

Adaptive phenotypic plasticity underlies Allen's rule, but not Bergmann's rule, in wild house mice

Mallory A. Ballinger and Michael W. Nachman[†]

Department of Integrative Biology
Museum of Vertebrate Zoology
University of California, Berkeley
Berkeley, CA 94702-3160

[†] To whom orrespondence should be addressed:
mnachman@berkeley.edu

Running title: Adaptive plasticity and Allen's rule

Keywords:

Article type: Major article

Total words:

Number of figures: 6

Number of supplementary figures: 4

The authors wish to be identified to the reviewers.

Abstract (200 words)

Distinguishing between genetic, environmental, and genotype-by-environment effects is 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 inter- and intraspecific evidence for both geographic patterns, 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 house mice (*Mus musculus domesticus*). We find a genetic basis for Bergmann's rule and Allen's rule in house mice, as mice from colder climates are larger with shorter tails than mice from tropical environments. We found very little plasticity associated with body size, suggesting that Bergmann's rule has been shaped by strong directional selection in house mice. Extremities show considerable plasticity in response to cold, as both tails and ears grow shorter in cold environments. The plastic responses of house mouse extremities mirror general evolutionary patterns of shorter extremities in colder environments, demonstrating an example of adaptive phenotypic plasticity. Together, our study suggests that adaptive plasticity underlying Allen's rule, in combination with strong selection for Bergmann's rule, likely facilitated the rapid expansion of house mice into new environments across the Americas.

Introduction

Clines in phenotypes have historically been attributed to natural selection and reflect adaptation to local environments (Huxley 1939; Endler 1977). Two of the most well described phenotypic clines are Allen's rule and Bergmann's rule. Allen's rule predicts shorter extremities in colder climates compared to warmer regions, producing latitudinal clines in extremity length (Allen 1877). Bergmann's rule predicts larger body sizes in colder climates compared to warmer habitats, generating latitudinal clines in body size (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 (Johnston and Selander 1964; James 1970; Laiolo and Rolando 2001; Romano et al. 2020) and mammals (Brown and Lee 1969; Griffing 1974; Yom-Tov and Nix 1986; Fooden and Albrecht 1999), including humans (Ruff 1994, 2002; Foster and Collard 2013; Betti et al. 2015). Moreover, various meta-analyses have supported (Ashton et al. 2000; Ashton 2002; Freckleton et al. 2003; Meiri and Dayan 2003; Blackburn and Hawkins 2004; Millien 2006; Olson 2009; Symonds and Tattersall 2010; Nudds and Oswald 2007) or refuted (McNab 1971; Geist 1987; Gohli and Voje 2016; Riemer et al. 2018) 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 inconsistencies 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 (Falconer and Mackay 1996; Lynch et al. 1998). 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 interactions (Conover and Schultz 1995; Alho et al. 2011). 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 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 and direction of plasticity they exhibit in response to environmental stimuli. Variation in plasticity and genotype-by-environment interactions may facilitate adaptation and divergence in polygenic traits (Via and Lande 1985; Gillespie and Turelli 1989; Gomulkiewicz and Kirkpatrick 1992; West-Eberhard 2003). However, controlling for environ-

66 mental effects and measuring the contributions of phenotypic plasticity is difficult, as transplant experi-
67 ments and common garden experiments are infeasible for many taxa. These limitations have impeded our
68 ability to make substantial progress on understanding the evolutionary and ecological mechanisms under-
69 lying Bergmann’s rule and Allen’s rule.

70 House mice (*Mus musculus domesticus*) are a tractable system to disentangle the genetic and environmental
71 contributions underlying complex traits. House mice have recently expanded their range from Western
72 Europe to the Americas, where they can be found from the tip of South America up to Alaska. Across
73 this broad latitudinal range, house mice are exposed to various environmental gradients, from cold, tem-
74 perate environments to warm, tropical and arid environments (Phifer-Rixey and Nachman 2015). Despite
75 house mice residing in these novel environments for only ~500 generations (Phifer-Rixey et al. 2018), there
76 is evidence for clinal adaptation across populations. Specifically, mice in eastern North America follow
77 Bergmann’s rule (Lynch 1992), with larger mice in more northern populations compared to their southern
78 conspecifics. These body size differences persist in a common environment and over many generations,
79 indicating a genetic basis for Bergmann’s rule in house mice (Lynch 1992; Phifer-Rixey et al. 2018). Exper-
80 imental evolution studies of house mice conducted in the laboratory recapitulate these clinal patterns, in
81 which mice bred at lower temperatures become larger and undergo genetic divergence in body size (Bar-
82 nett and Dickson 1984). Furthermore, earlier work revealed an environmental influence on tail length when
83 exposed to cold temperatures. Specifically, house mice reared in a cold environment grew significantly
84 shorter tails than mice reared at warm temperatures, consistent with Allen’s rule (Sumner 1909, 1915; Bar-
85 nett 1965). However, these earlier studies either only investigated a single population of wild house mice
86 or used traditional laboratory strains of mice, making it difficult to place the results in an explicit evolu-
87 tionary framework. We still have little understanding of the phenotypic variation of house mice across their
88 entire latitudinal distribution, and the subsequent contributions of genetics and the environment on these
89 complex traits.

90 Here, we use a combination of approaches to tease apart genetics from plasticity in Bergmann’s rule and
91 Allen’s rule in American house mice. First, we determined if house mice conform to both Bergmann’s rule
92 and Allen’s rule across their entire introduced range by analyzing phenotypic data from wild-caught indi-
93 viduals across North and South America. Second, because it is difficult to disentangle genetics from plas-
94 ticity using wild phenotypic data, we collected temperate and tropical populations of house mice from the
95 ends of their latitudinal distribution, brought them back to the lab, and established wild-derived colonies.
96 We analyzed phenotypic differences between populations and across generations in a common environ-
97 ment 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 extremity length. Measuring developmental plasticity within and between populations allows us to assess the influence of temperature on complex traits and the evolutionary mechanisms underlying these clinal patterns. 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 phenotypic plasticity.

Materials and Methods

Wild-caught phenotypic metadata

To determine if house mice conform to Allen's rule and Bergmann's rule, we tested for associations between body mass, tail length, ear length, and latitude in wild house mice collected across North and South America. We downloaded specimen data of all house mouse records from VertNet (Constable et al. 2010) on October 13, 2020, using the search query: *vn: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 errors. 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, we collected live animals 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 (Phifer-Rixey et al. 2018; 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 21°C with a 12-hr dark and 12-hr light cycle. Commercial rodent chow was provided ad libitum. 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.

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, we randomly assigned 3.5-week-old mice 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). We measured initial body weights and tail lengths and recorded subsequent weekly body mass and tail lengths once a week for each mouse. At the end of the experiment, we sacrificed mice at 75 ± 3 days of age, and recorded final body masses and tail lengths, in addition to standard museum measurements. Two final ear lengths were not included in analyses due to ear damage. We deposited skulls and skeletons of all mice 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)(Lüdtke 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 (i.e., 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 for Allen's rule by including body mass as an additional predictor in all statistical models (Freckleton 2002).

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: $(\text{trait}) \sim \text{Body Mass} + \text{Sex} * \text{Population} * \text{Environment}$). The significance of interactions were tested were evaluated 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 likelihood) 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: $(\text{trait}) \sim \text{Body Mass} + \text{Sex} * \text{Population} * \text{Environment}$). The model included line as a random effect (formula: $\sim 1 \mid \text{Line}$). Results were evaluated using `summary{lmerTest}` (v. 3.1.3)(Kuznetsova et al. 2017) and `Anova{car}`. We performed *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 extremity 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 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 1B,

185 1D, 1F; $n = 445$). We see strong evidence for both Bergmann's rule (Figure 1B) and Allen's rule (Figure 1D,
186 1F) across adult, male American house mice, highlighting how variation and noise encompassed in collated
187 museum metadata makes inferring broad ecogeographic patterns difficult.

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

189 Phenotypic clines observed across wild house mouse populations could represent genetic differences, phe-
190 notypic plasticity, or both. To disentangle genetics from plasticity, we collected live mice from the ends
191 of the latitudinal transect (Manaus, Amazonas, Brazil and Saratoga Springs, New York, USA) and brought
192 them into a common laboratory environment. Population-specific differences in body mass in wild-caught
193 mice (N0) persisted across the first two generations of laboratory-reared mice (N1 and N2; Figure 2A).
194 Specifically, mice from New York are larger than mice from Brazil (Kruskal-Wallis, $F_{1,439}=282.54$, $P<0.001$)
195 (Figure 2A; Figure S1), and these differences persisted across generations (Mann-Whitney U , $P < 0.001$).
196 Sex-specific differences in body mass were also seen across generations, with males larger than females
197 (Kruskal-Wallis, $F_{1,439}=50.79$, $P<0.001$) (Figure 2A; Figure S1), and the direction and magnitude of sexual
198 dimorphism was the same among New York and Brazil house mice. The maintenance of body mass differ-
199 ences in a common environment and across generations suggests a strong genetic basis in house mice.

