quantitative genetics; adaptive landscape; Phenotypic covariance; Genotypephenotype map

## Introduction

In order to persist over evolutionary time species must have the ability to respond in the direction of selection. Since organisms are formed by a combination of multiple traits organized into a coherent whole (a multidimensional system), understanding the interaction between the available phenotypic (co)variation and selection is crucial to understand species' responses to selection (Lande, 1979) and, consequently, species persistence over time. For instance, if a species lacks phenotypic (co)variation in a certain direction it can quickly become extinct when directional selection operates along that trajectory (Walsh & Blows, 2009). How the available phenotypic (co)variation shapes species evolution is, therefore, an important venue of research in evolutionary biology and is relatively well appreciated theoretically, empirically, and computationally (e.g. Lande, 1979; Marroig & Cheverud, 2005; Schluter, 1996). On the other hand, our understanding of how directional selection shapes the evolution of the available phenotypic (co)variation, although equally important, is only just beginning. Most studies addressing this issue have been simulations derived from theory (Arnold et al., 2008; Roff & Fairbairn, 2012) although some experimental evidence has emerged (Blows & Higgie, 2003; Delph et al, Steven et al., 2011; Roff & Fairbairn, 2012; Wilkinson et al., 1990).

In traditional evolutionary thinking, directional selection is thought to deplete genetic variation leading to a decrease in phenotypic (co)variation (Barton & Turelli, 1989; Walsh & Blows, 2009). Therefore, establishing plausible mechanisms that account for the widespread phenotypic (co)variation observed in nature became a priority in evolutionary biology (Barton & Turelli, 1989). Despite the inherent difficulties in pursuing answers to this issue (Arnold et al., 2008), recent advances in theoretical and computational studies have provided some benchmarks in how available phenotypic (co)variation evolution is expected to evolve under directional selection (Hansen, 2013; Jones et al., 2014; Jones et al., 2003; Pavlicev et al., 2010; Pavlicev et al., 2011). Those models are based in the evolution of the genotype-phenotype map (GP-map), which describes how genetic (co)variation is translated into the phenotypic (co)variation. If different genotypes differ in the genetic (co)variation among traits, in other words the degree of pleiotropic interaction (epistasis) among traits,
this leads to the possibility that genetic (co)variation among traits can evolve in response to selection (Pavlicev et al., 2010, 2011). In those models, selection might actually drive the evolution of the mutational machinery to align available phenotypic (co)variation with selection. Consequently, the impact of directional selection on the GP-map organization is thought to be substantial and can occur at a rapid pace (Jones et al., 2014; Pavlicev et al., 2010).

From these models, we can draw some predictions about changes in the available phenotypic (co)variation that could be easily testable by empirical data. First, we would expect an increase in genetic variation in the direction of selection (Figure 3.1). Second, the degree to which traits are correlated are expected to change, specifically, we expect an increase in correlations among the traits that are being co-selected (Roff & Fairbairn, 2012). Lastly, we expect a re-orientation of the patterns of available phenotypic (co)variation to match the expected direction of selection (Figure 3.1, Jones et al., 2014).

Validation of these predictions in a trait-multidimensional natural population scenario is essential to the further development of our comprehension about evolution itself and how species adapt to new selective pressures. It is especially relevant in a world where most natural environments are under some kind of stress due to anthropic pressures either directly, as changing in land use, or indirectly, as climate change. However, three main problems hinder the gathering of empirical data to assess these questions in natural populations. First, sampled populations should be separated by many generations, given that such changes are not expected in a short period of time. Second, populations must be well sampled in order to properly estimate statistical parameters. Third, the populations in question should have experienced directional selection. Here, we used a unique sample set that matches the first and second requirements, and which thus permits us to test the third requirement.

Our sample is composed of chipmunk specimens of two species, Tamias alpinus and T. speciosus, collected almost a century apart. These come from two independent transects separated by approximately 180 km along the Sierra Nevada of California. The first of these is in the central part of the Sierras, in Yosemite National Park; the other is in the Southern Sierras, within or just east of Sequoia-Kings Canyon National Parks. These species are phylogenetically close, but have responded in strikingly different manners to a century of climatic and associated environmental changes. While the alpine chipmunk, *T. alpinus*, has shifted its elevational distribution upwards and decreased its genetic diversity, the lodgepole chipmunk, *T. speciosus*, has changed neither its elevational distribution nor genetic diversity (Moritz et al., 2008; Rubidge et al., 2012; Rubidge et al., 2011).

In this contribution we tested for both species if evolution between the historical and modern periods was driven by directional selection, and since we found evidences favoring a directional selection scenario, we tested how the selective regime changed the available phenotypic (co)variation through time. To this end, we analyzed 35 skull traits using a quantitative genetics framework in those populations. Then we compared the phenotypic (co)variation matrices between periods (historical and modern) for each species and transect in order to assess if the specific selective regimes each population was subjected to had an impact in the overlying phenotypic (co)variation patterns.



Figure 3.1. The expected changes in the (co)variation patterns between 2 trais ( $Z_1$  and  $Z_2$ ) under a sustained directional selection scenario ( $\beta$ ). In this hypothetical example, prior to selection both traits were tightly negatively correlated. Each ellipse represents the (co)variation pattern of one generation, with the bluer representing ancestral and red representing derived populations. Since, selection was in the direction to increase both traits, we observe a change in the magnitude and pattern of covariation between both traits due to selection. More specifically, we observe an increase in the total amount of variation in the direction of selection; second, the pattern of correlation among traits changed in order

to mirror the selective regimen, in this case resulting in a positive tight correlation between both traits; and third a re-orientation of the (co)variation patterns matching the selective regimen occurred.

# Methods

# Samples

One of us (APAA) recorded three-dimensional coordinates for 27 landmarks on 193 adult skulls of T. alpinus and 321 adult skulls of T. speciosus (see Table 2.1 for sample sizes for each period). Adult specimens were defined by fully erupted permanent premolar 4 and a completely fused basisphenoid-basioccipital suture. Based on these landmarks we calculated 35 linear distances, which were used in the subsequent analyses (Figure S2.1).

We used historical samples collected as part of a California-wide survey of terrestrial vertebrate conducted by Joseph Grinnell and colleagues from 1911-1915. Modern specimens were collected as part of the Grinnell Resurvey project, an intensive resampling of Grinnell's historic sites that occurred from 2003-2013. All specimens are deposited in the Museum of Vertebrate Zoology (MVZ, UC Berkeley). Samples come from two independent transects, one through the Yosemite National Park located in the Sierra Nevada in central California and the other from the Southern Sierras, within and just east of Sequoia National Park approximately 180 km to the south. It has been previously shown (Walsh et al., accepted) that the T. alpinus population from Yosemite has changed its skull morphology in a pattern compatible with directional selection when tested in a univariate context, with most changes been concentrated in the facial region for both transects. Also, the Yosemite transect population increased in size over the past century while the Southern Sierras population decreased its size (Walsh et al. accepted). In contrast, comparable samples of T. speciosus had fewer traits changing in a pattern compatible with directional selection in both transects, although in Yosemite most changes were also concentrated in the facial region. Matrices similarity

Here we used phenotypic matrices ( $\mathbf{P}$ -matrices) as a proxy for its genetic counterpart, which is the evolutionary relevant parameter. Our decision to substitute the  $\mathbf{G}$ -

matrix by the **P**-matrix is based in considerable evidence supporting the interchangeability of the **G**- and **P**-matrices, at least for morphological characters and particularly in mammals (Cheverud, 1988; Marroig & Cheverud, 2001; Porto et al., 2009). Furthermore, to guarantee our **P**-matrices are similar to other estimate of **G**, we compared our **P**-matrices to *Calomys expulsus* **G**-matrix from Garcia et al. (2014). Also, we compared the overall similarity between the covariance matrices for all populations (historical and modern) using two methods, Random Skewers and Krzanowski (Blows et al., 2004; Cheverud & Marroig, 2007). Since matrices are estimated with error, we corrected the values of matrices similarity using a matrix repeatability estimates (Table S3.1, Cheverud & Marroig, 2007).

