Environmental and genetic contributions to ecogeographic rules in house mice

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Running title: Allen's rule and Bergmann's rule in house mice

Abstract (200 words)

Distinguishing between genetic, environmental, and genotype-by-environment effects are central to understanding geographic variation in phenotypic clines. Two of the best-documented phenotypic clines are Bergmann's rule and Allen's rule, which predict larger body sizes and shortened extremities in colder climates, respectively. Although numerous studies have found evidence for both ecogeographic patterns within and across taxa, we still have little understanding about whether these patterns are driven by genetics, environment, or both. Here, we measure the genetic and environmental contributions of Bergmann's rule and Allen's rule across introduced populations of American house mice (*Mus musculus domesticus*). First, we document patterns of Bergmann's rule and Allen's rule in wild-caught house mice across North and South America, with larger body sizes and shortened extremities present at higher latitudes. Next, we find genetically based differences in body mass and tail length between mice from upstate New York and equatorial Brazil, patterns consistent with Bermgann's rule and Allen's rule. We then assess the contributions of phenotypic plasticity to these ecogeographic patterns and found very little plasticity in body size across both populations. Unlike body size, we found considerable plasticity in extremity length in response to cold temperature. The plastic responses of tail length and ear length goes in the same direction as the evolved responses, highlighting an example of adpative phenotypic plasticity underlying Allen's rule.

Introduction

Clines in phentoypes have historically been attributed to natural selection and reflect adaptation to local environments (Huxley 1938; Endler 1977). Two of the most well described phenotypic clines are Allen's rule and Bergmann's rule. Allen's rule predicts that extremities of organisms occupying cold geographic regions are shorter compared to conspecifics occupying warm regions (Allen 1877). Similarly, Bergmann's rule predicts larger body sizes in colder climates compared to organisms occupying warmer climates (Bergmann 1847). Shortened extremities and larger body sizes minimize heat loss by reducing surface area to volume ratios and are viewed as thermoregulatory adaptations (Mayr 1956). Numerous studies have documented Bergmann's rule and Allen's rule within and across species of birds (Snow 1954; James 1970; Johnston and Selander 1971) and mammals (Brown and Lee 1969; Griffing 1974; Yom-Tov and Nix 1986), including humans (Ruff 1994; Foster and Collard 2013; Betti et al. 2015). Moreover, various meta-analyses have supported (**???**; **???**; Freckleton et al. 2003; Meiri and Dayan 2003; Blackburn and Hawkins 2004; Millien2006; Olson2009; Symonds and Tattersall 2010; Nudds and Oswald 2007; Alhajeri et al. 2020) or refuted (**???**; **???**; McNab 1971; Geist 1987) an evolutionary basis to these phenotypic clines. The contradicting results found across the literature are unsurprising given the variation within and among datasets, such as choice of taxonomic groups, environmental variables, and inconsitencies in morphological measurements. To date, we still have very little understanding of the mechanisms underlying Allen's rule and Bergmann's rule.

Missing from many of these discussions are careful analyses determining which traits are genetically encoded, environmentally influenced, or both. Most traits associated with Bergmann's rule and Allen's rule are quantitative, meaning they are polygenic and environmentally determined [Lynch and Walsh 1998; Falconer and Mackay 1996]. Disentangling genetics from environmental effects in natural populations is difficult when solely using phenotypic data collected from wild-caught specimens. Patterns consistent with Bergmann's rule and Allen's rule may exist underneath environmental effects and possible genotype-by-environment ineratctions (**???**; Conover and Schultz 1995). Phenotypic plasticity may also generate clinal patterns, giving a false indication for adaptive clines (**???**; James 1983). In fact, many temporal patterns of Bergmann's rule are driven by the environment (i.e. nonadaptive plasticity) and not genetic adaptation in birds (Teplitsky et al. 2008; Husby et al. 2011) and mammals (Ozgul et al. 2009, 2010). Furthermore, we have little understanding how populations conforming to these ecogeographic rules vary in the degree or direction of plasticity they exhibit in response to environmental stimuli. Variation in plasticity and gentoype-by-environment interactions may faciliate adaptation and divergence in polygenic traits such as body size (Via and Lande 1985; Gillespie and Turelli 1989; Gomulkiewicz and Kirkpatrick 1992). However, controlling for environmental effects and measuring the contributions of phenotypic plasticity is difficult,

as transplant experiments and common garden experiments are infeasible for many taxa. These limitations have impeded our ability to make substantial progress on understanding the evolutionary and ecological mechanisms underlying Bergmann's rule and Allen's rule.

House mice (*Mus musculus domesticus*) are a tractable system to dissentangle genetics from environment underlying complex traits. House mice have recently expanded their range from Western Europe to the Americas, where they can be found from the tip of South America up to Alaska. Across this broad latitudinal range, house mice are exposed to considerable variation in climatic conditions, including cold, temperate environments to warm, tropical and arid environments (**???**). Despite house mice residing in these novel environments for only ~500 generations, there is evidence for clinal adaptation across populations. Specifically, mice in eastern North America follow Bergmann's rule (Lynch 1992), with larger mice in more northern populations compared to their southern conspecifics. These body size differences persist in a commmon environment and over many generations, indicating a genetic basis for Bergmann's rule in house mice (**???**; Lynch 1992). Experimental evolution studies of house mice conducted in the laboratory recapitulate these clinal patterns, in which mice evolved at lower temperatures become larger and undergo genetic divergence in body size (Barnett and Dickson 1984). Furthermore, earlier work revealed an environmental influence on tail length when exposed to cold temperatures. Specfically, house mice reared in a cold environment grew significantly shorter tails than mice reared at warm temperatures, consistent with Allen's rule (**???**; **???**; Barnett 1965). However, these earlier studies either only investigated a single population of wild house mice or used traditional laboratory strains of mice, making it difficult to place the results in an explicit evolutionary framework. We still have little understanding of the phenotpic variation of house mice across their entire latitudinal distribution, and the subsequent contributions of genetics and the enviornment on these complex traits.