200 Extremity length (i.e. tail length and ear length) showed considerable variation across wild-caught mice
201 (N0) and laboratory-reared mice (N1 and N2; Figure 2B-C). Despite this variation, Brazil mice have longer
202 tails than New York mice (ANCOVA, $F_{1,419}=42.12$, $P<0.001$) (Figure 2B; Figure S1), and these differences
203 have persisted across generations in a common environment (Tukey's HSD, $P < 0.001$), suggesting a genetic
204 basis for tail length in house mice. Ear length showed the most variation, with no strong trends between
205 populations (Figure 2C; Figure S1). Variation in extremity length could be due to multiple investigators
206 measuring tail length and ear length across generations, or it could reflect plasticity in extremity length.
207 For example, although tail length decreases slightly across generations (Figure 2B), it is difficult to discern
208 if this is due to phenotypic plasticity or noise being captured in the metadata. Regardless, extremity length
209 shows greater variation than body mass, even when measured in a common garden experiment.

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

211 The results presented above identified phenotypic divergence in body mass and tail length in house mice,
212 with New York mice having shorter tails and larger body sizes than mice from Brazil, consistent with Allen's
213 rule and Bergmann's rule, respectively. To determine the influence of phenotypic plasticity on Bergmann's

rule and Allen's rule, we performed a second common garden experiment by rearing laboratory-born mice from both populations in a cold environment. We used temperature as the environmental variable as environmental temperature is highly correlated with latitude (Millien et al. 2006) and phenotypic variation in wild house mice across North and South America is explained most by temperature-related variables (Suzuki et al. 2020). Evolved differences in body mass were evident at weaning (Figure 3), with New York mice larger than Brazil mice. These body mass differences persisted across development and sex and were not influenced by temperature (Figure 3). At the end of the experiment, body size differences recapitulated patterns seen across generations, with New York mice larger than Brazil mice ($\chi^2=4$, $P<0.05$) and males larger than females ($\chi^2=42.15$, $P<0.001$) (Figure 5, Figure S2). The lack of plasticity in body mass is not a result of differences in fat accumulation, as body mass index (BMI) does not differ between populations ($\chi^2=0.50$, $P>0.05$) or environments ($\chi^2=1.28$, $P>0.05$) (Figure S3; Figure S4). This suggests that phenotypic plasticity plays a modest role in body size evolution of house mice.

In contrast to body mass, tail length is greatly influenced by developmental temperature, with the first few weeks post-weaning having the greatest influence on absolute tail length (Figure 4). Specifically, mice reared in a cold environment grew shorter tails than mice reared in a warm environment (ANCOVA, $\chi^2=86.05$, $P<0.001$; Tukey's HSD, $P<0.01$) (Figure 6A). Despite developmental temperature playing a significant role 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, $\chi^2=11.71$, $P<0.001$) (Figure 6A; Figure S2). Thus, tail length exhibits both evolved divergence and phenotypic plasticity. Similarly, cold-reared mice grew shorter ears than warm-reared mice (ANCOVA, $\chi^2=57.66$, $P<0.001$) (Figure 6B; Figure S2), with no population-specific differences. These temperature-growth responses of the extremities are not a simple consequence of body size modification, as body mass 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 natural selection (Ghalambor et al. 2007), 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 attenuated in comparison to Brazil's (Figure 6A), suggesting that New York house mice are close to the phenotypic optimum or there is a developmental limitation on tail length. 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), further illustrating adaptive phenotypic plasticity. The overall degree and directionality of plasticity in extremities mirror patterns associated with Allen's rule.

Discussion

We described phenotypic patterns consistent with Bergmann's rule and Allen's rule in house mice collected across the Americas. We also disentangled the contributions of genetics and plasticity to these ecogeographic patterns. First, we found that wild house mice conform to Bergmann's rule and Allen's rule, as house mice are larger in size with shortened extremities in northern latitudes. Second, persistent differences in body mass and tail length in a common environment across many generations indicated a genetic basis to Bergmann's rule and Allen's rule, reflecting thermoregulatory adaptations. 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 to cold temperatures appears adaptive, matching the direction and magnitude of the evolved response in temperate house mice. Adaptive plasticity associated with Allen's rule, in conjunction with strong selection for body size, likely promoted the rapid expansion of house mice into new environments across the Americas.

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 revealed a genetically determined difference in body mass, with mice from colder climates significantly larger than mice from tropical environments (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 suggest that there has been strong directional selection for body size in house mice. Selection over short time scales for Bergmann's rule has also been shown for other non-native species, such as *Drosophila*, 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, in common garden experiments, and through experimental evolution studies (Cavicchi et al. 1985; Coyne and Beecham 1987; Partridge et al. 1994; James et al. 1995; Land et al. 1999; Huey et al. 2000; Gilchrist et al. 2001, 2004). The formation of Bergmann's rule has also occurred rapidly across introduced populations of house sparrows (Johnston and Selander 1964, 1971) and starlings (Cardilini et al. 2016). Together, these results suggest that introduced species may experience strong selection as they expand their geographic range into new environments, allowing clines such as Bergmann's rule 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 northern latitudes (Figure 1C-D). These patterns are recapitulated in the lab and across generations, demonstrating a genetic basis for tail length (Figure 2B). Shorter tails in northern populations of house mice likely confer selection for heat conservation and adaptation to the cold, as tail length shows a positive correlation with temperature of the coldest month across rodents (Alhajeri et al. 2020). Similar trends and correlations have also been found for limb length and bill length in birds (Nudds and Oswald 2007; Symonds and Tattersall 2010; Danner and Greenberg 2015; Friedman et al. 2017). In addition to thermoregulatory advantages, alternative mechanisms for Allen's rule have been postulated to explain why longer tails are found in the tropics, such as enhanced climbing ability with increased arboreality (Alroy 2019; 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, we do not see a genetic basis for ear length in American house mice (Figure 2C). Although ear length shows a negative correlation with latitude (Figure 1E-F), this is likely driven by plasticity.

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 directional selection for body size in house mice. Lack of plasticity associated with Bergmann's rule is consistent with previous studies in laboratory mice (Sumner 1909, 1915; Ashoub 1958; Serrat et al. 2008; Serrat 2013) and, in addition to selection, may be due to a number of physiological factors. First, the environmental influence of temperature may need to occur pre-weaning or prenatal to elicit a plastic response (e.g., Weaver and Ingram 1969). Second, temperatures pushing endotherms outside their thermoneutral zone have repeatedly been shown to reduce body mass

(Ashoub 1958; Gordon 2012; Burness et al. 2013; Andrew et al. 2017), suggesting that both Brazil mice and New York mice would also show reductions in body mass. Lastly, traits that provide flexible and immediate heat conservation, such as increased fur insulation (e.g., Sumner (1915); Weaver and Ingram (1969)), may be an initial phenotypic response associated with Bergmann's rule, and not body size itself.

Unlike Bergmann's rule, Allen's rule can be generated via developmental phenotypic plasticity, as extremity length is highly sensitive to ambient temperature in both mammals and birds (Serrat 2014; Tattersall et al. 2017). Our results in wild house mice agree with previous studies in mammals, with mice growing shorter tails and ears in a cold environment (Figure 6; Ogle and Mills (1933); Harland (1960); Chevillard et al. (1963); Weaver and Ingram (1969)). In laboratory mice, temperature directly affects the growth of cartilage in both tails and ears, impacting extremity length (Serrat et al. 2008). Furthermore, the universal patterns of tail length plasticity in response to cold is also recapitulated at the skeletal level, with both the length and number of caudal vertebrae decreasing in response to cold temperatures in mice (Barnett 1965; Noel and Wright 1970; Thorington Jr 1970; Al-Hilli and Wright 1983). Although we did not measure skeletal differences between New York and Brazil 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. Moreover, ear length shows the greatest plasticity in both populations, with both New York and Brazil mice growing shorter ears in the cold. The pronounced plastic response of ears compared to tails may indicate that smaller appendages consisting entirely of cartilage are less developmentally canalized. Less constraint associated with extremities may also underlie the highly plastic nature of Allen's rule compared to Bergmann's rule.