#### Directional selection versus genetic drift of skull traits

Before exploring if directional selection had an impact in available phenotypic (co)variation, we tested if natural selection was responsible for the multivariate evolution between historical and modern periods. To do so, let  $\xrightarrow{}_{Z_0}$  represents a vector of means for mtraits in a given population at the initial time t=0. After t generations the expected trait means for *n* populations is equal to  $\overrightarrow{z_0}$  with variance given by  $\mathbf{D} = \mathbf{G} t/\mathrm{Ne}$ , where **D** is the divergence matrix, G represents the G-matrix of the ancestral population, and Ne is the effective population size (Lande, 1976, 1979). Using this theoretical framework, we can simulate multivariate evolution in the traits we measured. We used the **P**-matrix from the historical sample to estimate the **D**-matrix expected from drift with the upper and lower estimates of Ne for T. alpinus (Rubidge et al., 2014) and a generation time of one year (Reid, 2006). Then, we sampled from this expected distribution 1,000 populations and estimated the norm of the evolutionary change vector  $(\Delta z)$  for each simulated population. All those estimates were made taking into account the standard error of both historical and modern means. Finally, we compared the 95% probability distribution for these estimated  $\Delta z$ -norms to the empirical  $\Delta z$ -observed. We then concluded that directional selection was the main process responsible for observed divergence if the range of estimates from the magnitude of morphological divergence fell outside the 95% distribution expected through drift.

# Selection gradient estimate

In order to estimate the observed selection gradient ( $\beta$ ), the directional selection responsible for the morphological changes observed, we used the multivariate selection response equation (Lande, 1979)  $\beta = G^{-1}\Delta z$ , where  $\Delta z$  is the response to selection estimated as difference between modern and historical observed trait means;  $G^{-1}$  is the inverse of the **G**-matrix (substituted by the historical **P**-matrix). Due to the fact that inverted matrices are dominated by small eigenvalues usually estimated with a lot of noise, we controlled our **P**-matrices for noise using an eigenvalue extension method (Marroig et al., 2012). The calculated normalized  $\beta$  was then used as a benchmark to estimate the changes observed in the patterns of (co)variation, as described below. Moreover, to compare the strength of selection between transects and species we estimated the norm of the selection gradient standardized by trait means (Hansen & Houle, 2008).

#### Effects of directional selection on skull's P-matrices

Since our main purpose is to compare changes in the patterns of (co)variation between time periods, we have to understand the possible distribution of those parameters in each species/area sample. To do so, we estimated **P**-matrices separately for the historical and modern samples estimating a distribution of one thousand **P**-matrices through a Monte Carlo resampling for each period, transect and species (Melo et al., 2015). We then used these 1,000 estimated matrices in subsequent analyses and considered a result significant whenever the observed modern **P**-matrix value fell outside the 95% distribution of the historical estimates (Manly, 2006).

To quantify the impact of natural selection on the P-matrices, we compared historical and modern **P**-matrices in relation to three different matrix features: 1) size, which can be described as the total amount of variation in the matrix or in a certain direction (Hansen & Houle, 2008; Hohenlohe & Arnold, 2008); 2) shape, which provides an indication of eccentricity, or how tight the correlation among traits are; and 3) orientation in relation to the selection gradient.

In order to determine if the total amount of variance had changed from historical to modern samples, we estimated the trace of each covariance matrix from the Monte Carlo distribution. In addition, we determined if the amount of variance had changed in the direction of selection or in directions uncorrelated with the observed selection gradient. The amount of variance in any given direction was calculated as the evolvability in that direction (Hansen & Houle, 2008), a metric which captures the ability of a population to evolve in direction of a specified selection gradient. Evolvability can be measured as:

$$e(\beta) = \beta' G \beta$$

Where  $e(\beta)$  is the evolvability in the direction of a given selection gradient ( $\beta$ ) and G is the **G**-matrix. To compare the effects of directional selection, we estimated the evolvability in direction of the normalized observed selection gradient ( $\beta_{obs}$ ) for the distribution of **P**-matrices from the historical and modern periods. Moreover, we generated 1,000 random selection gradient vectors uncorrelated with  $\beta_{obs}$ . In order to obtain those sets of uncorrelated vectors we first sampled 1,000 normalized vectors form a normal distribution and applied the formula:

$$\beta_i = \beta_r - [\beta_{obs}(\beta_r \beta_{obs})]$$

where  $\beta_r$  is the random selection gradient sampled from a normal distribution and  $\beta_i$  is the uncorrelated resulting vector. We latter normalized to size one those random vectors and compared the evolvability potential of both the historical and modern **P**-matrices in those directions. Evolvability estimates were divided by the geometric means of all traits, thus accounting for scale differences between populations (Hansen & Houle, 2008).

We also compared changes in the overall magnitude of integration between periods by estimating the coefficient of determination  $(r^2)$  of the correlation matrices (Cheverud et al., 1989). This coefficient is simply the average of the squared correlation coefficients and measures how tightly the correlations among traits are, the higher the estimates the tighter the correlations.

Lastly, we compared changes in the orientation of the  $\mathbf{P}$ -matrix distributions between periods by estimating the correlation between the observed selection gradient and the first principal component of each of the 1,000 matrices from historical and modern times for both species and transects. All statistical analyses were done in the R Environment for Statistical Computing (R Core Team, 2014) using the EvolQG package (Melo et al., 2015).

# Results

# Matrices similarity

**P**-matrices comparisons with the **G**-matrix derived from *Calomys expulsus* (Garcia et al., 2014) showed a high similarity between them, with comparisons from Random Skewers method ranging from 0.61 to 0.81 (Table S3.2) and comparison from Krzanowski method ranging from 0.66 to 0.72 (Table S3.2). Those are all fairly high values indicating that our P-matrices are reasonable estimates of their genetic counterparts (Marroig & Cheverud, 2001; Prôa et al., 2013). Moreover, the comparisons of the **P**-matrices among populations showed that they are all structurally similar, with estimates for Random Skewers ranging from 0.81 to 0.95 (Table S3.3) and Krzanowski ranging from 0.81 to 0.87 (Table S3.3).

## Directional selection versus genetic drift of skull traits

To determine if the amount of divergence observed between historical and modern periods for each population was explained by genetic drift or natural selection, we simulated the amount of morphological divergence expected by drift and compared it to the empirically measured magnitude of morphological change. For any effective population size adopted, the magnitude of morphological change expected by drift was at least 5.7 times lower than the empirically measured magnitude of morphological change for T. speciosus and 10.95 times lower for T. alpinus in the Southern Sierras. For the Yosemite transect, the same pattern was observed, the magnitude of morphological change expected by drift was 7.5 and 19.6 times lower for T. speciosus and T. alpinus, respectively, than that empirically observed. Therefore, data for both species support directional selection as the primary mode underlying the observed temporal changes (Table 3.1).