Here, we use a combination of approaches to tease apart genetics from plasticity in Bergmann's rule and Allen's rule in American house mice. First, we determined if house mice conform to both Bergmann's rule and Allen's rule across their entire introduced range by analyzing phenotypic data from wild-caught individuals across North and South America. Second, because it is difficult to dissentangle genetics from plasticity using wild phenotypic data, we collected temperate and tropical populations of house mice from the ends of their latitudinal distribution, brought them back to the lab, and established wild-derived colonies. We analyzed phenotypic differences between populations and across generations in a common environment to identify a genetic basis for Allen's rule and Bergmann's rule. Third, to measure the influence of environment on body size and extremity length, we performed a second common garden experiment by rearing both populations of house mice in a cold environment and measured the effects on body size and

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extremity length. Measuring developmental plasticity in these traits allows us to assess the influence of temperature on Bergmann's rule and Allen's rule. Specifically, we show that unlike body size, extremity length is highly plastic, and this plastic response goes in the same direction as the evolved response, highlighting an example of adaptive phentoypic plasticity.

Materials and Methods

Wild-caught phenotypic metadata

To determine if house mice conform to Allen's rule and Bergmann's rule, we assessed the relationship between body mass, tail length, ear length, and latitude in wild house mice collected across North and South America. Specimen data of all house mouse records were downloaded from VertNet (a Database of Vertebrate Specimen Records) (http://vertnet.org)(Constable et al. 2010) on October 13, 2020, using the search query: *vntype*:specimen, *genus*:Mus. We obtained 62,139 museum records and retained records that included *Mus musculus* specimens collected in North or South America (excluding islands). We further excluded individuals listed as pregnant, juvenile, subadult, or immature, and kept those identified as adult, mature, or with no age class noted. We also manually coded females and males as 'adult' if they fulfilled any of the following criteria: females - presence of placental scars, parous, or lactating; males - presence of seminal vesicles, testes descended (TD), or testes scrotal (TS). Tail lengths shorter than 20mm and longer than 120mm ($n = 8$), and ear lengths greater than 30mm ($n = 1$) were considered extreme outliers (greater than 3.5 standard deviations from the mean) and were removed from downstream analyses, as these likely represent juveniles or measuring erros. Sample information for the final VertNet dataset (n = 3,018) is provided in Data S1.

Laboratory-reared mice - common garden experiment 1

For the first common garden experiment, live animals were collected from two locations that represent the ends of the latitudinal transect: Manaus, Amazonas, Brazil (MAN) and Saratoga Springs, New York, USA (SAR). Details of this common garden experiment are specified in (**???**; Suzuki et al. 2020). Briefly, live mice from both Brazil and New York were brought back to the lab at the University of California, Berkeley. Within each population, unrelated pairs of wild-caught mice were mated to produce first generation (N1) lab-reared mice, and these inbred lines have subsequently been maintained through sib-sib matings each generation for over 10 generations. Wild-caught mice and their descendants were housed in a standard laboratory environment at 21ºC with a 12-hr dark and 12-hr light cycle. Commerical rodent chow was provided ad libitium. Standard museum measurements were taken for all wild-caught, N1, and N2 mice from each population (see Data S3). Tail lengths less than 50mm ($n = 2$) and ear lengths less than 8mm ($n = 1$) were considered outliers (greater than three standard deviations away from the mean) and were removed from downstream analyses.

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Developmental phenotypic plasticity - common garden experiment 2

For the second common garden experiment, we used two wild-derived inbred lines each from Brazil (MANA, MANB) and New York (SARA, SARB). Each line was over 10 generations of inbreeding. Equal numbers of males and females were produced for each within-line comparison (n = 656; see Data S4). Full sibs were born at room temperature (21°C) and singly-housed at weaning (~21 days old). After a brief acclimation period, 3.5-week-old mice were randomly assigned into sized matched groups based on sex-specific body mass, and housed at either 5°C or remained at 21°C for the duration of the experiment (~50 days total). Initial body weights and tail lengths were measured, and subsequent weekly body mass and tail lengths were recorded once a week for each mouse. At the end of the experiment, mice were sacrificed at 75 ± 3 days of age, and final body masses and tail lengths were taken, in addition to standard museum measurements. Two final ear lengths were not included in analyses due to ear damage. Skulls and skeletons of all mice were deposited in the Museum of Vertebrate Zoology, University of California, Berkeley. All experimental procedures were in accordance with the UC Berkeley Institutional Animal Care and Use Committee (AUP-2017-08-10248).

Data Analysis

All data analyses and visualizations were completed in R (v. 4.0.3). Within R, we used the tidyverse (v. 1.3.0)(Wickham et al. 2019), performance (v. 0.7.1)(Lüdecke et al. 2021), cowplot (v. 1.1.1), here (v. 1.0.1), and rmarkdown (v. 2.7)(Allaire et al. 2021) packages, along with R base library. Tail length residuals and ear length residuals were calculated by regressing absolute length from body mass. Residuals calculated from body mass consistently performed better (lower Akaike's information criterion (AIC) values across all datasets than residuals calculated from body length. We controlled for the effect of body size on extremity length when testing Allen's rule by including body mass as an additional predictor in all statistical models (Freckleton 2002)). We controlled for sex-biased variation in morphological measurements by including sex as a predictor variable in all models.