Adaptive phenotypic plasticity and Allen's rule

Phenotypic plasticity is adaptive when it aligns with the direction of selection, moving traits closer to the local phenotypic optima (Baldwin 1896; West-Eberhard 2003; Ghalambor et al. 2007). We show evidence for adaptive phenotypic plasticity underlying Allen's rule, as plasticity produces shorter ears and tails in cold environments. We also observed an attenuated plastic response for tail length in New York house mice, suggesting New York mice are closer to the phenotypic optimum and are better adapted to colder environments. Overall, plasticity in house mouse extremities mirror general evolutionary patterns of shorter extremity lengths in colder climates and may play an important role in generating Allen's rule.

There are two outcomes by which adaptive phenotypic plasticity can facilitate adaptive evolution. Adaptive plasticity can incompletely move the trait value closer to the phenotypic optimum, with directional selection refining the trait value, leading to subsequent genetic changes (Price et al. 2003; Ghalambor et

al. 2007). Or, adaptive plasticity can slow or impede evolution by moving individuals completely to the phenotypic optimum, shielding genetic variation from natural selection (Price et al. 2003; Ghalambor et al. 2007). Intriguingly, we find evidence for each of these two scenarios in different house mouse extremities. In tails, we see evidence for the first scenario, with both genetic and plastic contributions generating shorter tails in colder environments. Despite the plastic response of tail length in Brazil mice recapitulating the magnitude of the evolved response of New York mice, we see clear evidence of genetic differences in tail length between New York and Brazil house mice. This suggests that phenotypic plasticity moves tail length close to the local optimum but does not shield it from subsequent selection. However, in ears, we see no evidence for genetic differences among tropical and temperate house mouse populations, indicating that the observed plastic response to cold temperatures may shield ear length from selection and fully account for observed ear length differences among populations and across latitude. Overall, both scenarios of adaptive phenotypic plasticity with Allen’s rule, in addition to strong, directional selection underlying Bergmann’s rule, likely facilitated the rapid expansion of house mice into new environments across the Americas.

Acknowledgements

We are extremely thankful to Kathleen Ferris, Gabriela Heyer, Dana Lin, Felipe Martins, Megan Phifer-Rixey, Michael Sheehan, and Taichi Suzuki for collecting wild house mice, establishing wild-derived mouse colonies, and maintaining colonies. M.A.B was supported by a National Science Foundation Graduate Research Fellowship (DGE 1106400), Junea W. Kelly MVZ Graduate Fellowship, and UC Berkeley Philomathia Graduate Fellowship. This work was supported by MVZ and IB graduate student research funds to M.A.B and an NIH grant to M.W.N. (R01GM127468).

References

- Alhajeri, B. H., Y. Fourcade, N. S. Upham, and H. Alhaddad. 2020. A global test of Allen’s rule in rodents. *Global Ecology and Biogeography* 29:2248–2260.
- Al-Hilli, F., and E. Wright. 1983. The effects of changes in the environmental temperature on the growth of bone in the mouse. Radiological and morphological study. *British Journal of Experimental Pathology*

64:43.

- Alho, J., G. Herczeg, A. Laugen, K. Räsänen, A. Laurila, and J. Merilä. 2011. Allen's rule revisited: Quantitative genetics of extremity length in the common frog along a latitudinal gradient. *Journal of Evolutionary Biology* 24:59–70.
- Allaire, J., Y. Xie, J. McPherson, J. Luraschi, K. Ushey, A. Atkins, H. Wickham, et al. 2021. Rmarkdown: Dynamic documents for r.
- Allen, J. A. 1877. The influence of physical conditions in the genesis of species. *Radical Review* 1:108–140.
- Alroy, J. 2019. Small mammals have big tails in the tropics. *Global Ecology and Biogeography* 28:1042–1050.
- Andrew, S., L. Hurley, M. Mariette, and S. Griffith. 2017. Higher temperatures during development reduce body size in the zebra finch in the laboratory and in the wild. *Journal of Evolutionary Biology* 30:2156–2164.
- Ashoub, M. E.-R. 1958. Effect of two extreme temperatures on growth and tail-length of mice. *Nature* 181:284–284.
- Ashton, K. G. 2002. Patterns of within-species body size variation of birds: Strong evidence for bergmann's rule. *Global Ecology and Biogeography* 11:505–523.
- Ashton, K. G., M. C. Tracy, and A. de Queiroz. 2000. Is bergmann's rule valid for mammals? *The American Naturalist* 156:390–415.
- Baldwin, J. M. 1896. A new factor in evolution. *The American Naturalist* 30:441–451.
- Barnett, S. 1965. Genotype and environment in tail length in mice. *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences: Translation and Integration* 50:417–429.
- Barnett, S., and R. Dickson. 1984. Changes among wild house mice (*mus musculus*) bred for ten generations in a cold environment, and their evolutionary implications. *Journal of Zoology* 203:163–180.
- Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67:1–48.
- Bergmann, C. 1847. Über die verhältnisse der wärmeökonomie der thiere zu ihrer grösse. *Gottinger Studien* 3:595–708.
- Betti, L., S. J. Lycett, N. von Cramon-Taubadel, and O. M. Pearson. 2015. Are human hands and feet affected by climate? A test of allen's rule. *American Journal of Physical Anthropology* 158:132–140.

388 Blackburn, T. M., and B. A. Hawkins. 2004. Bergmann's rule and the mammal fauna of northern north
389 america. *Ecography* 27:715–724.

390 Brown, J. H., and A. K. Lee. 1969. Bergmann's rule and climatic adaptation in woodrats (*neotoma*). *Evolu-*
391 *tion* 329–338.

392 Burness, G., J. R. Huard, E. Malcolm, and G. J. Tattersall. 2013. Post-hatch heat warms adult beaks: Ir-
393 reversible physiological plasticity in japanese quail. *Proceedings of the Royal Society B: Biological*
394 *Sciences* 280:20131436.

395 Cardilini, A. P., K. L. Buchanan, C. D. Sherman, P. Cassey, and M. R. Symonds. 2016. Tests of ecogeograph-
396 ical relationships in a non-native species: What rules avian morphology? *Oecologia* 181:783–793.

397 Cavicchi, S., D. Guerra, G. Giorgi, and C. Pezzoli. 1985. Temperature-related divergence in experimental
398 populations of *drosophila melanogaster*. I. Genetic and developmental basis of wing size and shape
399 variation. *Genetics* 109:665–689.

400 Chevillard, L., R. Portet, and C. M. 1963. Growth rate of rats born and reared at 5 and 30 c. *Federation*
401 *Proceedings* 22:699–703.

402 Conover, D. O., and E. T. Schultz. 1995. Phenotypic similarity and the evolutionary significance of counter-
403 gradient variation. *Trends in Ecology & Evolution* 10:248–252.

404 Constable, H., R. Guralnick, J. Wieczorek, C. Spencer, A. T. Peterson, V. S. Committee, and others. 2010.
405 VertNet: A new model for biodiversity data sharing. *PLoS Biology* 8:e1000309.

406 Coyne, J. A., and E. Beecham. 1987. Heritability of two morphological characters within and among natural
407 populations of *drosophila melanogaster*. *Genetics* 117:727–737.

408 Danner, R. M., and R. Greenberg. 2015. A critical season approach to allen's rule: Bill size declines with
409 winter temperature in a cold temperate environment. *Journal of Biogeography* 42:114–120.

410 Endler, J. A. 1977. *Geographic variation, speciation, and clines*. Princeton University Press.

411 Falconer, D. S., and T. F. Mackay. 1996. *Introduction to quantitative genetics* (Vol. 1). Pearson.

412 Ferris, K. G., A. S. Chavez, T. A. Suzuki, E. J. Beckman, M. Phifer-Rixey, K. Bi, and M. W. Nachman. 2021.
413 The genomics of rapid climatic adaptation and parallel evolution in north american house mice. *PLoS*
414 *Genetics* 17:e1009495.

415 Fooden, J., and G. H. Albrecht. 1999. Tail-length evolution in fascicularis-group macaques (*cercopithecidae*:
416 *Macaca*). *International Journal of Primatology* 20:431–440.

417 Foster, F., and M. Collard. 2013. A reassessment of bergmann's rule in modern humans. *PloS One* 8:e72269.

418 Fox, J., and S. Weisberg. 2019. *An R companion to applied regression* (Third.). Sage, Thousand Oaks CA.

419 Freckleton, R. P. 2002. On the misuse of residuals in ecology: Regression of residuals vs. Multiple regression.
420 *Journal of Animal Ecology* 71:542–545.