Next, we estimated the standardized magnitude of morphological change ( $z\mu$ ) and selection gradient ( $\beta\mu$ ) for both species to gauge the strength of selection. For *T. alpinus* from the Yosemite transect, we obtained  $z\mu = 0.178$  and  $\beta\mu = 39.388$ ; comparable numbers for *T. speciosus* were  $z\mu=0.064$  and  $\beta\mu = 15.457$ , which is 2.8 and 2.6 lower than those of *T. alpinus*. In the Southern Sierras, we obtained  $z\mu = 0.099$  and  $\beta\mu = 27.062$  for *T. alpinus* and  $z\mu = 0.063$  and  $\beta\mu = 20.279$  for *T. speciosus*, 1.57 and 1.33 lower than those of *T. alpinus*. In both cases, directional selection was stronger in *T. alpinus* than in *T. speciosus*.

	observed (i.e. empirically measured) and expected under drift							
		T. alpinus			T. speciosus			
		Yosemite	Southern Sierras	Yosemite	Southern Sierras			
		Δz Cl		Δz Cl	Δz CI			
	Observed	0.942 - 1.190	0.558 - 0.795	0.454 - 0.631	0.403 - 0.607			
	Ne = 230019	0.020 - 0.051	0.020 - 0.051	0.026 - 0.062	0.025 - 0.071			
	Ne= 430625	0.014 - 0.036	0.014 - 0.037	0.019 - 0.046	0.018 - 0.052			
	Ne= 648513	0.011 - 0.030	0.011 - 0.030	0.015 - 0.037	0.014 - 0.042			

**Table 3.1.** Magnitude of morphological change  $(\Delta z)$  of skull traits of *T. alpinus* and *T. speciosus* observed (i.e. empirically measured) and expected under drift

Ne: effective population size based in (Rubidge et al., 2014).  $\Delta z$  CI corresponds to the 95% confidence interval for the magnitude of morphological change

# Effect of directional selection on morphological P-matrices

To search for the effect of directional selection on the **P**-matrices we investigated the following changes between the historical and the modern **P**-matrices: 1) the total amount of variation estimated by the matrices traces; 2) the amount of variation associated to the direction of selection and to directions uncorrelated with selection, calculated as evolvability divided by the geometric mean of all traits; 3) the overall magnitude of correlation among traits estimated as the coefficient of determination of the correlation matrices  $(r^2)$ ; and 4) the orientation of the axis of greatest variation in relation to the selection gradient estimated by the correlations between PC1 and the selection gradients.

For the Yosemite transect, the historical matrix trace of *T. alpinus* was  $2.79 \pm 0.22$  s.d. and for the modern sample was  $3.33 \pm 0.34$  s.d.; the historical trace for *T. speciosus* was  $4.34 \pm 0.28$  s.d. and modern trace was  $4.12 \pm 0.23$  s.d. For the Southern Sierras transect, we obtained historical and modern matrix traces for *T. alpinus* of  $2.63 \pm 0.24$  s.d. and  $2.60 \pm 0.25$  s.d., respectively; for *T. speciosus* the historical trace was  $4.68 \pm 0.30$  s.d. and modern  $4.47 \pm 0.31$  s.d.. Thus, the total amount of variation in each species-population comparison

did not change temporally. However, the amount of variation in the direction of selection did increase for *T. alpinus* in both transects (Figure 3.2 upper right panel, Yosemite observed modern estimate = 0.033, 95% historical distribution= 0.0128- 0.031; Southern Sierras observed modern estimate = 0.018, historical distribution = 0.006- 0.015), but not for *T. speciosus* (Figure 3.2- lower right panel, Yosemite observed modern estimate = 0.024, 95% historical distribution= 0.015-0.029; Southern Sierras observed modern estimate = 0.011, historical distribution = 0.009-0.017). Importantly, there was no change in the amount of variation in either species or transect in directions uncorrelated with the selection gradient (Figure 3.2- left panels).

The overall magnitude of integration increased over time for *T. alpinus* in Yosemite, as the observed  $r^2$  index for the modern population (0.108) did not overlap with the historical distribution (0.072-0106). For *T. alpinus* from the Southern Sierras, however, the overall magnitude of integration remained unaltered across time (observed modern  $r^2$ index=0.094, 95% historical distribution= 0.079-0.140). We also observed idiosyncratic changes in *T. speciosus*, as the Yosemite population decreased its overall magnitude of integration (the observed modern value, 0.073 does not overlap the historical 95% distribution, 0.078-0.131) while the Southern Sierras population remained unaltered from historical to modern periods (observed modern value, 0.118, overlapping the historical 95% distribution, 0.085-0.133; Figure 3.3).

Lastly, the orientation of the axis of greatest variation in relation to the selection gradient, estimated by the correlations between PC1 and the selection gradients did not change for most populations (Figure 3.4). The only population where we observed an increased in the correlation among PC1 and the selection gradient was T. alpinus from the Southern Sierras where the modern observed correlation of 0.31 was larger than the historical 95% distribution of 0.027-0.23.



Figure 3.2. Amount of variation in the direction of selection ( $\beta$ - direction) and in directions uncorrelated (direction  $\neq \beta$ ) with selection for *T. alpinus* and *T. speciosus* in historical and modern periods for both transects. The boxplots correspond to evolvability estimates from 1,000 matrices through Monte Carlo sampling for the empirical selection gradient (right panel) and for random selection gradients uncorrelated with the empirical (left panel). \* denotes significant changes from historical to modern periods.



Figure 3.3. Overall magnitude of integration among traits for *T. alpinus* and *T. speciosus* in historical and modern periods for both transects. The boxplots correspond to coefficient of determination of the correlation matrices  $(r^2)$  estimated from 1,000 matrices through Monte Carlo sampling. \* denotes significant changes from historical to modern periods.



Figure 3.4. Orientation of the axis of greatest variation (PC1) in relation to the selection gradient  $(\beta)$  for *T. alpinus* and *T. speciosus* in historical and modern periods for both transects. The boxplots correspond to the correlation between both vectors (PC1 and  $\beta$ ) estimated from 1,000 matrices through Monte Carlo sampling. \* denotes significant changes from historical to modern periods.

# Discussion

How species adapt to their environment is a fundamental issue in biology, one dependent not only upon changes in species' environments (i.e. directional selection) but also in the amount of available phenotypic (co)variation. Our study investigated how these two interact over a period of approximately 100 generations in two co-distributed chipmunk species. We observed that some features of the available phenotypic (co)variation in cranial dimensions changed in response to directional selection, but in idiosyncratic ways.

Directional selection was a major component in skull evolution for both T. alpinus and T. speciosus, although the strength of selection, estimated as the selection gradient, for T. alpinus was stronger than for T. speciosus. The stronger selection gradient observed in T. alpinus populations supports the hypothesis that this species is more sensitive to the environmental changes observed at their habitat than T. speciosus (Hammond et al., 2015; Moritz et al., 2008; Rubidge et al., 2012). Since a species can become extinct when directional selection is too strong, one might think that T. alpinus is at a higher risk of extinction than T. speciosus. Indeed, theoretical work determined the threshold between the amount of sustained environmental changes, translated as the selection gradient, to the amount of variance available in a population above which the risk of extinction increases (Burger & Lynch, 1995), and T. alpinus from both transects presented a value greater than this threshold (Chapter 2). However as discussed below, available phenotypic (co)variation in T. alpinus has been redistributed between the historical and modern sampling periods to match the selection gradient, and potentially enhancing the survival of this chipmunk.

The difference in selection strength between populations of T. alpinus and T. speciosus allowed us to further narrow our predictions about how the available phenotypic (co)variation is expected to change in a directional selection scenario under a model that allows for the evolution of the genotype-phenotype map (Jones et al., 2014; Pavlicev et al., 2010; Wolf et al., 2005). Since T. alpinus faced a stronger directional selection regime than T. speciosus, we hypothesize that any changes in the available phenotypic (co)variation would be more pronounced in T. alpinus. Our first prediction proposes that the amount of phenotypic variation would increase in the direction of the selective regimen but not necessarily in other directions. Indeed, *T. alpinus* showed both an increased variance in the direction of selection but not in other directions and in the total amount of phenotypic variation for both populations examined. This was not the case for both populations of *T. speciosus*. In principle, an increased (co)variation in the direction of selection is compatible with a hypothesis of an increased frequency of rare alleles, which could be explained solely by additive genetic variance (Barton & Turelli, 1989). However, variance increase caused by rare alleles is thought to be transient and mostly to impact traits determined by a small number of alleles (Jain & Stephan, 2015), which is unlikely to be the case for skull traits. Therefore the impact of rare alleles is likely to be limited. Alternatively, a model accounting for epistatic interactions among genes could lead to this increased variation in the direction of selection, as indicated in Figure 3.1. Under this model, selection acting in a given direction will favor alleles influencing the degree of correlation favored by selection, which in turn will lead to changes in the amount of variation in this direction.