We tested for clinal patterns of body mass and extremity length across latitude in wild-caught house mice using Spearman correlations. For common garden experiment one, we fitted a linear model using lm{stats} to predict body mass, tail length, and ear length with sex, population, and generation (formula: (trait) ~ Body Mass + Sex * Population * Environment). The significance of interactions were tested were evaluated using using summary{base} and analysis of variance (ANOVA) based on type III (partial) sums of squares, implemented in the CAR library (v. 3.0.10)(Fox and Weisberg 2019). For common garden experiment two, we fitted a linear mixed model (estimated using restricted maximum like-

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lihood) using lmer{lme4} (v. 1.1.26)(Bates et al. 2015) to predict body mass, tail length, and ear length with sex, population and environment (formula: (trait) ~ Body Mass + Sex * Population * Environment). The model included line as a random effect (formula: ~1 | Line). Results were evaluated using summary{lmerTest} (v. 3.1.3)(Kuznetsova et al. 2017) and Anova{car}. We peformed *post hoc* comparisons on significant two-way interactions using Tukey's HSD tests or Mann-Whitney *U* tests. The code to perform analyses for this study are available as a git-based version control repository on GitHub (https://github.com/malballinger/Ballinger_allenbergmann_XXXX_2021). The analysis can be reproduced using a GNU Make-based workflow with built-in bash tools (v. 3.2.57(1)-release) and R (v. 4.0.3).

Results

Evidence for Bergmann's rule and Allen's rule in wild American house mice

We assessed the relationship between tail length, ear length, body mass, and latitude in mice collected across North and South America to determine if populations of house mice conform to Allen's rule and Bergmann's rule. Using a large dataset downloaded from VertNet (n = 3,018, Data S1), we find weak evidence for Bergmann's rule, as body mass showed a non-significant, positive correlation with latitude across both males and females (Figure 1A). In contrast, we found stronger evidence for Allen's rule in American house mice, with tail length (Figure 1C) and ear length (Figure 1E) showing a significant, negative correlation with latitude. These patterns of extremeity length largely hold true across both sexes (Figure 1C, 1E).

Lack of significant evidence for Bergmann's rule in wild house mice is likely due to the influence of uncontrolled factors (e.g. age, diet, health), environmental effects, or phenotypic plasticity. Although we minimized the inclusion of records of non-adult specimens by removing recorded pregnant females, juveniles, and subadults, we still see large variation across all three traits, likely due to various factors that were not recorded. To reduce this variation, we filtered the VertNet dataset to only include adult males (Figure 1; n = 445). We see strong evidence for both Bergmann's rule (Figure 1B) and Allen's rule (Figure 1D, 1F) across adult, male American house mice, highlighting how variation and noise encompassed in metadata speaks to the difficulty of collating museum metadata to infer broad ecogeographic patterns.

Differences in body mass and tail length persist in a common environment

Phenotypic clines observed across wild house mouse populations could represent genetic differences, phenotypic plasticity, or both. To dissentagnle genetics from plasticity, we collected live mice from the ends of the latitudinal transect (Manaus, Amazonas, Brazil and Saratoga Springs, New York, USA) and brought them into a common laboratory environment. Population-specific differences in body mass in wild-caught mice (N0) persisted across the first two generations of laboratory-reared mice (N1 and N2; Figure 2). Specifically, mice from New York are larger than mice from Brazil (Kruskal–Wallis, $F_{1,439}$=282.54, P<0.001) (Figure 2; Figure S2), and these differences persisted across and within generations (Mann-Whitney U, P < 0.001). Sex-specific differences in body mass were also seen across generations, with males being larger than females (Kruskal–Wallis, $F_{1,439}$=50.79, P<0.001) (Figure 2; Figure S2), and the direction and magnitude of sexual dimorphism was the same among populations of American house mice (stats). The maintance of body mass differences in a common environment and across generations suggests a strong genetic basis in

193 house mice.

194 Extremity length (i.e. tail length and ear length) showed considerable variation across wild-caught mice
195 (N0) and laboratory-reared mice (N1 and N2; Figure 2A). Despite this variation, Brazil mice have longer
196 tails than New York mice (ANCOVA, $F_{,419}$=42.12, P<0.001) (Figure 2B; Figure S2), and these differences
197 have persisted across generations in a common environment (Tukey's HSD, P < 0.001), suggesting a genetic
198 basis for tail length in house mice. Ear length showed the most variation, with no strong trends between
199 populations (Figure 2C; Figure S2). Variation in extremity length could be due to multiple investigators
200 measuring tail length and ear length across generations, or it could reflect the inherent plastic nature of
201 extremity length. For example, although tail length decreases slightly across generations (Figure 2B), it is
202 difficult to discern if this is due to phenotypic plasticity or noise being captured in the metadata. Regard-
203 less, extremity length shows greater variation than body mass, even when measured in a common garden
204 experiment.

205 *Extremity length, and not body size, is greatly influenced by temperature*

206 The results presented above identified phenotypic divergence in body mass and tail length in house mice,
207 with New York mice having shorter tails and larger body sizes than mice from Brazil, consistent with
208 Allen's rule and Bergmann's rule. To determine the influence of phenotypic plasticity on Bergmann's rule
209 and Allen's rule, we performed a second common garden experiment by rearing laboratory-born mice from
210 both populations in a cold environment. We used temperature as the environmental variable as differences
211 in temperature explain the majority of phenotypic variation in house mice across North and South America
212 (Suzuki et al. 2020). Evolved differences in body mass were evident at weaning (Figure 3), with New
213 York mice larger than Brazil mice. These body mass differences persisted across development and sex, and
214 were not influenced by temperature (Figure 3; Figure 5; Figure S3), and recapitulate patterns seen across
215 generations, with New York mice larger than Brazil mice (χ^2=4, P<0.05), and males are larger than females
216 (χ^2=42.15, P<0.001). The lack of plasticity in body mass is not a result of differences in fat accumulation, as
217 body mass index (BMI) does not differ between populations (χ^2=0.50, P>0.05) or environments (χ^2=1.28,
218 P>0.05) (Figure S4; Figure S5). This suggests that phenotypic plasticity plays a modest role in body size
219 evolution of house mice.