421 Freckleton, R. P., P. H. Harvey, and M. Pagel. 2003. Bergmann's rule and body size in mammals. *The*
422 *American Naturalist* 161:821–825.

423 Friedman, N. R., L. Harmáčková, E. P. Economo, and V. Remeš. 2017. Smaller beaks for colder winters:
424 Thermoregulation drives beak size evolution in australasian songbirds. *Evolution* 71:2120–2129.

425 Geist, V. 1987. Bergmann's rule is invalid. *Canadian Journal of Zoology* 65:1035–1038.

426 Ghalambor, C. K., J. K. McKay, S. P. Carroll, and D. N. Reznick. 2007. Adaptive versus non-adaptive
427 phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional*
428 *Ecology* 21:394–407.

429 Gilchrist, G. W., R. B. Huey, J. Balanyà, M. Pascual, and L. Serra. 2004. A time series of evolution in action:
430 A latitudinal cline in wing size in south american drosophila subobscura. *Evolution* 58:768–780.

431 Gilchrist, G. W., R. B. Huey, and L. Serra. 2001. Rapid evolution of wing size clines in drosophila subob-
432 scura. *Genetica* 273–286.

433 Gillespie, J. H., and M. Turelli. 1989. Genotype-environment interactions and the maintenance of polygenic
434 variation. *Genetics* 121:129–138.

435 Gohli, J., and K. L. Voje. 2016. An interspecific assessment of bergmann's rule in 22 mammalian families.
436 *BMC Evolutionary Biology* 16:1–12.

437 Gomulkiewicz, R., and M. Kirkpatrick. 1992. Quantitative genetics and the evolution of reaction norms.
438 *Evolution* 46:390–411.

439 Gordon, C. 2012. Thermal physiology of laboratory mice: Defining thermoneutrality. *Journal of Thermal*
440 *Biology* 37:654–685.

441 Griffing, J. P. 1974. Body measurements of black-tailed jackrabbits of southeastern new mexico with impli-
442 cations of allen's rule. *Journal of Mammalogy* 55:674–678.

443 Harland, S. 1960. Effect of temperature on growth in weight and tail-length of inbred and hybrid mice.
444 *Nature* 186:446.

- 445 Huey, R. B., G. W. Gilchrist, M. L. Carlson, D. Berrigan, and L. Serra. 2000. Rapid evolution of a geographic
446 cline in size in an introduced fly. *Science* 287:308–309.
- 447 Husby, A., S. M. Hille, and M. E. Visser. 2011. Testing mechanisms of bergmann’s rule: Phenotypic de-
448 cline but no genetic change in body size in three passerine bird populations. *The American Naturalist*
449 178:202–213.
- 450 Huxley, J. S. 1939. Clines: An auxiliary method in taxonomy. *Bijdragen tot de Dierkunde* 27:491–520.
- 451 James, A. C., R. Azevedo, and L. Partridge. 1995. Cellular basis and developmental timing in a size cline of
452 *drosophila melanogaster*. *Genetics* 140:659–666.
- 453 James, F. C. 1970. Geographic size variation in birds and its relationship to climate. *Ecology* 51:365–390.
- 454 ———. 1983. Environmental component of morphological differentiation in birds. *Science* 221:184–186.
- 455 Johnston, R. F., and R. K. Selander. 1964. House sparrows: Rapid evolution of races in north america.
456 *Science* 144:548–550.
- 457 ———. 1971. Evolution in the house sparrow. II. Adaptive differentiation in north american populations.
458 *Evolution* 1–28.
- 459 Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. lmerTest package: Tests in linear mixed
460 effects models. *Journal of Statistical Software* 82:1–26.
- 461 Laiolo, P., and A. Rolando. 2001. Ecogeographic correlates of morphometric variation in the red-billed
462 chough *pyrrhocorax pyrrhocorax* and the alpine chough *pyrrhocorax graculus*. *Ibis* 143:602–616.
- 463 Land, J. V. ‘., P. V. Putten, and W. V. Delden. 1999. Latitudinal variation in wild populations of *drosophila*
464 *melanogaster*: Heritabilities and reaction norms. *Journal of Evolutionary Biology* 12:222–232.
- 465 Lüdecke, D., M. S. Ben-Shachar, I. Patil, P. Waggoner, and D. Makowski. 2021. Assessment, testing and
466 comparison of statistical models using r. *Journal of Open Source Software* 6:3112.
- 467 Lynch, C. B. 1992. Clinal variation in cold adaptation in *mus domesticus*: Verification of predictions from
468 laboratory populations. *The American Naturalist* 139:1219–1236.
- 469 Lynch, M., B. Walsh, and others. 1998. Genetics and analysis of quantitative traits (Vol. 1). Sinauer Sunder-
470 land, MA.
- 471 Mayr, E. 1956. Geographical character gradients and climatic adaptation. *Evolution* 10:105–108.
- 472 McNab, B. K. 1971. On the ecological significance of bergmann’s rule. *Ecology* 52:845–854.

- Meiri, S., and T. Dayan. 2003. On the validity of bergmann's rule. *Journal of Biogeography* 30:331–351.
- Millien, V., S. Kathleen Lyons, L. Olson, F. A. Smith, A. B. Wilson, and Y. Yom-Tov. 2006. Ecotypic variation in the context of global climate change: Revisiting the rules. *Ecology Letters* 9:853–869.
- Mincer, S. T., and G. A. Russo. 2020. Substrate use drives the macroevolution of mammalian tail length diversity. *Proceedings of the Royal Society B* 287:20192885.
- Noel, J. F., and E. Wright. 1970. The effect of environmental temperature on the growth of vertebrae in the tail of the mouse. *Development* 24:405–410.
- Nudds, R., and S. Oswald. 2007. An interspecific test of allen's rule: Evolutionary implications for endothermic species. *Evolution: International Journal of Organic Evolution* 61:2839–2848.
- Ogle, C., and C. Mills. 1933. Animal adaptation to environmental temperature conditions. *American Journal of Physiology-Legacy Content* 103:606–612.
- Ozgul, A., D. Z. Childs, M. K. Oli, K. B. Armitage, D. T. Blumstein, L. E. Olson, S. Tuljapurkar, et al. 2010. Coupled dynamics of body mass and population growth in response to environmental change. *Nature* 466:482–485.
- Ozgul, A., S. Tuljapurkar, T. G. Benton, J. M. Pemberton, T. H. Clutton-Brock, and T. Coulson. 2009. The dynamics of phenotypic change and the shrinking sheep of st. Kilda. *Science* 325:464–467.
- Partridge, L., B. Barrie, K. Fowler, and V. French. 1994. Evolution and development of body size and cell size in *drosophila melanogaster* in response to temperature. *Evolution* 48:1269–1276.
- Phifer-Rixey, M., K. Bi, K. G. Ferris, M. J. Sheehan, D. Lin, K. L. Mack, S. M. Keeble, et al. 2018. The genomic basis of environmental adaptation in house mice. *PLoS Genetics* 14:e1007672.
- Phifer-Rixey, M., and M. W. Nachman. 2015. The natural history of model organisms: Insights into mammalian biology from the wild house mouse *mus musculus*. *Elife* 4:e05959.
- Price, T. D., A. Qvarnström, and D. E. Irwin. 2003. The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270:1433–1440.
- Riemer, K., R. P. Guralnick, and E. P. White. 2018. No general relationship between mass and temperature in endothermic species. *Elife* 7:e27166.
- Romano, A., R. Séchaud, and A. Roulin. 2020. Geographical variation in bill size provides evidence for allen's rule in a cosmopolitan raptor. *Global Ecology and Biogeography* 29:65–75.
- Ruff, C. 2002. Variation in human body size and shape. *Annual Review of Anthropology* 31:211–232.

502 Ruff, C. B. 1994. Morphological adaptation to climate in modern and fossil hominids. *American Journal of*
503 *Physical Anthropology* 37:65–107.

504 Serrat, M. A. 2013. Allen’s rule revisited: Temperature influences bone elongation during a critical period
505 of postnatal development. *The Anatomical Record* 296:1534–1545.

506 ———. 2014. Environmental temperature impact on bone and cartilage growth. *Comprehensive Physiol-*
507 *ogy* 4:621–655.

508 Serrat, M. A., D. King, and C. O. Lovejoy. 2008. Temperature regulates limb length in homeotherms by
509 directly modulating cartilage growth. *Proceedings of the National Academy of Sciences* 105:19348–
510 19353.

511 Sumner, F. B. 1909. Some effects of external conditions upon the white mouse. *The Journal of Experimental*
512 *Zoology* 7:97–155.