Our second prediction was that co-selected traits would increase their correlations. Indeed, Yosemite T. alpinus did show an increase in the overall phenotypic integration among traits, conforming to this prediction. This pattern is also in accordance with an epistatic model, where coordinate selection across multiple traits will lead to tighter correlations among them (Roff & Fairbairn, 2012). On the other hand, T. alpinus from the Southern Sierras did not exhibit an increase in the overall correlations among traits. On possible explanation for these different spatial responses would be the direction that selection acted, since selection in the Yosemite population was already in a dimension of relatively high variance, meaning that most traits were involved in the response to selection, while in the Southern Sierra population selection was not along a high variance dimension, as can be appreciated by comparing the evolvability boxplot distributions (Figure 3.2). Therefore, we would expect fewer traits in the Southern Sierra transect to be co-selected, which lead to the stability in the overall phenotypic integration among traits we observed. Furthermore, Yosemite T. speciosus showed the opposite response decreasing its overall degree of correlation, a pattern that would be expected in a drift scenario (Jones et al., 2003).

Lastly, we predicted a re-alignment of the (co)variation patterns with the selection gradient. Even though this was not the case for three of the populations analyzed (Figure 3.4), the Southern Sierra sample of *T. alpinus* did exhibit an increase in the correlation between PC1 and the selection gradient. Once again, this pattern might be linked to the direction where selection was operating, since in this specific population selection was not in a dimension of relatively high variation. On the other hand, for T. alpinus from Yosemite, the selection gradient was in a direction of high (co)variation, which indicates that a reorientation of the patterns of (co)variation would not have been necessary, since sufficient variation was available for selection to act upon. Macroevolutionary studies in mammals showed that the overall phenotypic correlation among traits and the amount of variance are between groups, whereas the orientation is more conserved in labile very a macroevolutionary time scale (Lofsvold, 1986; Marroig & Cheverud, 2001, p. 200; Oliveira et al., 2009; Porto et al., 2009; Steppan, 1997; Steppan et al., 2002). Since we showed that a reorientation of the phenotypic (co)variation could be easily achieved under a model of sustained directional selection, a possible explanation for the widespread stability of the (co)variation orientation on a macroevolutionary time scale could be a concordance between the adaptive landscape and the patterns of (co)variation. An interesting next step would be to investigate more cases where selection has not acted along an axis of major variance to see if the pattern reported here is robust.

An interesting aspect raised by our analysis is the striking contrast observed between species. While T. alpinus has changed the three aspects of their phenotypic (co)variation analyzed, T. speciosus has remained fairly stable, with the only aspect changing, the overall phenotypic correlation among traits, in a direction opposite to what would be expected in our predictions. It is possible that the differences in both species are related to the discrepant strength of selection observed between them. Even though we did observe a pattern consistent with directional selection for all populations, the selection observed in T. speciosus might have been weaker than that necessary to produce changes in phenotypic structure.

There are some caveats to our study that should be acknowledged. First, we worked with phenotypic instead of genetic (co)variances, because of the difficulties in estimating the latter (McGuigan, 2006; Steppan et al., 2002). Although evolutionary quantitative genetics theory is based on genetic (co)variation, we assumed that our phenotypic estimates are appropriate substitutes to their genetic counterparts based on a substantial body of evidence showing that they are structurally similar, at least for morphological traits and particularly for mammals (Cheverud, 1988, 1996; House & Simmons, 2005; Marroig & Cheverud, 2001; Porto et al., 2009; Reusch & Blanckenhorn, 1998; Roff, 1995). Furthermore, comparisons of phenotypic (co)variation in the historical and modern samples of both chipmunk species and the genetic (co)variation of a third rodent species (*Calomys expulsus*) are structurally similar, a result that supports our assumptions (for details on the reasoning behind this analysis see Marroig & Cheverud, 2001). Second, we were only able to estimate the net selection gradient between the end points of the approximately one hundred generations that spanned the historic to the modern periods. Although not ideal (Jones et al., 2004), this is the best approximation we have for the level of directional selection operating on both species between these sample periods.

Our study has several strengths. First, we examined well-sampled natural populations separated by multiple generations. Second, the large, measured effective population size of our samples allowed us to overcome some of the caveats expected when working in experimental settings, which are frequently hampered by small effective sizes. Indeed, small effective sizes will affect any study designed to analyze the effects of directional selection because of the likelihood of substantial genetic drift. Moreover, the populations we examined have encountered different selective regimens, in both direction and strength, over time. This allowed us to narrow the predictions and match our expectations to the different populations.

Our study examined a largely neglected aspect of the evolutionary dynamics: the interaction between selective regimes and available phenotypic (co)variation. We suggest that the available multidimensional phenotypic (co)variation of a species can evolve quickly in natural populations under relatively strong directional selection, a hypothesis supported by both theoretical and simulation studies (Jones et al., 2014; Melo & Marroig, 2015; Pavlicev et al., 2010). Since species under strong directional selection tend to be more prone to extinction, our study coupled with previous theoretical, computational, and experimental

knowledge highlights one mechanism by which species may enhance their survival in the face of environmental change: namely a rapid reorganization of their available phenotypic (co)variation. This is especially relevant in an ever-growing environmentally vulnerable Earth, where many species are threatened.

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# Supplementary material: Chapter 3

		Random Skewers	Krzanowski	
T. alpinus - Yosemite	Historical	0.92	0.88	
	Modern	0.92	0.89	
T. alpinus - Southern Sierras	Historical	0.95	0.89	
	Modern	0.90	0.86	
T. speciosus - Yosemite	Historical	0.95	0.88	
	Modern	0.98	0.92	
T. speciosus - Southern Sierras	Historical	0.96	0.89	
	Modern	0.97	0.89	

Table S3.1. P-matrices repeatability estimates for each population.

**Table S3.2.** Comparisons between P-matrices and the G-matrix from Calomys expulsus, byRandom Skewers and Krzanowski.

		Random Skewers	Krzanowski
T. alpinus - Yosemite	Historical	0.61	0.69
	Modern	0.67	0.66
T. alpinus - Southern Sierras	Historical	0.74	0.66
	Modern	0.67	0.68
T. speciosus - Yosemite	Historical	0.73	0.70
	Modern	0.77	0.71
T. speciosus - Southern Sierras	Historical	0.79	0.69
	Modern	0.81	0.72

 Table S3.3.
 Random Skewers (upper diagonal) and Krzanowski (lower diagonal) P-matrices comparisons among populations.

			1	2	3	4	5	6	7	8
1	T. alpinus - Yosemite	Historical		0.85	0.83	0.85	0.83	0.88	0.83	0.81
2		Modern	0.81		0.86	0.85	0.89	0.91	0.90	0.90
3	T. alpinus- Southern Sierras	Historical	0.87	0.81		0.83	0.89	0.93	0.89	0.88
4		Modern	0.82	0.82	0.82		0.81	0.87	0.84	0.88
5	T. speciosus- Yosemite	Historical	0.86	0.85	0.82	0.82		0.95	0.93	0.92
6		Modern	0.84	0.87	0.84	0.83	0.87		0.95	0.94
7	T. speciosus - Southern Sierras	Historical	0.87	0.82	0.85	0.82	0.86	0.85		0.95
8		Modern	0.84	0.86	0.85	0.83	0.84	0.87	0.86	

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Conclusões gerais

"Todas as manhãs a gazela acorda sabendo que tem que correr mais veloz que o leão ou será morta. Todas as manhãs o leão acorda sabendo que deve correr mais rápido que a gazela ou morrerá de fome. Não importa se és um leão ou uma gazela: quando o Sol desponta o melhor é começares a correr."