220 In contrast to body mass, tail length is greatly influenced by developmental temperature, with the first few
221 weeks post-weaning having the greatest influence on absolute tail length (Figure 4). Specifically, mice
222 reared in a cold enviornment grew shorter tails than mice reared in a warm environment (ANCOVA,
223 χ^2=86.05, P<0.001; Tukey's HSD, P<0.01). Despite developmental temperature playing a signficant role

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in tail length, evolved differences in tail length were evident at the end of the experiment, with Brazil mice growing longer tails than New York mice (ANCOVA, χ^2=11.71, *P*<0.001) (Figure 6; Figure S3). Thus, tail length exhibits both divergence between populations and phenotypic plasticity between environments. Similarly, cold-reared mice grew shorter ears than warm-reared mice (ANCOVA, χ^2=57.66, P<0.001) (Figure 6; Figure S3), with no population-specific differences. These temperature-growth reponses of the extremeites are not a simple consequence of body size modification, as body size does not differ between treatments (Figure 5). Overall, unlike body size, extremity length showed significant plasticity in response to temperature, with mice growing shorter extremities in a cold environment, consistent with patterns of Allen's rule.

Adaptive phenotypic plasticity in extremity length

Differences between warm- and cold-reared mice revealed a strong plastic response to temperature in extremity length. Because plasticity is considered adaptive when a phenotype is altered in the same direction as under selection (**???**), we next asked whether phenotypic plasticity of Brazil mice goes in the same or opposite direction as the evolved response of New York mice. For tail length, the plastic response in Brazil house mice recapitulates the evolved tail length of New York mice (Figure 6A), highlighting an example of adaptive phenotypic plasticity. Interestingly, the plastic response of New York tail length is blunted in comparison to Brazil's (Figure 6A), suggesting that New York house mice are closer to the phenotypic optimum and confer a greater evolved capacity to cold temperatures. Lastly, plasticity in ear length of Brazil house mice goes in the same direction as the plastic response of New York house mice (Figure 6B), again highlighting an example of adaptive phenotypic plasticity. The overall degree and directionality of plasticity in extremities mirrors patterns associated with Allen's rule.

Discussion

We describe phenotypic patterns consisten with Bergmann's rule and Allen's rule in house mice collected across the Americas. We also dissentangle the associated contributions of genetics and plasticity to these ecogeographic patterns. First, we found that wild house mice conform to Bermgann's rule and Allen's rule, as house mice were smallest with the longest extremities in the tropics and increased in size with shortened extremities with increasing latitude. Second, comparisons between temperate and tropical house mice reared in a common environment revealed phenotypic differences corresponding to Bergmann's rule and Allen's rule. Because differences in body mass and tail length persisted in a common environment across many generations indicates a genetic basis and represent divergence between the two populations. These phenotypic differences presumably have arisen as an adpative response to novel thermal environments. Finally, we measured the contributions of phenotypic plasticity underlying these ecogeographic patterns and revealed that extremity length is highly plastic in response to cold temperature, while body size is unaffected. This plastic response in extremity length seems to be adaptive as it goes in the same direction as the evolved response seen in temperate house mice.

Genetic contributions to ecogeographic rules

Parallel phenotypic clines across multiple transects provide strong evidence for natural selection (Endler 1977). Body mass in house mice increases as latitude increases (Figure 1A-B), resembling patterns seen in eastern North America (Lynch 1992; Phifer-Rixey et al. 2018), South America (Suzuki et al. 2020), and Australia (Tomlinson and Withers 2009). These clinal patterns are consistent with Bergmann's rule and reflect an evolved response to differences in environmental temperature. Phenotypic measurements of second- and tenth-generation lab-reared mice from temperate and tropical populations demonstrated a genetically determined difference in body mass (Figure 2A). These results agree with previous studies also finding a genetic basis for Bergmann's rule in house mice from eastern North America (Lynch 1992; Phifer-Rixey et al. 2018), western North America (Ferris et al. 2021), and South America (Suzuki et al. 2020), and suggests that there has been strong selection for body size in house mice. The strong selection over short time scales for Bergmann's rule has also been shown for other non-native species, such as *Drosophila*, which is one of the best-documented examples for a genetic basis of Bergmann's rule. Specifically, body size clines in *Drosophila* have been repeated across continents (refs), in common garden experiments (refs), and through experimental evolution studies (Anderson 1966, 1973; Cavicchi et al. 1985; Partridge et al. 1994a,b). The formation of Bergmann's rule has also occurred rapidly across introduced populations of house sparrows [(???); Packard1967; Baker1980; Fleischer and Johnston 1982; Murphy1885] and starlings (???). These results

suggest that introduced species may experience strong selection as they rapidly expand their geographic range into new environments, and thus clines such as Bergmann's rule are able to establish rapidly.

Patterns for Allen's rule were consistent for tail length only, with mice from the equator having longer tails than mice from higher latitudes (Figure 1C-D). These patterns are recapitualed in the lab and across generations, demonstrating a genetic basis for tail length (Figure 2B). Shorter tails in northern populations of house mice likely confers selection for heat conservation and adaptation to the cold, as tail length shows a positive correlation with tempeature of the coldest month across rodents (Alhajeri et al. 2020). Similar trends and correlations have also been found for appendage length and bill length in birds (REFs). Alternative mechanisms for Allen's rule have been postulated to explain why longer tails are found in the tropics, such as increased climbing ability associated arboreal environments (**???**; Mincer and Russo 2020). Because house mice are commensal with humans, it is unlikely that longer tails confer a climbing advantage in the tropics, though this warrants future investigation. Furthermore, unlike tail length, we do not see a genetic basis for ear length in American hosue mice (Figure 2C). Although ear length shows a negative correlation with latitude (Figure 1E-F), this is likely driven by geographic variation in precipitation rather than temperature (Alhajeri et al. 2020). Again, this result highlights alternative mechanisms of Allen's rule, such as primary productivity, and not just thermoregulation (James 1970; Alhajeri and Steppan 2016).