513 ———. 1915. Heredity, correlation and growth, in the white mouse. *The Journal of Experimental Zoology*
514 18:325.

515 Suzuki, T. A., F. M. Martins, Phifer-Rixey Megan, and M. W. Nachman. 2020. The gut microbiota and
516 bergmann’s rule in wild house mice. *Molecular Ecology* 29:2300–2311.

517 Symonds, M. R., and G. J. Tattersall. 2010. Geographical variation in bill size across bird species provides
518 evidence for allen’s rule. *The American Naturalist* 176:188–197.

519 Tattersall, G. J., B. Arnaout, and M. R. Symonds. 2017. The evolution of the avian bill as a thermoregulatory
520 organ. *Biological Reviews* 92:1630–1656.

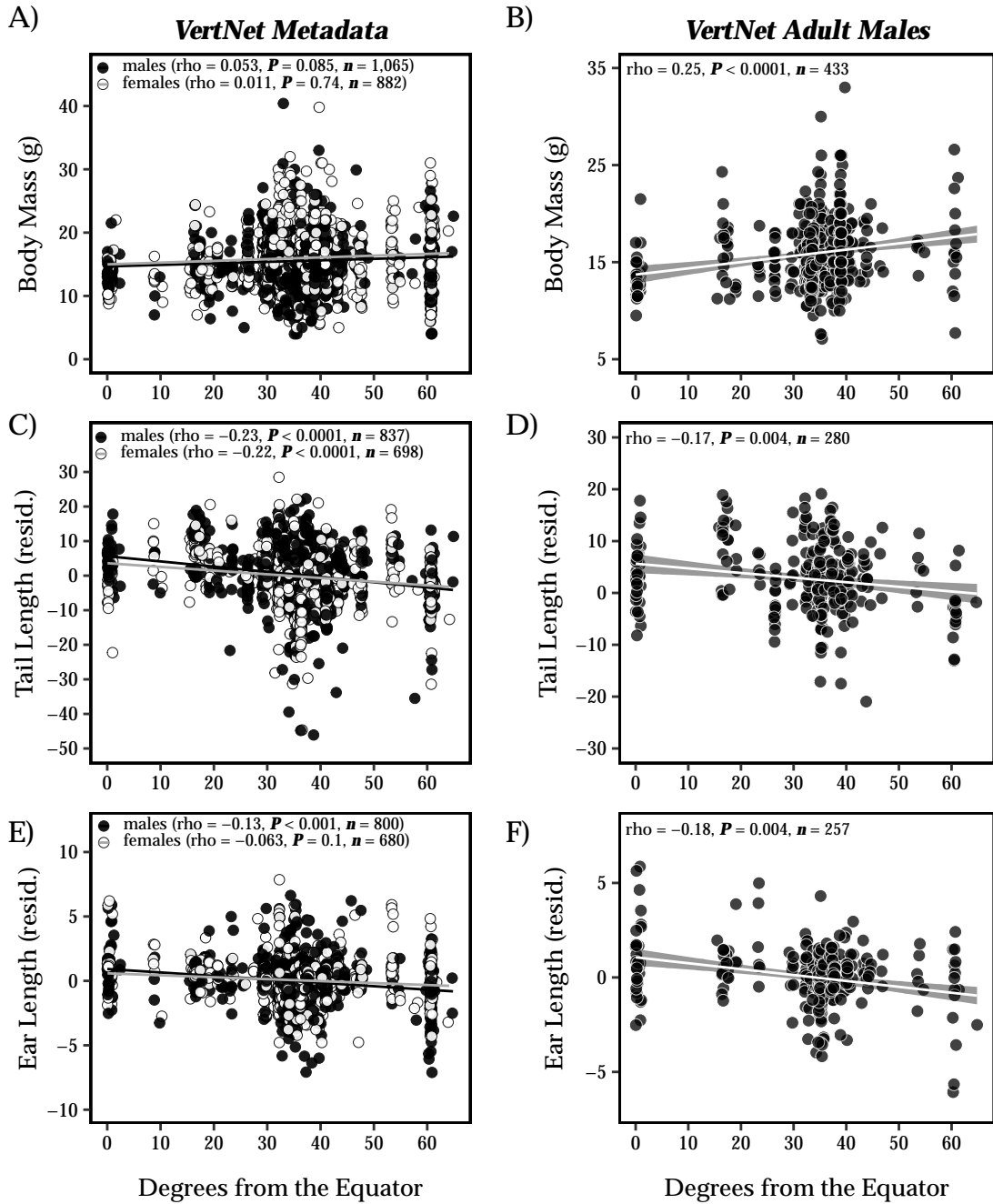
521 Teplitsky, C., J. A. Mills, J. S. Alho, J. W. Yarrall, and J. Merilä. 2008. Bergmann’s rule and climate change
522 revisited: Disentangling environmental and genetic responses in a wild bird population. *Proceedings*
523 *of the National Academy of Sciences* 105:13492–13496.

524 Thorington Jr, R. W. 1970. Lability of tail length of the white-footed mouse, *peromyscus leucopus novebo-*
525 *racensis*. *Journal of Mammalogy* 51:52–59.

526 Tomlinson, S., and P. C. Withers. 2009. Biogeographical effects on body mass of native australian and
527 introduced mice, *pseudomys hermannsburgensis* and *mus domesticus*: An inquiry into bergmann’s
528 rule. *Australian Journal of Zoology* 56:423–430.

529 Via, S., and R. Lande. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity.
530 *Evolution* 39:505–522.

- 531 Weaver, M. E., and D. L. Ingram. 1969. Morphological changes in swine associated with environmental
532 temperature. *Ecology* 50:710–713.
- 533 West-Eberhard, M. J. 2003. *Developmental plasticity and evolution*. Oxford University Press.
- 534 Wickham, H., M. Averick, J. Bryan, W. Chang, L. D. McGowan, R. François, G. Grolemund, et al. 2019.
535 Welcome to the tidyverse. *Journal of Open Source Software* 4:1686.
- 536 Yom-Tov, Y., and H. Nix. 1986. Climatological correlates for body size of five species of australian mammals.
537 *Biological Journal of the Linnean society* 29:245–262.



539

Figure 1. Bergmann's rule and Allen's rule in American house mice. Associations 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 in black and females denoted in white. Results from Spearman correlations are presented in each plot, along with sample sizes. For clar-

