Environmental and genetic contributions to ecogeographic rules in house mice

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Abstract (200 words)

Introduction

Clines in phentoypes have historically been attributed to natural selection that 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 (Allen 1877) predicts that organisms have shorter appendages at higher latitudes than those closer to the equator. Similarly, Bergmann's rule (Bergmann 1847) proposes that organisms at higher latitudes are larger in size than those closer to the equator. Although explanatations for these ecogeographic rules continues to be debated (REFs), the overarching hypothesis is that of heat conservation in colder climates by decreasing surface area to volume ratios (REFs). Numerous studies have documented Bergmann's rule and Allen's rule within and across species of mammals (REFs), birds (REFs), and even in humans (REFs). Various meta-analyses have also been conducted, either supporting (REFs) or refuting (REFs) an evolutionary basis to these phenotypic clines. These contradicting results are unsurprising, given the variation in datasets and choice of environmental correlates used in each study. To date, we still have very little understanding of the mechanisms underlying these broad phenotypic clines.

Missing from these discussions are careful analyses determining which traits are genetically encoded and/or environmentally influenced. Many of the traits underlying Bergmann's rule and Allen's rule are quantitative, thus they are polygenic and environmentally determined (Harpak and Przeworski 2021). Dissentangling genetics from environmental effects is impossible with phenotypic data collected on wild-caught specimens. Clines in phenotypes may be masked in the wild by environmental effects and possbile genotype-by-environment interactions (REFs). Thus, cryptic variation in traits consistent with either Bergmann's rule or Allen's rule can exist underneath environmental effects, which are hidden in studies relying soley on wild-caught phenotypic data. Moreover, less attention has been given to the environmental influences of these traits (but see (James 1983)), likely due to the difficulty in controlling for environmental effects via transplant experiments or common garden experiments. These limitations have impeded our ability to make substantial progress on understanding the mechanisms behind 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 are endemic to Western Europe, but through European colonization, have expanded their range across broad latitudinal distributions of the Americas. They can be found from the tip of South America up to Alaska, spanning temperate and tropical environments (Phifer-Rixey and Nachman 2015), making them an ideal system to test for ecogeographic rules. Although house mice have only resided in these novel environments for ~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. Moreover, 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; Phifer-Rixey et al. 2018). Additionally, earlier work in Australian house mice revealed an environmental influence on tail length when exposed to cold temperatures (Barnett and Dickson 1984). Specficially, wild mice housed at cold temperatures grew significantly shorter tails than mice reared at warm temperatures (Barnett and Dickson 1984). However, this study only investigated a single population of house mice, making it difficult to place these results in an explicit evolutionary framework. Overall, we still know very little regarding 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 the environment on body size and extremity length, we performed a common garden experiment by rearing both populations of house mice in warm and cold temperatures and measuring the effects on body size and tail length. Measuring developmental plasticity in these traits allows us to assess the influence of developmental temperature on Bergmann's rule and Allen's rule. Lastly, using classic reaction norm analyses, we show that unlike body size, tail length is highly plastic and the 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

Specimen data from wild house mice collected across several North and South American latitudinal transects were downloaded from the "Environmental adaptation in introduced populations of house mice, Mus musculus, across the Americas" project in Arctos (http://arctosdb.org). Details regarding the collection of these data have been previously described (Phifer-Rixey et al. 2018; Suzuki et al. 2019*a*, 2019*b*, 2020). Briefly, house mice were collected across two transects in North America in summer 2012 (Phifer-Rixey et al. 2018; Suzuki et al. 2020), and along two transects in South America in 2013 and 2014 (Suzuki et al. 2019*a*, 2020). Mice were collected during the warmest months of the year for each transect. Standard museum measurements were recorded for each mouse, including sex, reproductive status, total body length, tail length, hind foot length, ear length, and body weight. Pregnant females, juveniles, subadults, and individuals collected over 1,500m in elevation were removed from downstream analyses. Tail lengths that were shorter than 20mm were considered outliers and removed from downstream analyses. Sample information for the Arctos dataset (n = 215) is provided in Table SX.

To obtain a larger dataset of wild house mice, we obtained records of all house mice collected across the Americas by downloading museum specimens from VertNet (http://vertnet.org) in October of 2020, using the search query: *vntype*:specimen, *genus*:Mus. We filtered records to only include specimens that were collected in North or South America (excluding islands). We removed all known pregnant females, juveniles, and subadults. Sample information for the VertNet dataset (n = 3,016) is provided in Table SXX.