Contributions of phenotypic plasticity to ecogeographic rules

Body size in house mice shows very little plasticity in response to cold temperature (Figures 3 and 5), reaffirming that there has likely been strong selection for body size in house mice. Lack of plasticity associated with Bergmann's rule in house mice is consistent with previous studies (Sumner refs; Ashoub; Serrat 2008; Serrat 2013) and may be due to a number of physiological factors. First, the environmental influence of cold temperature may need to occur pre-weaning or prenatal to elicit a plastic response, as seen in previous studies of mammals and birds (refs in serrat paper). Second, temperatures pushing enodtherms outside their thermoneutral zone have repeatadly been shown to reduce body mass [(**???**); (**???**);]. Although the plastic response to warm temperatures will likely be different in Brazil mice compared to New York mice, since Brazil mice are small in body size and thus likely better at dissipating heat (James1970). Furthermore, environmental factors other than temperature, such as food availability, likely elicits a plastic response for body size in house mice (refs), again highlighting alternative mechanisms undelrying Bergmann's rule other than temperature (refs). Finally, traits that confer immediate heat conservation, such as increased fur insulation, may be an intial plastic response associated with Bergmann's rule and not body size itself (Scholander 1955).
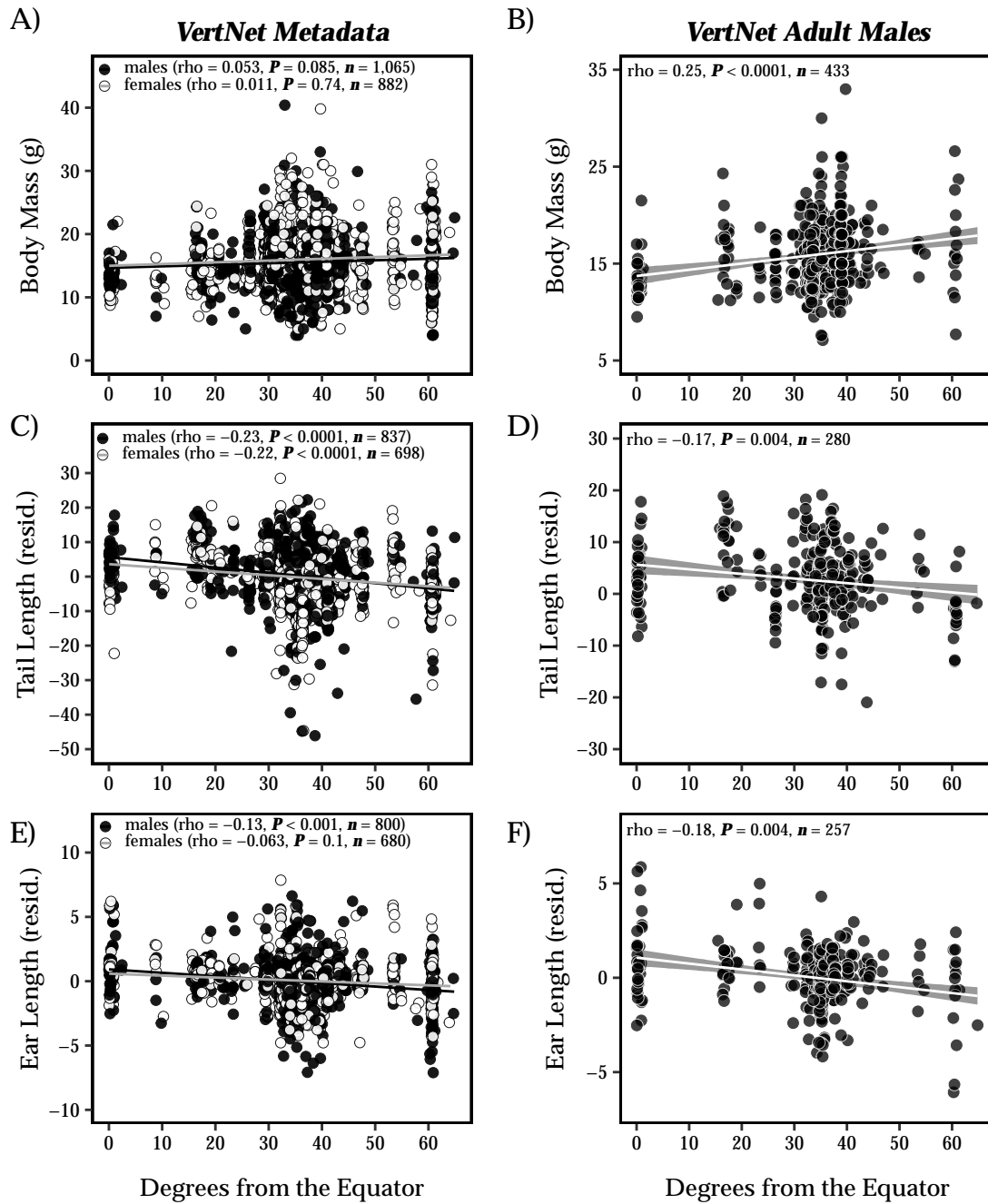
Unlike Bergmann's rule, Allen's rule can solely be generated via phenotypic plasticity, as extremity length is highly senstive to ambient temperature in both mammals and birds (REFs). For example, bill length, a major thermoregulatory organ of birds (Tattersal 2009), responds negatively to cold temperatures across populations (refs), presumably as a strategy for heat conservation. Additionally, numerous studies have documented the plastic nature of tail length and ear length in laboratory mice, with both traits responding negatively to cold temperatures (refs). Our results in wild house mice agree with previous studies on laboratory mice, with mice growing shorter tails and ears in a cold environment (Figure 6). This plastic nature of tail length is also seen at the skeletal level, with both the length and number of caudal vertebrae decreasing in response to cold temperatures (refs). Cold temperature direclty effects the growth of cartilage in both tails and ears, and thus modulates temperature within developing cartilage, impacting extremity length (???). Although we did not measure skeletal differences among mice, it is likely that the tail length plasticity we observed is a result of plasticity in both number and length of individual caudal vertebrae, as seen in other studies of wild mice (Thorington 1977; Ashboud).