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

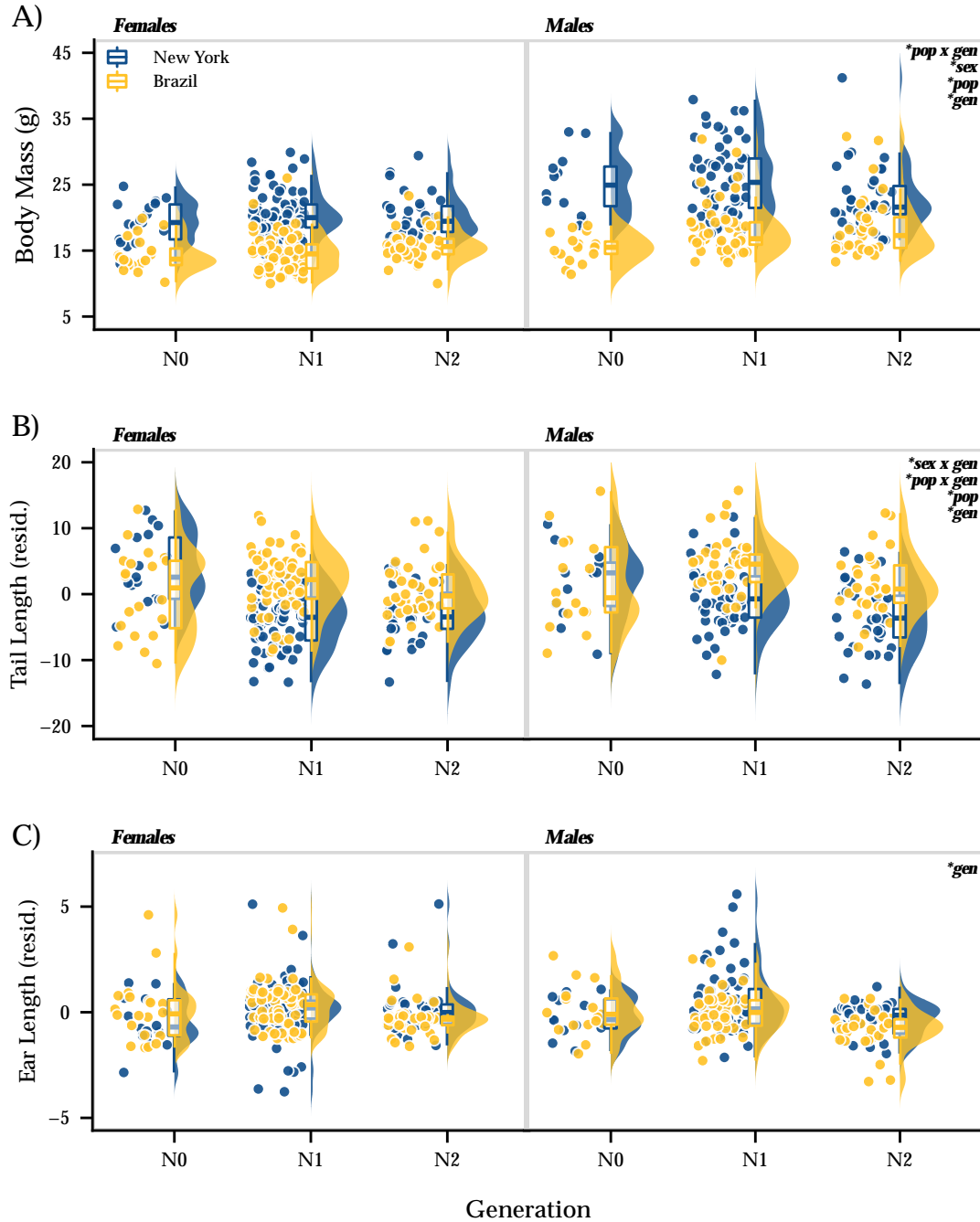


Figure 2. Body mass and tail length differences among populations persist over generations in a common lab environment. Differences in body mass (A), tail length (B), and ear length (C) between New York mice (blue) and Brazil mice (gold) across generations. Tail length and ear length are plotted as the residuals of a regression of body mass on extremity length. Population-level data are depicted as boxplots overlaid on density plots, with boxplot vertical lines denoting 1.5x the interquartile range. Individuals are represented as individual points, and the horizontal variation within each generation determined randomly to separate

554 points. Results from linear models are presented in each plot. Sample sizes: (A) $n = 441$; (B) $n = 432$; (C) n
555 $= 434$.

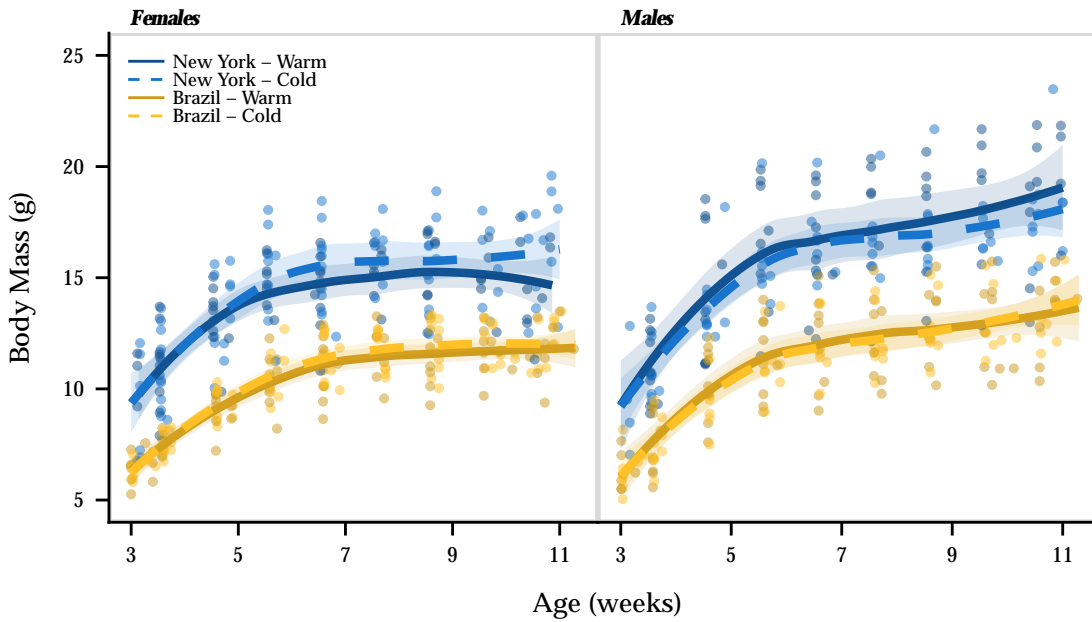


Figure 3. Evolved differences in body mass across development. Body mass growth trajectories across environments in New York (blue) and Brazil (gold) house mice. Cold-reared mice are denoted as dotted lines and warm-reared mice are denoted as solid lines. Individuals are plotted as semi-transparent points ($n = 80$), with population means depicted as smoothed regression fits, with standard error shading. The same individuals depicted here are also depicted in Figure 5.

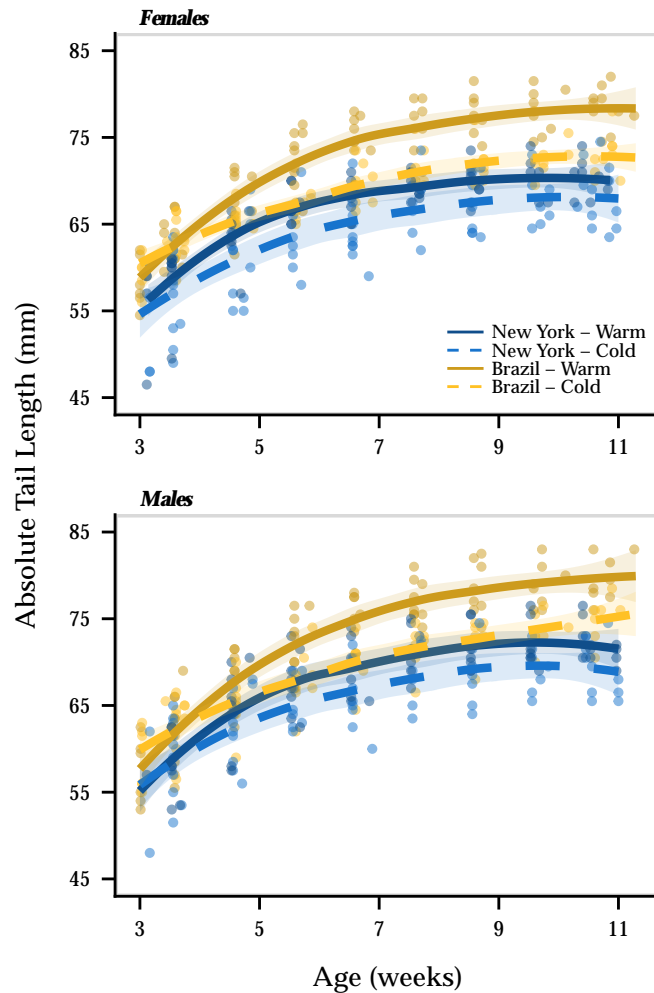


Figure 4. Tail length is highly influenced by cold temperature across development. Absolute tail length growth trajectories across environments in New York (blue) and Brazil (gold) house mice. Cold-reared mice are denoted as dotted lines and warm-reared mice are denoted as solid lines). Individuals are 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.

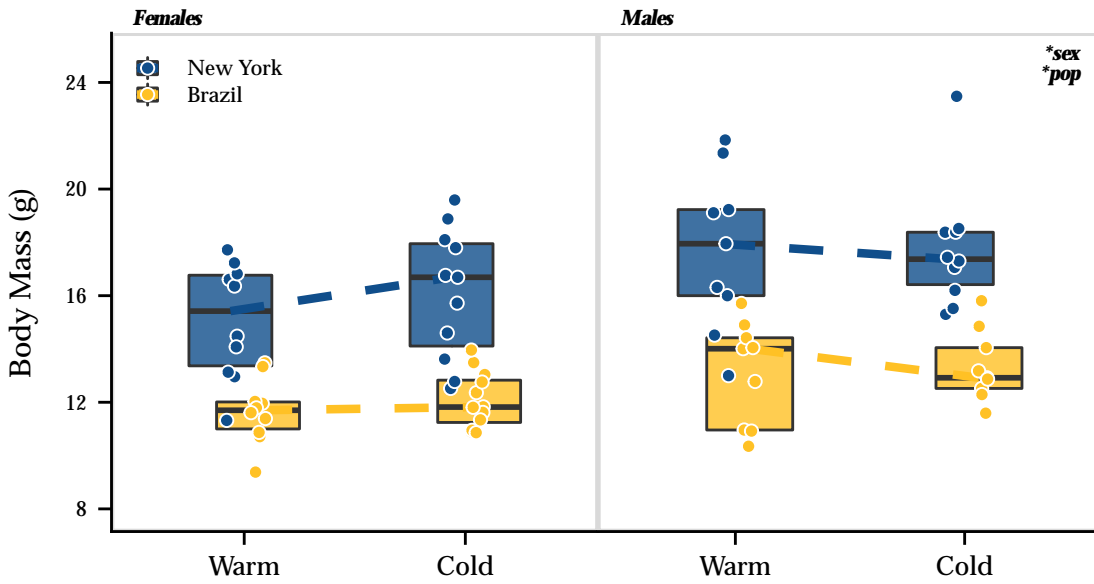


Figure 5. Evolved differences and very little plasticity in body size among New York and Brazil house mice. Individuals are represented as individual points ($n = 80$), with New York mice denoted in blue and Brazil mice denoted in gold. Boxplots indicate the 25th, median, and 75th quartiles. Results from linear mixed models are presented in each plot. The same individuals depicted here are also depicted in Figure 3.