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 house mouse latitudinal distribution: 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 ten generations. Wild-caught mice and their descendants were housed in a standard laboratory environment at 21^{o}C with a 12-hr dark and 12-hr light cycle. Standard rodent chow was provided ad libitium. Standard museum measurements were taken for all wild-caught, N1, and N2 mice from each population (see Table SXXX).

Developmental phenotypic plasticity - common garden experiment 2

For the second common garden experiment, we used two wild-derived inbred lines each from Brazil and New York (Brazil: MANA/Nach, MANB/Nach; New York: SARA/Nach, SARB/Nach). Each line was over 10 generations of inbreeding. Equal numbers of males and females were produced for each within-line comparison (n = 80 total; see Table SXXXX). Full sibs were born at room temperature (21ºC) and singly-housed at weaning (~21 days old). After a brief acclimation period, 3.5-week-old mice were randomly assigned into two-sized matched groups based on sex-specific body mass, and housed at either 5ºC or remained at 21ºC 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 ~xx days of age, and final body masses and tail lengths were taken, in addition to traditional standard museum measurements. 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 were completed using R (v. 4.0.3). Tail length residuals were calculated by regressing absolute tail length from body mass. We tested for clinal patterns of body mass and tail length in wild-caught house mice using Pearson and Spearman correlations between metadata and latitude. Corrected *p*-values are indicated in Table S1 and Figures 1 and S1. We tested for differences in body mass and tail length between lab-reared mice from Brazil and New York by fitting linear models with lm{stats} with a generation by population by sex interaction. Results were evaluated using the anova function and are indicated in Figure 2 and Table xxx. We tested for differences in body mass and tail length between experimental treatments by fitting linear models with lmer{lme4, v. 1.1-26} with a population by treatment by sex interaction, with line as a random variable. Results were evaluated using summary(lmerTest) and anova functions and are indicated in Figure 3, Figure 4, and Table xxx. The code to perform analyses for this study are available as a git-based version control repository on GitHub (https://github.com/Nachmanlab/Ballinger_allenbergmann_XXXX_2021). The analysis can be regenerated using a GNU Make-based workflow that made use of built-in bash tools (v. 3.2.57(1)-release) and R (v. 4.0.3).

Results

Stronger evidence for Bergmann's rule than Allen's rule in wild-caught American house mice.

To determine if introduced populations of house mice conform to Allen's rule and Bergmann's rule, we assessed the relationship between tail length, body mass, and latitude in mice collected across North and South America (Supplementary Table X; n = 215). Body mass postively correlated with absolute latitude (STATS; Table S1), with larger mice found at higher latitudes, consistent with Bergmann's rule (Figure 1). This body size pattern was also seen across males (xxx) and females (xxx; Table xxxx). Tail length did not correlate with absolute latitude (STATS; Table S1), inconsistent with Allen's rule (Figure 1). Overall, these patterns hold when using a larger dataset of American house mice (Supplementary Table XX; n = 3,016), although the correlation was weaker for body mass and absolute latitude (STATS), and tail length showed greater variation (Figure S1).

Differences in body mass persist in a common environment; tail length shows considerable variation.

Phenotypic clines observed across wild house mouse populations could be genetically determined or represent phenotypic plasticity. 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; Table 1). Specifically, mice from New York are larger than mice from Brazil (STATS; Figure 2). Sex-specific differences in body mass were also seen across populations and generations, with males larger than females (STATS; Table 1). The maintance of body mass differences in a common environment and across generations suggests a strong genetic basis in house mice.

Unlike body mass, tail length showed considerable variation across wild-caught mice (N0) and laboratory-reared mice (N1 and N2; Figure 2). This variation could be due to multiple investigators measuring tail length across generations, or it could reflect the inherent plastic nature of tail length. For example, although tail length decreases slightly across generations (STATS; Figure 2B), it is difficult to discern if this is due to phenotypic plasticity or noise being captured in the metadata. Regardless, tail length shows more variation than body mass, even when measured in a common garden experiment.

Tail length, but not body mass, is greatly influenced by developmental temperature.