Adaptive phenotypic plasticity and Allen's rule

Measuring phenotypic plasticity within and among locally adapted populations allows us to discern the directionality of plasticity underlying Allen's rule. Because extremities are strongly influenced by ambient temperature, plasticity may play an initial role in generating Allen's rule. Adaptive plasticity is expected to align with the direction of selection, moving traits closer to the optima (Ghalambor 2007). Our finding that plastic responses of tail length and ear length in Brazil house mice align with evolved responses of New York house mice is within the context of adaptive phenotypic plasticity (Ghalamobor 2007; Partridge 1994; Evolution). Specifically, both selection and plasticity produce shorter tails and ears at lower temperatures. The plastic response of Brazil tail length matches the evolved response of New York mice, suggesting that phenotypic plasticity moves mice to the optimum trait value. Although there has likely been directional selection in both Brazil mice and New York mice, the plastic responses observed here are likely similar to the ancestral plastic response, given the universal response of extremities to temperature in mice (reviewed in Serrat, 2014).

Furthermore, we expected that New York house mice would have an attentuted response to the cold environment as they have evolved in a temperate environment, regularly undergoing seasonal exposure to regular periods of cold during the winter. In line with this, we observed a canalized plastic response for tail length in New York house mice, likely reflecting New York mice being close to the phenotypic optimum. This canalized response may also be due to skeletal constraint, as the number and length of caudal verte-

brae have been shown to be affected by temperature (refs). Moreover, genetic assimilation may have likely played a role in generating shorter tails in colder climates (EXPAND UPON). Overall, the plastic responses of extremity length mirror evolved patterns of Allen's rule, and thus may play an important first step in generating ecogeographic pattern and allowing house mice and other non-native species are able to expand their geographic range into new enviornments rapidly.
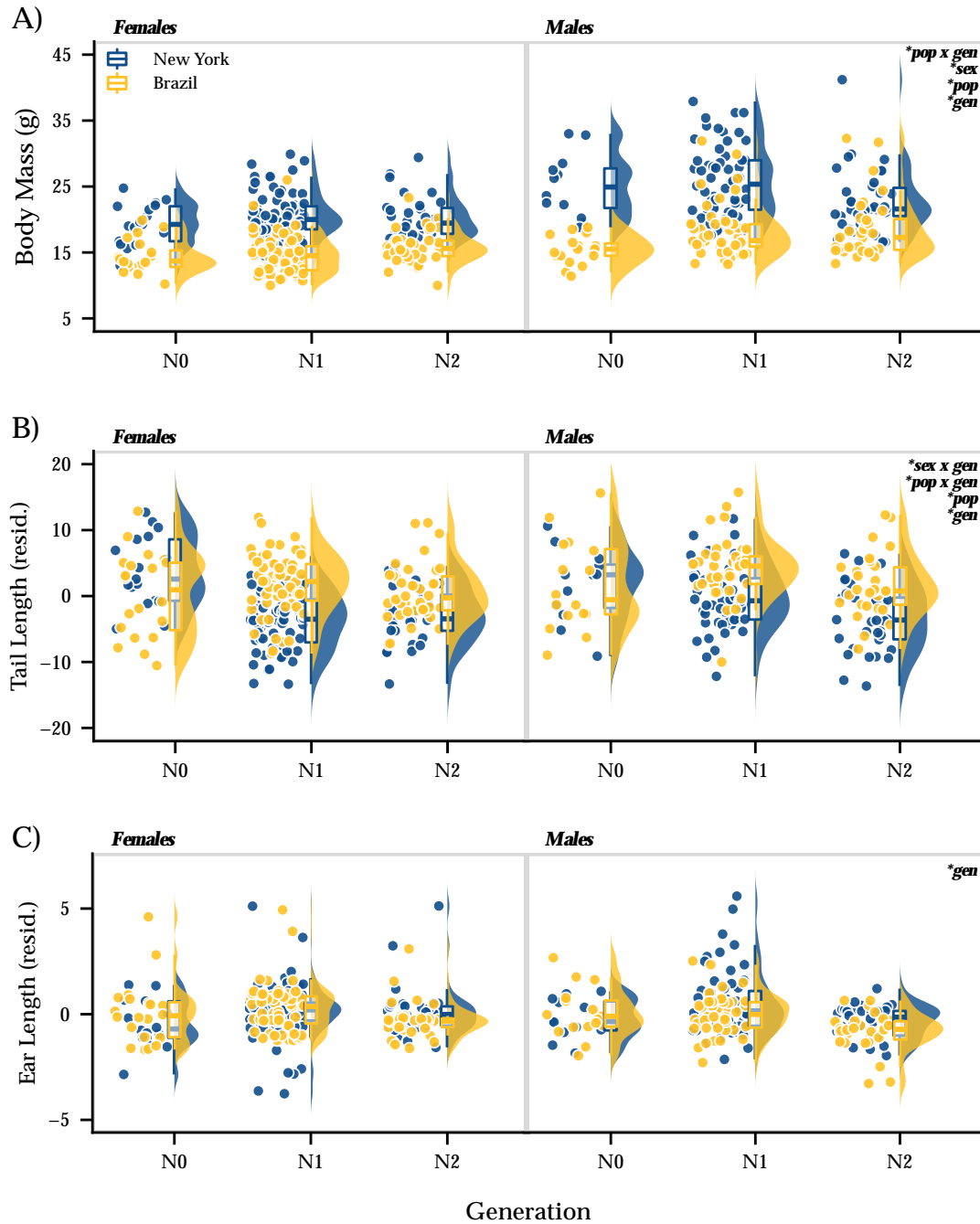
Figures

A)

VertNet Metadata

- males (rho = 0.053, *P* = 0.085, *n* = 1,065)
- females (rho = 0.011, *P* = 0.74, *n* = 882)

B)

VertNet Adult Males

rho = 0.25, *P* < 0.0001, *n* = 433

C)

- males (rho = −0.23, *P* < 0.0001, *n* = 837)
- females (rho = −0.22, *P* < 0.0001, *n* = 698)

D)

rho = −0.17, *P* = 0.004, *n* = 280

E)

- males (rho = −0.13, *P* < 0.001, *n* = 800)
- females (rho = −0.063, *P* = 0.1, *n* = 680)

F)

rho = −0.18, *P* = 0.004, *n* = 257

Degrees from the Equator

Degrees from the Equator

Figure 1. Bergmann's rule and Allen's rule in American house mice. The relationship between body mass (A-B), tail length (C-D), ear length (E-F) and absolute latitude across wild-caught North and South American house mice. Tail length and ear length are plotted as the residuals of a regression of body mass on extremity length. Individuals are represented as individual points, with males denoted as black and females denoted as white. Results from Spearman correlations are presented in each plot, along with sample sizes.