- Mia Couto, A Confissão da Leoa

# Conclusões gerais

Um dos principais desafios para biólogos contemporâneos é combater a perda de diversidade biológica causada por ações antrópicas (Barnosky et al., 2011). Essencial para essa tarefa é compreendermos como as espécies se adaptam e quais fatores afetam o ritmo e direção do processo adaptativo. É dentro deste contexto que essa tese se insere. Com esse trabalho busquei compreender como seleção natural atuou na diversificação de um grupo de espécies de esquilos do subgênero *Neotamias*. A escolha por esse grupo não foi aleatória, uma vez que diversas características deste grupo o transformam em um ótimo modelo para o estudo da dinâmica evolutiva, tanto no chamado tempo ecológico (microevolutivo), como em tempo geológico (macroevolutivo). Desta forma, a principal contribuição do meu trabalho é contribuir para um maior entendimento da relação entre variação morfológica e variáveis climáticas durante a evolução do grupo.

No primeiro capítulo estudei a relação entre variação morfológica e variáveis climáticas de um ponto de vista da diversificação do subgênero em uma escala macroevolutiva. O subgênero Neotamias é composto de 23 espécies que se diversificaram no início do Pleistoceno (há cerca de 2,75 milhões de anos, Sullivan et al., 2014)). Estas espécies ocupam os mais variados hábitats no oeste da América do Norte, de áreas costeiras até 4.000 metros de altitude. Os resultados apontam, primeiramente, que seleção natural foi um importante processo na diversificação craniana dentro do grupo e, em segundo lugar, que a variação morfológica estava alinhada com diversas variáveis climáticas. Dessa forma, demonstrei que variáveis climáticas podem ter sido fontes de pressões seletivas importantes dentro deste grupo.

No segundo capítulo mudei o foco para uma escala microevolutiva, estudando especificamente mudanças dentro de populações da comunidade de esquilos *Neotamias* que habitam a Sierra Nevada na Califórnia, Estados Unidos. Neste capítulo, utilizei espécimes coletados em dois períodos distintos com cerca de um século de diferença. Busquei responder se as mudanças climáticas observadas nos hábitats destas espécies, durante este período, seriam boas preditoras da direção da mudança fenotípica e/ou da força de seleção, dado que tal relação havia sido encontrada em tempo geológico. Nenhuma relação entre graus de mudancas em variáveis climáticas e mudancas fenotípicas foi encontrada. Este resultado ilustra o quão complexo é compreender o impacto das mudanças climáticas e/ou ambientais em espécies e populações diferentes. Um ponto crítico deste capítulo foi mostrar que seleção directional variou consideravelmente entre as diferentes populações analisadas. Foram obtidas estimativas de forças de seleção bastante distintas até mesmo dentro de uma mesma espécie (tanto em magnitude quanto em relação a sua direção). Isto demonstra que as mudanças climáticas e de uso da terra observadas no último século afetam de maneiras distintas diferentes populações, mesmo de espécies filogeneticamente próximas. Ainda, chama atenção o fato de que três das populações estudadas estão sofrendo mudanças fenotípicas em uma taxa suficientemente alta, que teoricamente resultaria na extinção destas populações caso a pressão seletiva se mantivesse ao longo de diversas gerações (Burger & Lynch, 1995). No entanto, este resultado parte do pressuposto de que a variação genética na população é estática e não interage com a pressão seletiva, o que não é o caso, como mostrado no capítulo 3 da tese.

O capítulo 3 investigou como a variação (e covariação) fenotípica em caracteres complexos, como o crânio, responde à seleção natural, utilizando duas espécies (e quatro populações) nas quais as forças seletivas estimadas foram mais discrepantes. Neste capítulo, usei a espécie *T. alpinus*, na qual a maior pressão seletiva foi observada, e a espécie *T. speciosus*, onde as menores pressões seletivas foram constatadas (mudanças em cerca de 100 gerações). A evolução por seleção natural é fruto da interação entre duas características: a força e direção da seleção propriamente dita e a quantidade de variação presente na população (e como esta está estruturada na população). Dessa forma, este capítulo focou nestas duas espécies, buscando compreender se (e como) a (co)variação fenotípica respondeu à seleção direcional observada. Os resultados mostram que seleção direcional afetou a estrutura de (co)variação fenotípica, como esperado por modelos teóricos, em especial nas populações onde seleção foi mais forte (Jones et al., 2014; Pavlicev et al., 2010). Nestas populações, observamos uma reorganização da (co)variação fenotípica na direção da seleção, indicando que a própria estrutura de (co)variação responde à seleção natural.

Essa tese demonstra que embora o clima seja uma força seletiva importante para a variação morfológica em *Neotamias*, isto não ocorre de maneira linear e facilmente identificada. Mais do que isso, mostrei que embora a pressão seletiva parece ser superior ao que uma população conseguiria suportar sem se extinguir, há potencial de adaptação e reorganização da própria variação fenotípica, o que aliviaria o possível efeito de extinção causado por seleção à longo prazo. Dessa forma, este trabalho chama atenção para várias caraterísticas essenciais para entendermos as diferentes respostas de populações às mudanças climáticas, não só em relação à pressão seletiva propriamente dita, mas também no que tange a sua interação com a arquitetura genética subjacente, que determina como a variação fenotípica se distribui na população.

Por fim, este estudo reforça a ideia de que entender como as populações responderão às mudanças climáticas é algo complexo, sendo necessário considerar o potencial evolutivo das populações (Etterson & Shaw, 2001; Gienapp & Brommer, 2014), assim como a forma como estas interagem no ambiente em que vivem. Nesse sentido, estudos de longo termo, como o apresentado aqui, devem ser promovidos, já que podem auxiliar na prevenção e/ou mitigação dos efeitos antrópicos em populações naturais. Mais do que isso, é essencial promover iniciativas interdisciplinares como o Projeto Grinnell, para uma melhor compreensão do porquê as respostas de cada população são tão discrepantes e únicas. Apenas ao compreendermos o ambiente e a biologia de cada população envolvida de maneira integrada, abarcando diferentes aspectos de sua biologia e ecologia, seremos capaz de responder estas questões.

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# Anexo

"Whatever doesn't kill you simply makes you... stranger." — The Joker; Batman: The Dark Knight Rises

# Anexo – Amostras utilizadas

Lista completa das amostras utilizadas, separadas por espécie e capítulos nos quais foram usadas. As amostra utilizadas nos capítulos 2 e 3 foram também separadas por período de coleta (histórico ou moderno) e população (Norte, Central e Sul). Sigla das instituições: NMNH- National Museum of Natural History - Washington D.C.; MVZ- Museum of Vertebrate Zoology- Berkeley.

# Tamias amoenus

Capítulo 1:

Capítulos 1 e 2:

Histórico

**MVZ** - 30463, 33950, 33952, 33954, 33955, 33956, 33958, 33960, 33964, 33972, 33973, 33976, 33980, 33981, 33983, 33984, 33985, 33986, 33987, 33988, 33989, 33990, 33991, 33993, 33994, 33995, 33996, 33997, 34000, 34001, 34003, 34772, 34773, 34775, 34776, 34778, 34779, 35110, 35113, 35114, 35115, 35119, 35120, 35121, 35123, 35124, 35125, 35126, 35127, 35128, 35129, 35130, 35131, 35132

## Moderno

**MVZ** - 196671, 200485, 200497, 200518, 200522, 200524, 200525, 200526, 200609, 200621, 206854, 208702, 208706, 215530, 217634, 217635, 217636, 217637, 217645, 217649, 217651, 217657, 217659, 217661, 217663, 217664, 217681, 217683, 217684, 217685, 217686, 217779, 217782, 217783, 217784, 217787, 217788, 217793, 217805, 217808, 217816, 218069, 218070, 218257, 218258, 218259, 218260, 218261, 218762, 218763, 218767, 220175, 220176, 220178, 220179, 220180, 220191, 220207, 220210, 220501, 220502, 220503, 220506, 220507, 220509, 220510, 220511, 220512, 220513, 220514, 220515, 220517, 220518, 220519, 220523, 220526, 220527

# Tamias quadrimaculatus

Capítulo 1:

MVZ - 32397, 32398, 65207, 88265, 105917, 105918, 151333

Capítulos 1 e 2:

Distribuição Norte - histórico<br/>  $\mathbf{MVZ}$  - 18645, 18646, 18654, 18656, 18658, 18872, 18873, 18874, 18875, 19441, 19442, 19443, 19444, 19445, 19446, 19447, 19452, 24265, 24268, 88187, 88188

Distribuição Norte - moderno **MVZ** - 221761, 221762, 221763, 221764, 224622, 224623, 224624, 224625

#### Distribuição Central - histórico

MVZ - 22780, 22782, 22784, 22785, 22786, 22787, 22789, 22790, 22791, 22793, 22794, 22795, 22796, 22797, 22798, 22802, 22803, 22804, 22805, 22807, 23303, 23304, 23305, 23306, 23307, 23308, 23309, 23311, 23312, 23314, 23315, 23316

#### Distribuição Central - moderno

**MVZ** - 201431, 201433, 201434, 201435, 201436, 201437, 201438, 201439, 201440, 201443, 201444, 201445, 201446, 201447, 201448, 201449, 207214, 216311, 216312, 216313, 216314, 216315, 216316, 216317, 216318, 216319, 216320, 216323

# Tamias sonomae

#### Capítulo 1:

 $\mathbf{MVZ}$  - 56830, 74322, 118557, 152182, 152187, 152188, 152212, 152216, 152218, 152219, 152223, 152225, 152226, 152232, 152237, 152259, 152260, 152262, 152263, 152268, 152269, 152273, 152277, 152281, 152284, 152285, 152286, 152288, 152291, 152313, 152361, 152362, 152364, 152365, 152404, 152409, 152433, 152478, 152479, 152527, 152559, 152562, 152563, 152564, 152595, 152596, 152615, 152687, 152786

# Tamias senex

Capítulo 1:

 $\mathbf{MVZ} - 101510, 10738, 10935, 10936, 10939, 10940, 116231, 121154, 123211, 125090, 126114, 13185, 13186, 13188, 13189, 13195, 13196, 13197, 13198, 13199, 13202, 13204, 13206, 13207, 13208, 13213, 13214, 13215, 13216, 13217, 13218, 13219, 13220, 13221, 13223, 13224, 13225, 13228, 13229, 13231, 13232, 13233, 13234, 13236, 13237, 13238, 13240, 13242, 13244, 13246, 13248, 13249, 13251, 13252, 13253, 13254, 13255, 13257, 13258, 13259, 13260, 13262, 13263, 13264, 13265, 13266, 13267, 13270, 13271, 13272, 13273, 13274, 13275, 13277, 13278, 141290, 151511, 151512, 151513, 151514, 151517, 151518, 151521, 151522, 151524, 151525, 151526, 151529, 151535, 151537, 151543, 151544, 151546, 161302, 182925, 20366, 20367, 207216, 208623, 223054, 223056, 223057, 224103, 22792, 22808, 22809, 22810, 22811, 22812, 22813, 22814, 22815, 22817, 22818, 22819, 22820, 22821, 22823, 22824, 22825, 22827, 22830, 22831, 22832, 22833, 22834, 22837, 22838, 23275, 23276, 23277, 23278, 23299, 23299, 23300, 23301, 299542, 30023, 3317, 3318, 3322, 3325, 3326, 59731, 59732, 65457, 65459, 74328$ 

Capítulos 1 e 2:

#### Histórico

 $\begin{array}{l} \mathbf{MVZ} & - \ 27951, \ 27952, \ 27953, \ 30466, \ 30467, \ 34068, \ 34070, \ 34072, \ 34076, \ 34082, \ 34083, \ 34084, \ 34089, \ 34090, \ 34092, \ 34093, \ 34095, \ 34096, \ 34097, \ 34100, \ 34104, \ 34105, \ 34106, \ 34107, \ 34108, \ 34109, \ 34110, \ 34113, \ 34114, \ 34115, \ 34785, \ 34786, \ 34787, \ 34788, \ 34789, \ 35015, \ 35016, \ 35133 \end{array}$ 

# Moderno

**MVZ** - 196676, 196677, 196679, 199195, 199196, 200527, 200539, 200540, 200626, 208612, 208639, 208640, 208649, 208700, 208703, 208705, 215528, 217687, 217688, 217690, 217693, 217694, 218071, 218075, 218092, 218093, 218094, 218095, 218096, 218107, 218108, 218109, 218110, 218111, 218114, 218115, 218119, 218120, 218121, 218122, 218123, 218124, 218125, 218126, 218127, 218128, 218129, 218130, 218131, 218132, 218133, 218134, 218135, 218137, 218138, 218139, 218140, 218141, 218142, 218144, 218147, 218586, 218587, 218588, 218596, 218597, 218601, 218604, 219783, 219787, 220197, 220198, 220199, 220200, 220203, 220204, 220205, 220208, 220209, 220211, 220430, 220431, 220432, 220540, 220675, 220676, 220677, 220678, 220680, 220682, 220683, 220684, 220685, 220686, 220687, 220688, 220689, 220690, 220691, 220692, 220693, 220694, 220695, 220696, 220697, 220698, 222790, 222793, 222799, 222807, 223051

# Tamias siskiyou

# Capítulo 1:

 $\mathbf{MVZ}$  - 56890, 56891, 56893, 56894, 56898, 56899, 56900, 56916, 60329, 99548, 151638, 151643, 151645, 151650, 151652, 151654, 151660, 151661, 151662, 151663, 151665, 151668, 151669, 151672, 151675, 151680, 151681, 151683, 151684, 151685, 151689, 151690, 151691, 151693, 151696, 151698, 151699, 151701, 161312, 182730, 182731, 182733, 182734, 182735, 182737, 220417, 220418, 220423, 220424, 220426

# Tamias townsendii

# Capítulo 1:

**MVZ** - 53865, 63475, 83363, 88878, 88880, 88882, 94224, 94233, 94238, 94239, 94240, 94248, 94250, 94252, 94255, 94256, 94257, 94269, 94271, 94272, 94273, 95947, 95948, 95949, 95950, 95951, 102980, 104576, 108110, 114319, 151709, 151710, 151715, 151721, 151724, 151730, 151733, 151736, 151738, 151739, 151740, 151741, 151743, 151745, 181528, 190016, 190017, 190018, 190022, 190026

# Tamias ruficaudus

Capítulo 1:

**NMNH** - 26993, 26999, 27274, 27275, 66637, 66638, 66640, 66641, 66642, 66644, 66645, 66646, 66647, 66648, 68821, 73963, 73982, 73983, 73994, 73999, 74000, 74004, 74308, 74309, 74310, 74311, 74317, 91316, 169188, 236149, 236150, 236151, 236152, 236488, 236489, 236491, 236495, 236496, 236500, 236501, 236502, 236503, 236505, 236507, 275781, 275782, 275783, 285111

MVZ - 275784, 275785

# Tamias minimus

#### Capítulo 1:

**MVZ** - 10930, 10942, 10944, 10945, 10948, 31679, 32389, 78558, 85248, 87086, 95292, 95293, 98993, 98995, 98996, 105408, 105411, 113705, 119313, 125187, 126497, 126498, 142231, 183745, 217077, 217078, 222660, 222662, 222665, 222666, 222667, 222670, 222671, 222673, 223050, 224485

Capítulos 1 e 2:

#### Histórico

**MVZ** - 17574, 17575, 23357, 23376, 24118, 24122, 24125, 24130, 24136, 27317, 27318, 27319, 27320, 27321, 27322, 27323, 27325, 27330, 27332, 27333, 27338, 27340, 27341, 27343, 27346, 27347, 27351, 27353, 27354, 27356, 27363, 27364, 27365, 27370, 27372, 27374, 27375, 27376, 27378