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. Evolved differences in body mass were evident at weaning (Figure 3A), with New York mice larger than Brazil mice. These body mass differences persisted across development and were not influenced by temperature. Clear sexual dimorphism is seen in both populations, with males larger than females (Figure 3A). Overall, population- and sex-specific differences in body mass are not influenced by temperature (Figure 4A; Table 2), suggesting phenotypic plasticity plays a modest role in body size evolution of house mice.

Unlike body mass, tail length is greatly influenced by developmental temperature, with the first few weeks post-weaning having the greatest influence on overall tail length (Figure 3B). Specifically, mice reared in a cold environment grow shorter tails than mice reared in a warm environment, and these differences are revealed at 5.5 weeks of age (STATS; Figure 3B). Overall, temperature-specific differences in tail length persist across development, regardless of population or sex (Figure 3B).

Evidence for adaptive phenotypic plasticity in tail length.

Conducting a second common garden experiment with only one investigator taking phenotypic measurements revealed evolved differences in tail length between Brazil mice and New York mice (Figure 4B). Specifically, Brazil mice have longer tails than New York mice, and these differences have persisted after more than ten generations of breeding in a common environment, suggesting a genetic basis for tail length. In addition, differences between warm- and cold-reared mice reflect a strong plastic response to temperature in tail length (Figure 4B). Although both populations grow significantly shorter tails in the cold (STATS; Figure 4B), Brazil house mice show a signficant population by environment interaction in tail length (STATS; Figure 4B; Table 2). Furthermore, the plastic response of tail length in Brazil house mice goes in the same direction as the evolved response (i.e. New York tail length at room temperature; Figure 4B), highlighting an example of adaptive phenotypic plasticity.

Discussion

Figures & Tables

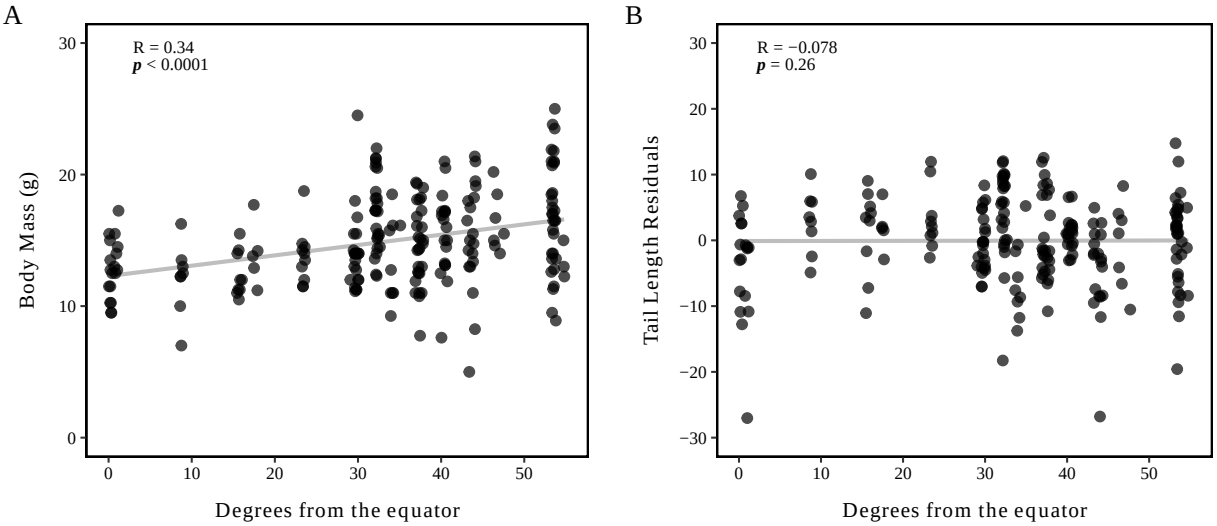


Figure 1. The relationship between body weight, tail length, and absolute latitude in North and South American house mice. Significant coorelation between body mass (A) and latitude, but not tail length (B) and latitude in wild-caught house mice. Tail length residuals were calculated by regressing tail length from body mass. Both plots include males and females, with individuals represented as individual point. Results from Spearman correlations are presented in each plot. Sample sizes: (A) n = 215; (B) n = 212.