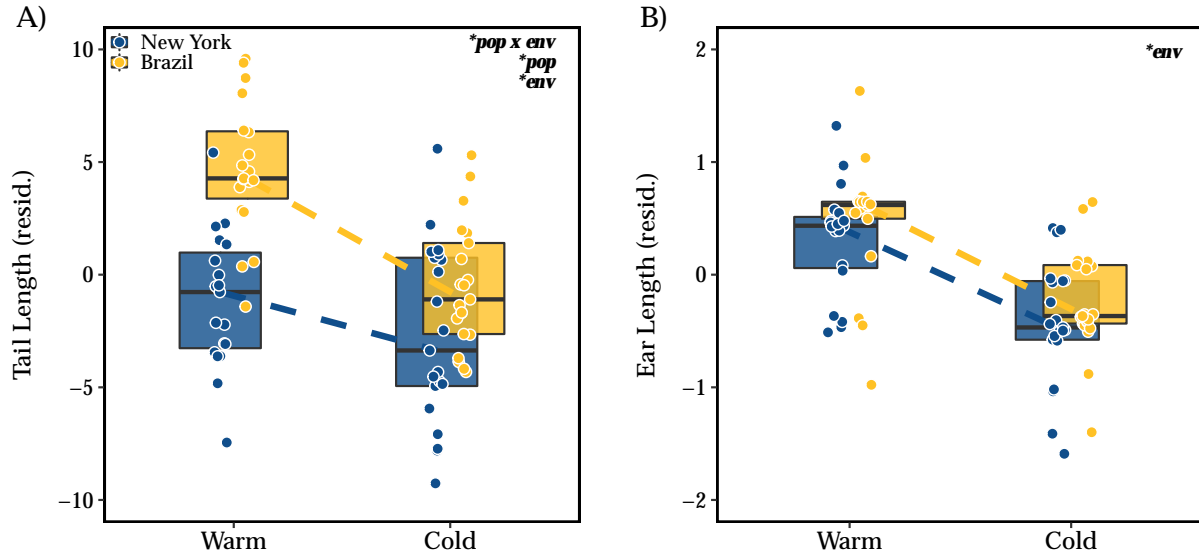


Figure 6. Adaptive phenotypic plasticity in extremity length among New York and Brazil house mice.

Tail length (A) and ear length (B) residuals were calculated by regressing from body mass. Individuals are represented as individual points (tail length residuals: $n = 80$; ear length residuals: $n = 78$), with New York mice denoted in blue and Brazil mice denoted in gold. Boxplots indicate 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.

Supplemental Figures

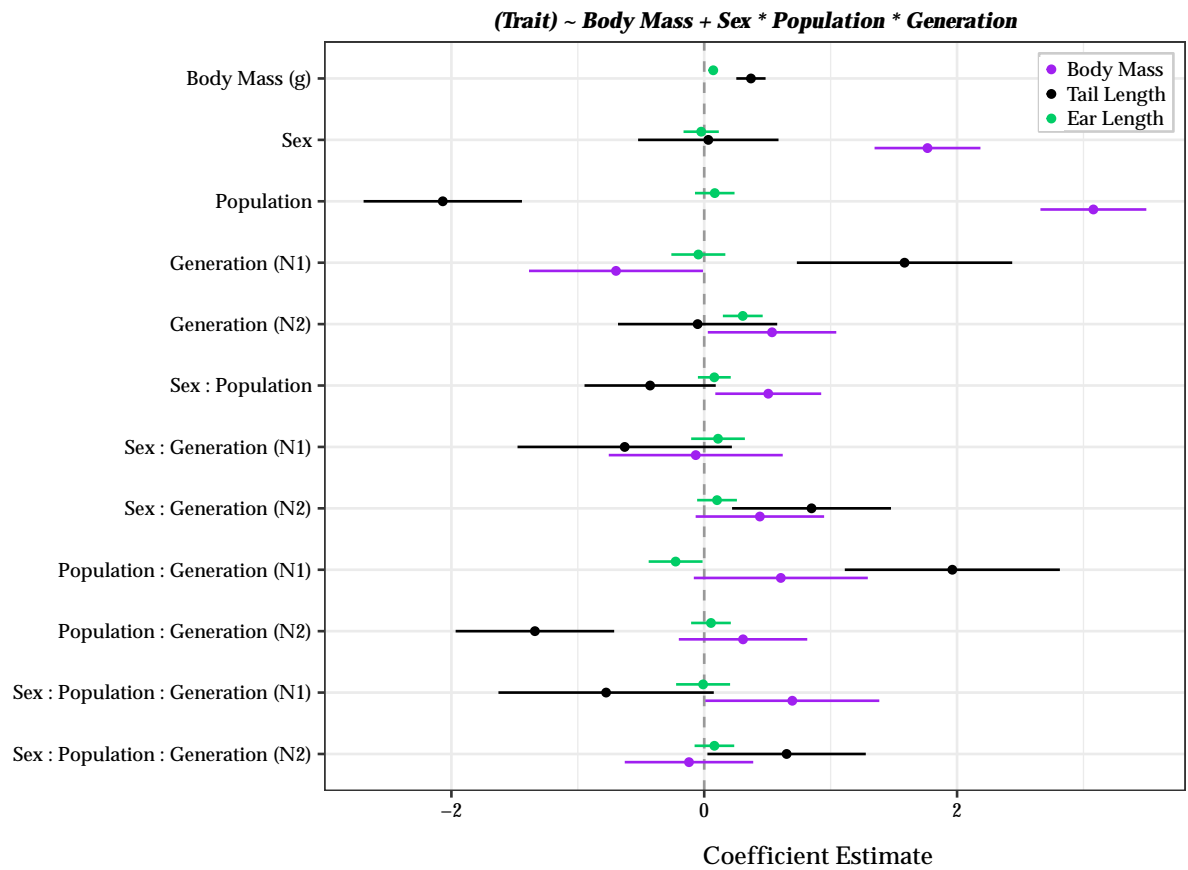


Figure S1. Effect sizes for common garden experiment one, investigating the fixed effects of sex, population, and generation on body mass, tail length, and ear length. Points and ranges represent model estimates and 95% credibility estimates for the linear model, with color indicating the phenotypic trait (purple: body mass; black: tail length; green: ear length). Solid lines that do not cross the dotted, vertical line are significant.