For clarity, standard error shading is ommitted from linear regression lines associated with the VertNet Metadata panels (A,C,E).

Figure 2. Body mass and tail length differences among populations persist over generations in a common lab environment. Tail length and ear length are plotted as the residuals of a regression of body mass on extremity length. Population-level data are depticted as boxplots overlayed on density plots, with boxplot vertical lines denoting 1.5x the inerquartile range. Individuals are represented as individual points, and the horizontal variation within each generation determined randomly to separate points. The New York population is represented in blue and the Brazil population represented in gold. Results from linear models

359 are presented in each plot. Sample sizes: (A) n = 441; (B) n = 432; (C) n = 434.
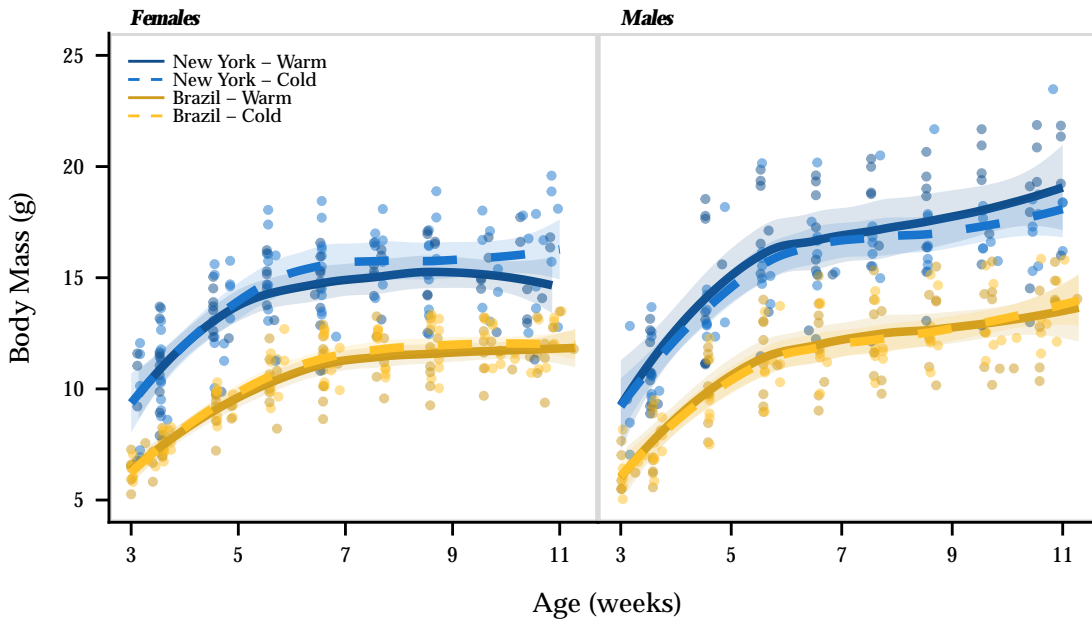
Figure 3. Evolved differences in body mass across development. Body mass growth trajectories across environments in New York (blue) and Brazil (gold) house mice. Within populations, cold-reared mice (dotted lines) and warm-reared mice (solid lines) have the same body mass across development, while males grow larger than females. Individuals are plotted as semi-transparent points (n = 80), with population means depicted as smoothed regression fits, with standard error shading. The same individuals depicted here are also depicted in Figure 5.
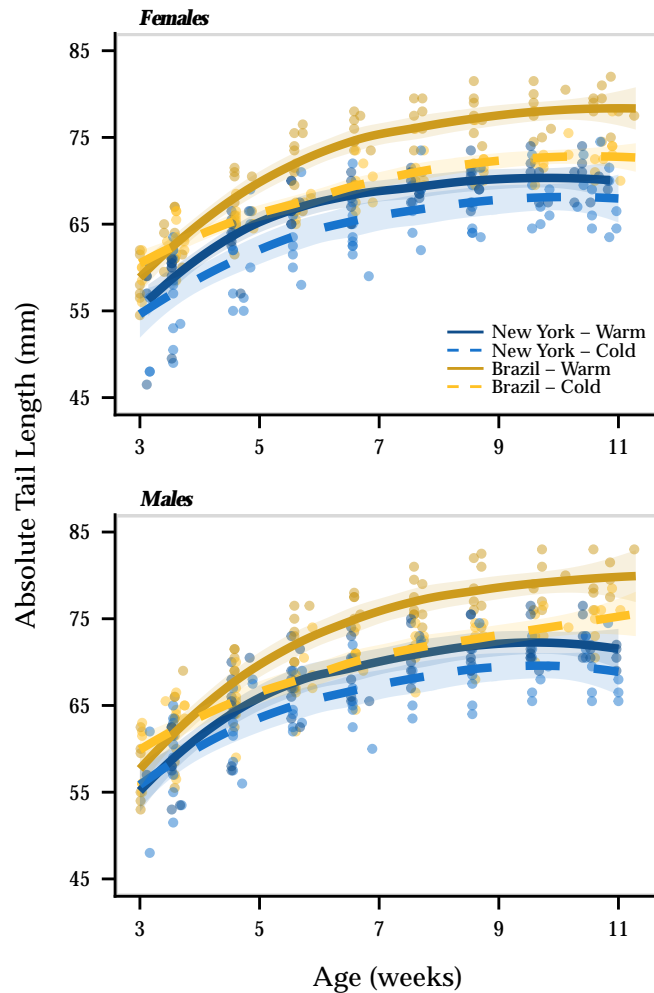
Figure 4. Tail length is highly influenced by cold temperature across development. Abslute tail length growth trajectories across environments in New York (blue) and Brazil (gold) house mice. Cold-reared mice (dotted lines) grow shorter tails compared to warm-housed mice (solid lines). Both New York and Brazil house mice show plasticity in response to temperature across tail development. Individuals are plotted as semi-transparent points (n = 80), with population means depicted as smoothed regression fits, with standard error shading. The same individuals depicted here are also depicted in Figure 6.