#### Moderno

**MVZ** - 208320, 208321, 208322, 208323, 208324, 208325, 208327, 208329, 208330, 208331, 208332, 208333, 208543, 208544, 208545, 208552, 208554, 216299, 216300, 216301, 216302, 216303, 216304, 216306, 216310, 217106, 217107, 217111, 217112, 217113, 217115, 217116, 217117, 217118, 217271, 219225, 219226, 219227, 219228, 219229, 219230, 219231, 219232, 219233, 219234, 219235, 219907, 219908, 219909, 219910, 219911, 219912, 219913, 219914, 219915, 219916, 219917, 219918, 219919, 219920, 219921, 219922, 219923, 219924, 220258, 220259, 220260, 220261, 220262, 220263, 220264, 220265, 220266, 221244, 221245, 221246, 221247, 221248, 221249, 221250, 221251, 221252, 221253, 221254, 221255, 221256, 221257, 221258, 221259, 221260, 221261, 221262, 221263, 221264, 221265, 221266, 221267, 221268, 221269, 221270, 221271, 221272, 221273, 221274, 221275, 221276, 221277, 221278, 221279, 221280, 222157, 222158, 222159, 222160, 222161, 222163, 222164, 221265, 221441, 224142, 224143, 224144, 224146, 224147, 224148, 224149, 224150, 224151, 224152, 224153, 224154, 224850, 224851, 224852, 224853, 224854

# Tamias alpinus

#### Capítulo 1:

**NMNH** - 28463, 29018, 29132, 29137, 29142, 29143, 29144, 29145, 29183, 29927, 29929, 29930, 30359, 30360, 30361, 30362, 30365, 30366, 30368, 30369, 30377, 30448, 30454, 30499, 30501, 30814, 30818, 30819, 30820, 30823, 31049, 40560, 109038, 109041, 109047, 109048, 109156, 109158, 109159, 109161, 109162, 109164, 109165, 109168, 109169, 109253, 109254, 109257, 109647, 109648, 109654, 109667, 109686, 110299, 110305, 116024, 250081, 274839

Capítulos 1, 2 e 3:

#### Distribuição Central (Yosemite) - histórico

**MVZ** - 22663, 22665, 22667, 22668, 22669, 22671, 22672, 22673, 22674, 22675, 22676, 22677, 22678, 22679, 22680, 22681, 22682, 22684, 22685, 22686, 22687, 22689, 22690, 22692, 22697, 22699, 22700, 22702, 22703, 22705, 23320, 23322, 23323, 23327, 23329, 23330, 23331, 23332, 23334, 23335, 23337, 23338, 23340, 23342, 23343, 23344, 23345, 23346, 23348, 23350

#### Distribuição Central (Yosemite) - moderno

**MVZ** - 201430, 207199, 207200, 207201, 207202, 207203, 207204, 207205, 207206, 207207, 207208, 216270, 216272, 217178, 217179, 217180, 217181, 217182, 217183, 217184, 217185, 217186, 219986, 219987, 219989, 219990, 219991, 219992, 219993, 219997, 219998, 219999, 220002, 220010, 220019, 222199, 222200, 222203

# Distribuição Sul (Southern Sierras) - histórico

**MVZ** - 14890, 14903, 14904, 14905, 14911, 14914, 14915, 14916, 14918, 14922, 14923, 14924, 14927, 14929, 14930, 14931, 14936, 14939, 14942, 14945, 14946, 14948, 14949, 14950, 14957, 14958, 14959, 14962, 14970, 14973, 14975, 17576, 17579, 17581, 17585, 17586, 17587, 17589, 17590, 17592, 17593, 17594, 17595, 17596, 17597, 17598, 17599, 17600, 17601, 17602, 17603, 17604, 17605, 17606, 17607, 17608, 17609, 17611, 17615, 17617, 17618, 17619, 17621, 17622, 25189, 25190, 25193, 25199, 25200, 25204, 25209, 25213, 30074, 30076, 108999

#### Distribuição Sul (Southern Sierras) - moderno

**MVZ** - 206396, 206397, 224075, 224077, 224078, 224481, 224483, 224484, 224502, 225304, 225305, 225306, 225307, 225308, 225309, 226162, 226163, 228177, 228178, 228179, 228180, 228182, 228183, 228185, 228186, 228187, 228188, 228189, 228190, 229676, 229678, 229679, 229681

# Tamias umbrinus

#### Capítulo 1:

# *Tamias rufus*

#### Capítulo 1:

**MVZ** - 6888, 43948, 43949, 43950, 43951, 43954, 43955, 43956, 43957, 43959, 55355, 55356, 55357, 55358, 58566, 62915, 62916, 62917, 62918, 62919, 95135, 199281

**NMNH** - 57152, 66546, 148129, 148130, 148131, 148135, 148136, 148137, 148195, 148197, 149081, 149949, 149951, 149952, 149955, 250979, 485496, 485497, 485498, 485499, 485500, 485501, 498509, 498510, 512847, 564126, 564127

# Tamias quadrivittatus

## Capítulo 1:

**MVZ** - 6895, 60459, 60460, 60461, 60464, 60467, 60468, 60469, 60470, 60471, 60472, 60473, 60474, 60475, 60477, 60478, 60485, 69627, 70161, 93102, 115549, 116386, 116388, 116389, 116514, 121620,

132773, 132775, 132776, 132777, 142042, 142043, 142044, 142045, 142046, 190011, 190013, 190014, 190015

NMNH - 23019, 23127, 54127, 54128, 129801, 133652, 150729

# Tamias cinereicollis

Capítulo 1:

**NMNH** - 22505, 23383, 24519, 24520, 24521, 24522, 24523, 24525, 24527, 24529, 24530, 24531, 24532, 24637, 32092, 32094, 32096, 32097, 32098, 32099, 53709, 53710, 53711, 53713, 53714, 53716, 53718, 53721, 53725, 53727, 53728, 53731, 181359, 202120, 202555, 205353, 205590, 205594, 205870, 205874, 205875, 205876, 205876, 205877, 208620, 208621, 208627, 208628

## *Tamias canipes*

Capítulo 1:

MVZ - 50332, 84525, 151220, 151224, 190000, 190001, 190002

**NMNH** - 97318, 97319, 97321, 109228, 109230, 119031, 120774, 127388, 128093, 128094, 128101, 128104, 129005, 129006, 129007, 129008, 129009, 129010, 130094, 130096, 130103, 130756, 130757, 130758, 130759, 130760, 130763, 130765, 130766, 130768, 130770, 130771

# Tamias dorsalis

Capítulo 1:

**MVZ** - 47937, 47938, 47939, 47940, 47941, 47943, 47944, 47947, 47949, 47950, 47957, 47964, 47968, 47976, 47977, 47979, 47996, 52155, 52156, 57579, 59442, 59443, 59444, 59445, 59447, 59448, 59449, 67408, 67907, 67909, 67911, 67912, 67915, 67916, 67919, 67920, 93104, 93105, 93106, 93107, 93108, 93109, 93110, 93111, 93112, 93113, 93114, 93115, 132213, 132214, 151239, 151240, 151241, 151242, 151243, 151245, 151247, 197180, 197182

# Tamias obscurus

Capítulo 1:

**NMNH** - 18049, 24956, 61132, 63564, 66234, 66236, 66239, 66240, 66241, 66242, 66245, 66246, 66248, 66253, 126594, 137867, 138614, 138615, 138617, 138629, 138633, 138635, 138637, 138639, 138640, 138641, 138642, 193199

# Tamias merriami

Capítulo 1:
**MVZ** - 1756, 1873, 1878, 1991, 1994, 2093, 2096, 2239, 6908, 9472, 13809, 13810, 21857, 23610, 25269, 25270, 25272, 25273, 25274, 25275, 25277, 25278, 25279, 25280, 25282, 25285, 29146, 29151, 29153, 29155, 29156, 30267, 30268, 30269, 30270, 30272, 30274, 30275, 30276, 30278, 30279, 42132, 42135, 55035, 63008, 98045, 99339, 99340, 103983, 103984, 103986, 103987, 104959, 114005, 114793, 123565, 125683, 207213, 216298, 223960