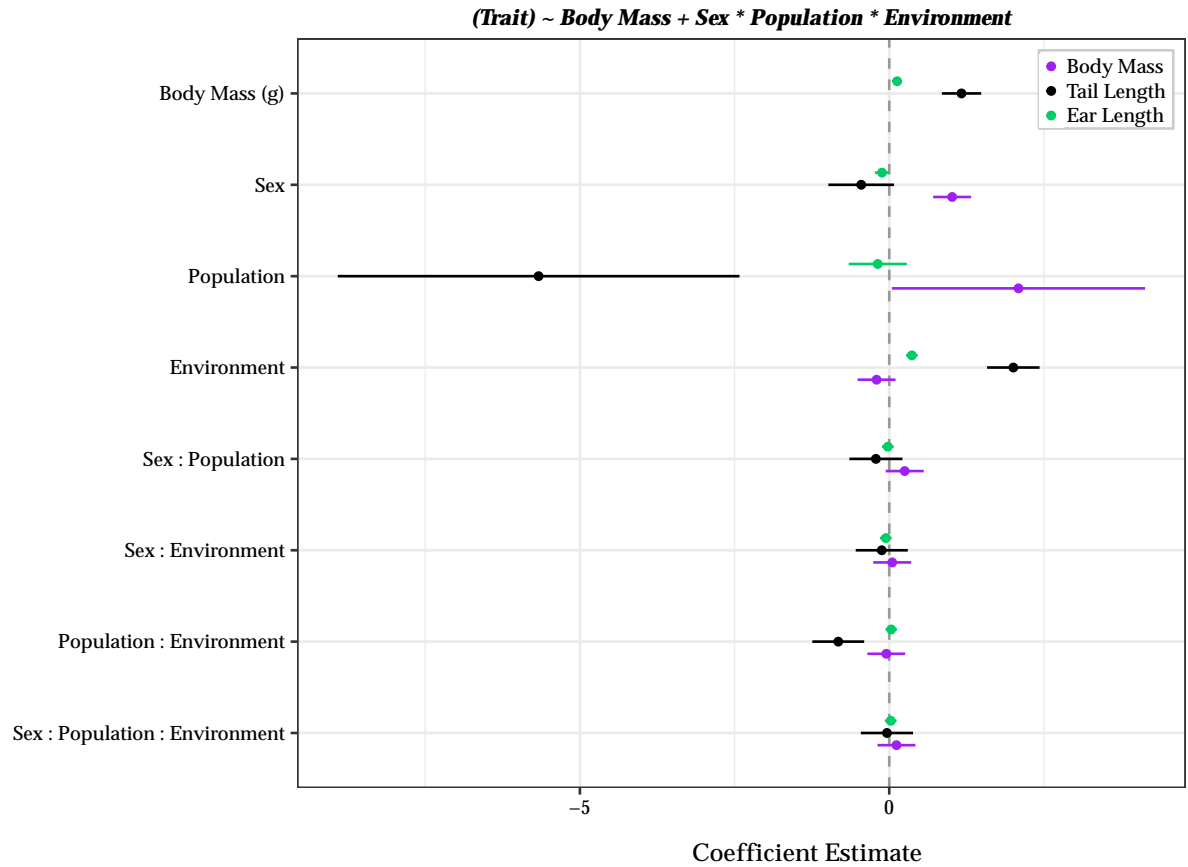
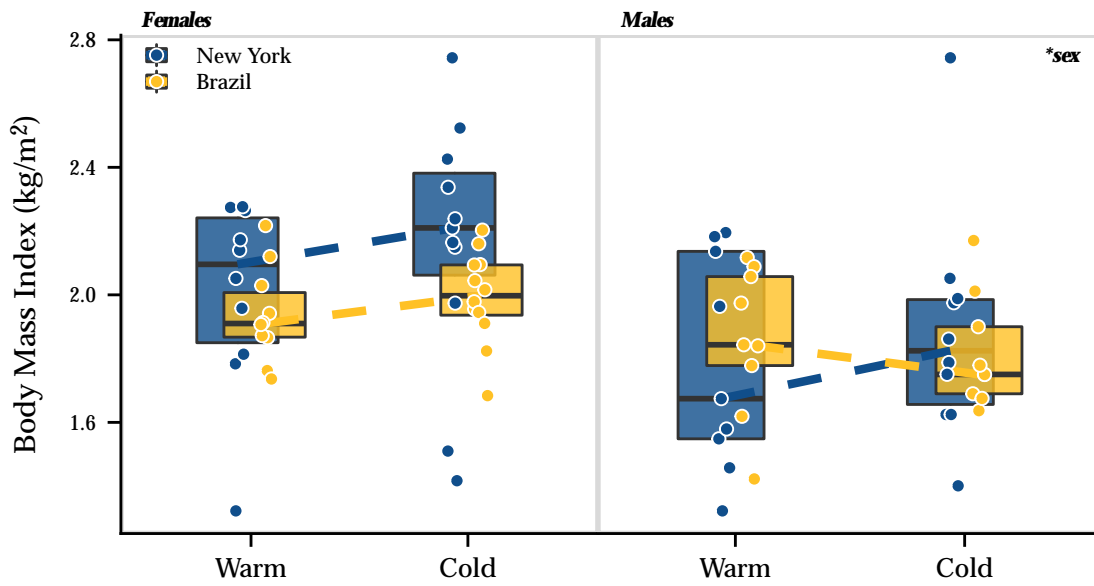


Figure S2. Effect sizes for common garden experiment two, investigating the fixed effects of sex, population, and environment on body mass, tail length, and ear length. Points and ranges represent model estimates and 95% credibility estimates for the linear mixed model, with color indicating the phenotypic trait (purple: body mass; black: tail length; green: ear length). Solid lines that do not cross the dotted, vertical line are significant.



594

595 **Figure S3. No differences in body mass index (BMI) among New York mice and Brazil mice.** No evolved
 596 differences or plasticity in BMI between New York (blue) and Brazil (gold) house mice. Individuals are
 597 represented as individual points ($n = 80$).

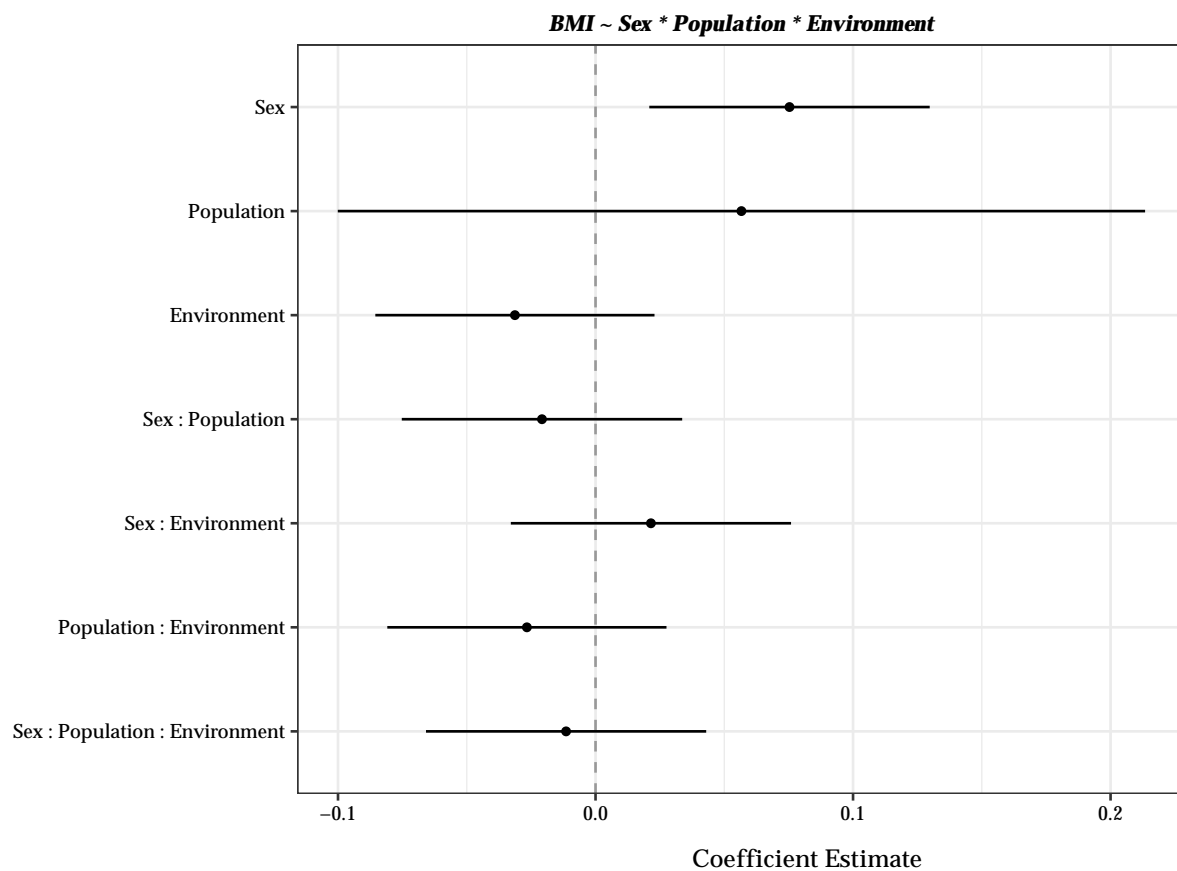


Figure S4. Effect sizes associated with body mass index (BMI). Points and ranges represent model estimates and 95% credibility estimates for the linear mixed model associated with common garden experiment two. Solid lines that do not cross the dotted, vertical line are significant.