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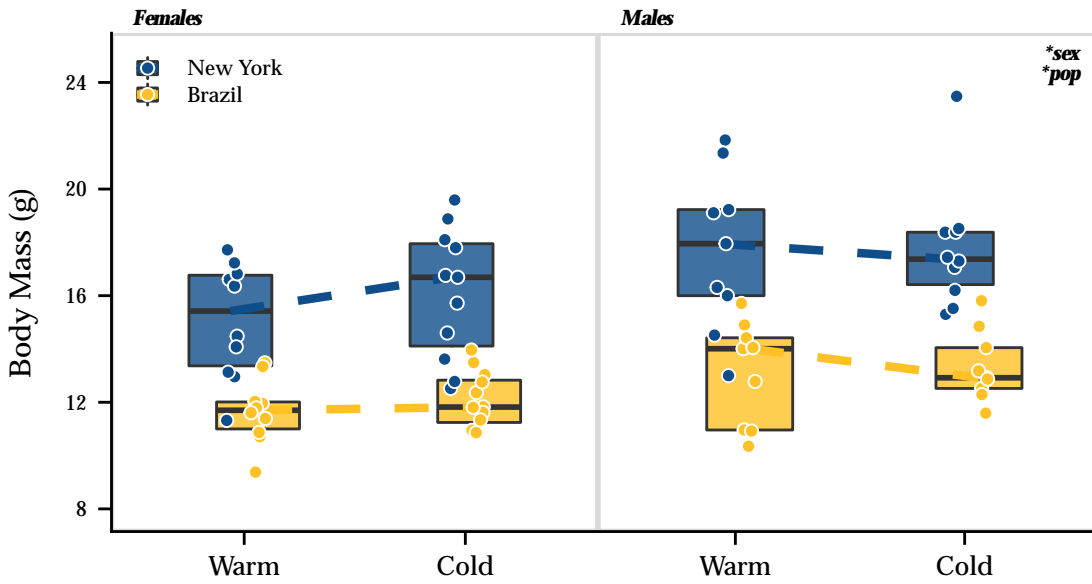
Figure 5. Evolved variation and very little plasticity in body size among New York and Brazil house mice. Sexual dimorphism and strong genetic components underlie body size differences among New York (blue) and Brazil (gold) hosue mice. There is very little plasticity in body mass in response to cold temperature. Individuals are represented as individual points (n = 80). Boxplots denote the 25th, median, and 75th quartiles. Results from linear mixed models are presented in each plot. The same individuals depicted here are also depicted in Figure 3.

22
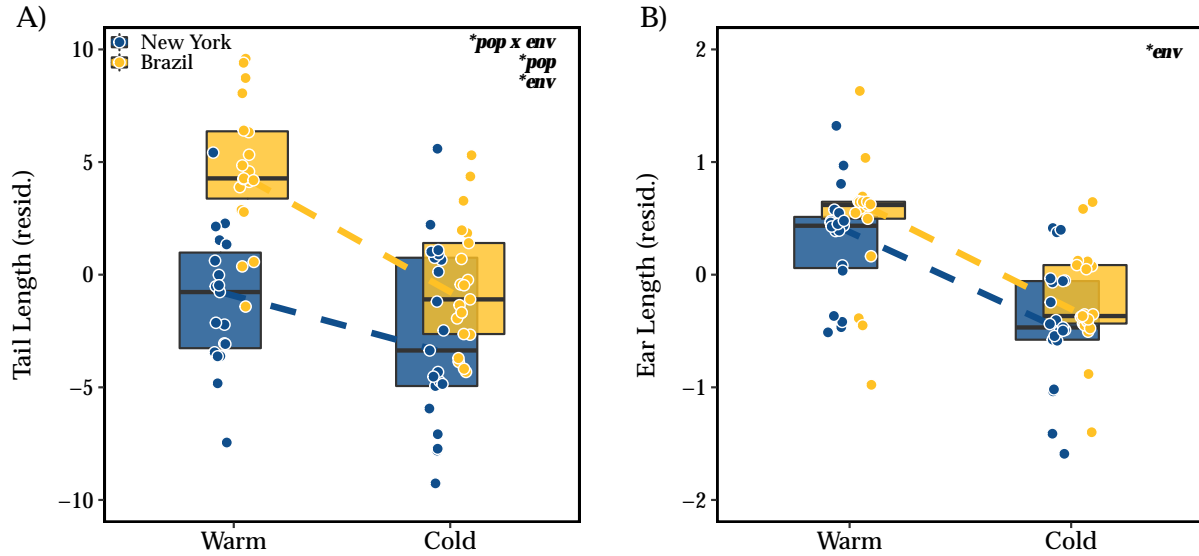
Figure 6. Adaptive phenotypic plasticity in extremity length among New York and Brazil house mice.
Evolved differences in tail length among New York (blue) and Brazil (gold) house mice, with warm-reared
Brazil mice having longer tails than warm-reared New York mice. Both tail length and ear length show
significant plasticity in both populations, with tails and ears growing shorter in the cold. The plastic re-
sponse of Brazil house mice goes in the same direction as the evovled and plastic responses of New York
house mice, highlighting an example of adaptive plasticity in extremity length. Tail length and ear length
residuals were calculated by regressing from body mass. Individuals are represented as individual points
(tail length residuals: n = 80; ear length residuals: n = 78). Boxplots denote the 25th, median, and 75th
quartiles. Both sexes were combined as there were no sex-specific differences in extremity length. Results
from linear mixed models are presented in each plot. The same individuals depicted here are also depicted
in Figure 4.

Acknowledgements

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