## Tamias speciosus

#### Capítulo 1:

**MVZ** - 21338, 32926, 68989, 85250, 85251, 85252, 88184, 88185, 88186, 94860, 94861, 99010, 99011, 99012, 99014, 99015, 99016, 109001, 109002, 109003, 109005, 109008, 119131, 151375, 151378, 151379, 151380, 151381, 151382, 151383, 151783, 161305, 165877, 201265

Capítulos 1,2 e 3:

### Distribuição Central (Yosemite) - histórico

 $\begin{array}{l} \mathbf{MVZ} & - 11931, \ 11933, \ 22707, \ 22708, \ 22709, \ 22710, \ 22712, \ 22713, \ 22714, \ 22715, \ 22716, \ 22717, \ 22719, \ 22720, \ 22721, \ 22724, \ 22725, \ 22726, \ 22729, \ 22730, \ 22731, \ 22734, \ 22735, \ 22736, \ 22737, \ 22738, \ 22740, \ 22741, \ 22742, \ 22743, \ 22744, \ 22745, \ 22747, \ 22748, \ 22749, \ 22750, \ 22752, \ 22754, \ 22761, \ 22762, \ 22763, \ 22764, \ 22766, \ 22772, \ 22773, \ 23383, \ 23384, \ 23388, \ 23390, \ 23391, \ 23395, \ 23396, \ 23397, \ 23400, \ 23401, \ 23402, \ 23404, \ 23409, \ 23410, \ 23411, \ 23412, \ 23414, \ 23415, \ 23416, \ 23418, \ 23420, \ 23421, \ 23422, \ 23423, \ 23424, \ 23425, \ 23426, \ 23427, \ 23428, \ 24385, \ 24387 \end{array}$ 

#### Distribuição Central (Yosemite) - moderno

MVZ - 216338, 201450, 201451, 201452, 201453, 201454, 201455, 201456, 201457, 201458, 201459, 201460, 201461, 201462, 201463, 201464, 201466, 201467, 201468, 201471, 201472, 201473, 201474, 201476, 201477, 201478, 201479, 201480, 201481, 201482, 201483, 201484, 201485, 201486, 201487, 201488, 201489, 201490, 201492, 201493, 201494, 201495, 201496, 201497, 201498, 201499, 201500, 201502, 201503, 201504, 201505, 201506, 201508, 201509, 201510, 201512, 201513, 201514, 201515, 201516, 201517, 201518, 201522, 201523, 201527, 201528, 201529, 201530, 201531, 201532, 201533, 201548, 201549, 201551, 201553, 201556, 201557, 201558, 201560, 201561, 201565, 207224, 207237, 207238, 207240, 207241, 207242, 207244, 207245, 207246, 207247, 207248, 207254, 207258, 207259, 207260, 207261, 207264, 207265, 207266, 207268, 207269, 207271, 207272, 207273, 207274, 207275, 207276, 207277, 207279, 207280, 207281, 207283, 207284, 207285, 208335, 216019, 216020, 216021, 216324, 216325, 216326, 216327, 216328, 216330, 216333, 216334, 216335, 216336, 216337, 216339, 216340, 216342, 216343, 216344, 216347, 216348, 216349, 216350, 216351, 216352, 216353, 216358, 216361, 216362, 216363, 216365, 216366, 216367, 216373, 216374, 217191, 217192, 217193, 217196, 217197, 217198, 220025, 220026, 220027, 220029, 220055, 220064, 220066, 220067, 220070, 222211, 222212, 222216, 224158, 224159, 224160, 224161, 224162, 224163, 224164, 224165, 224166, 224167, 224168, 224169, 224170, 224171, 224172, 224173, 224174, 224175, 224176, 224177, 224178, 224179, 224180, 224181, 224182, 224183, 224184, 224185, 224186, 224187, 224188, 224189, 224190, 224191, 224192, 224193, 224194, 224195, 224196, 224197, 224198, 224199, 224200, 224202, 224203, 224204, 224205, 224206, 224207, 224226, 224227, 224228

### Distribuição Sul (Southern Sierras) - histórico

 $\begin{array}{l} \mathbf{MVZ} & - 14775, \ 14776, \ 14780, \ 14784, \ 14786, \ 14790, \ 14791, \ 14792, \ 14793, \ 14801, \ 14810, \ 14815, \ 14820, \\ 14822, \ 14823, \ 14824, \ 14826, \ 14827, \ 14828, \ 14831, \ 14835, \ 14836, \ 14841, \ 14843, \ 14844, \ 14847, \ 14852, \\ 14855, \ 14856, \ 14857, \ 14858, \ 14861, \ 14863, \ 14865, \ 14869, \ 14870, \ 14872, \ 14875, \ 14876, \ 14877, \ 14879, \\ \end{array}$ 

 $14880,\ 14881,\ 14882,\ 14885,\ 14892,\ 14894,\ 14895,\ 14896,\ 14897,\ 14901,\ 25215,\ 25216,\ 25220,\ 25221,\ 25223,\ 25225,\ 25226,\ 25226,\ 25230,\ 25231,\ 25232,\ 25236,\ 25237,\ 25242,\ 25245,\ 25247,\ 25248,\ 25250,\ 25252,\ 25253,\ 25254,\ 25257,\ 25259,\ 25261,\ 25262,\ 25264,\ 30078,\ 30079,\ 30080,\ 30081,\ 30083,\ 30087$ 

# Distribuição Sul (Southern Sierras) - moderno

**MVZ** - 206412, 219224, 222502, 222503, 222504, 222505, 222506, 222507, 222508, 222509, 222510, 222511, 222512, 222513, 222514, 222516, 222518, 222519, 222520, 222674, 222675, 222676, 222677, 222681, 222687, 222689, 223552, 223553, 223961, 223963, 223964, 223966, 223968, 223969, 223971, 223972, 224079, 224080, 224081, 224082, 224083, 224084, 224085, 224087, 224209, 224210, 224211, 224212, 224213, 224214, 224215, 224216, 224217, 224218, 224219, 224220, 224221, 224222, 224223, 224224, 224225, 224279, 224280, 224281, 224282, 224283, 224284, 224285, 224291, 224293, 224295, 224298, 224299, 224432, 224434, 224488, 224490, 224491, 224492, 224493, 224495, 224496, 224497, 224498, 224499, 224499, 224501, 225310, 225311, 225313, 225314, 225316, 225317, 225318, 225319, 225320, 225321, 225323, 225324, 225325, 225326

### *Tamias panamintinus*

Capítulo 1:

**MVZ** - 14978, 14979, 14980, 14982, 14983, 14985, 14986, 14987, 14991, 14993, 27186, 27187, 27190, 27191, 27192, 27195, 27196, 27197, 27200, 27201, 27202, 27203, 27206, 27209, 27211, 27213, 27214, 27215, 27216, 27217, 27222, 27223, 27278, 27282, 27284, 27286, 27287, 27290, 86158, 86161, 86163, 92600, 99007, 151323, 151786, 160847, 216375, 220267, 220268, 220269, 220270, 220271, 221281, 224266, 224273

# Tamias durangae

Capítulo 1:

**NMNH** - 94628, 94630, 94631, 94634, 94635, 94636, 94637, 94638, 95214, 95226, 95333, 95337, 95338, 95341, 116881, 116883, 116885, 116887, 116889, 116890, 116891

## *Tamias striatus*

Capítulo 1:

**NMNH** - 20790, 22759, 22767, 22769, 22778, 30425, 72844, 77508, 80779, 82874, 82884, 82885, 82890, 97723, 116804, 118663, 118664, 135547, 135549, 135550, 135553, 135557, 142124, 143961, 171905, 171908, 193377, 193378, 193398, 193413, 199534, 253960, 253967, 258325, 271569, 271574, 271577, 276433, 276637, 277596, 282674, 398176, 505612, 505613, 505614, 567660, 568006, 568354, 568355, 568358

MVZ - 568362, 568369, 569092, 570491