"Body mass in wild mice is linearly correlated with latitude (female: n = xxx, rho = xxxx, p = xxxx"

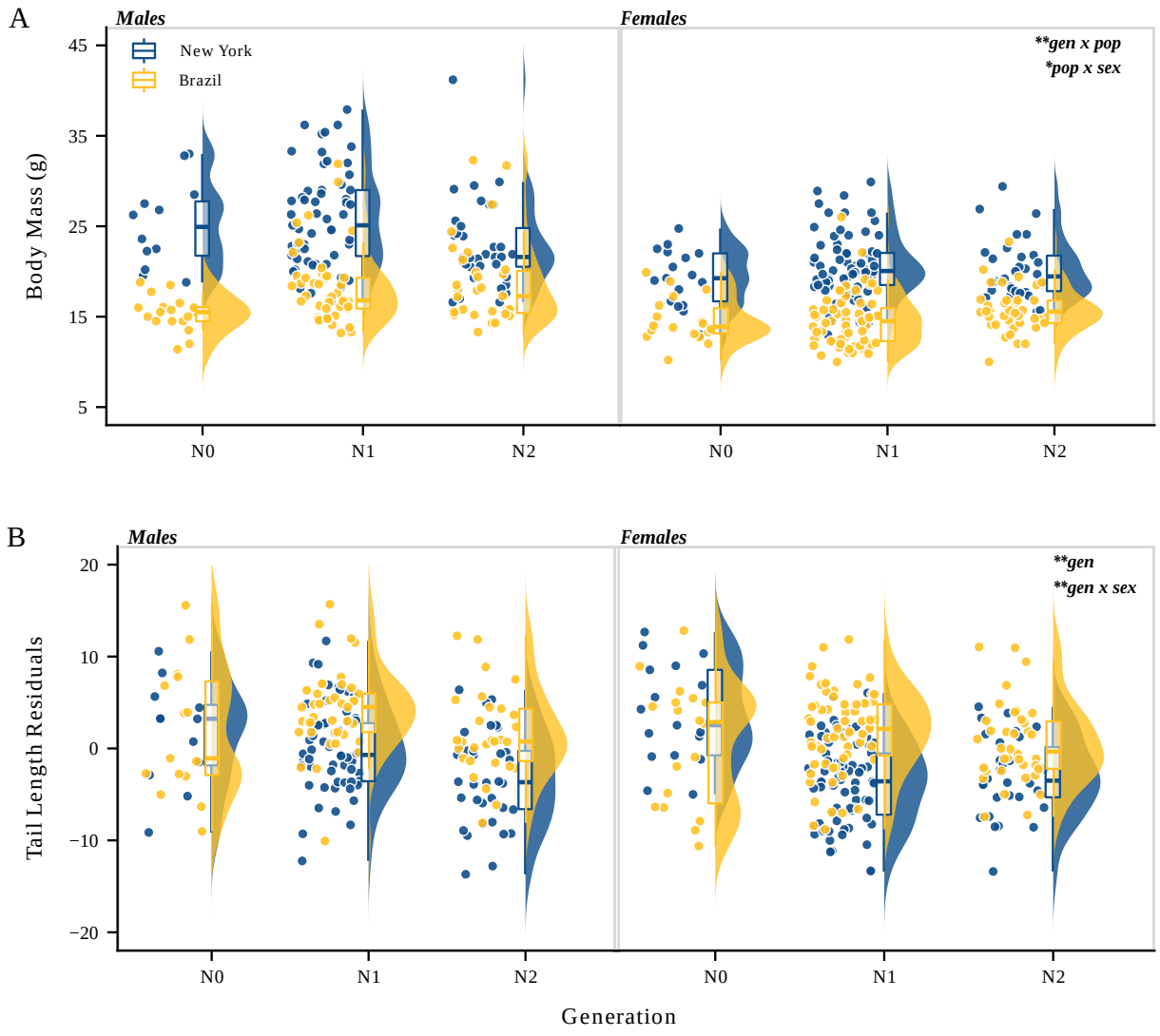


Figure 2. Body mass and tail length differences between populations and across generations in a common lab environment. "Body mass differences among populations persist over two generations in lab"' (A), indicating a genetic basis. No clear differences in tail length (B) are seen between populations, suggesting the inherent "plastic"/"noisy" nature of tail length. Tail length residuals were calculated by regressing tail length from body mass. 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. Results from linear mixed models are presented in each plot. Only signifiant interactions are depicted in (A). Sample sizes: (A) n = 438; (B) n = 427. *P<0.05, ***P <0.01.

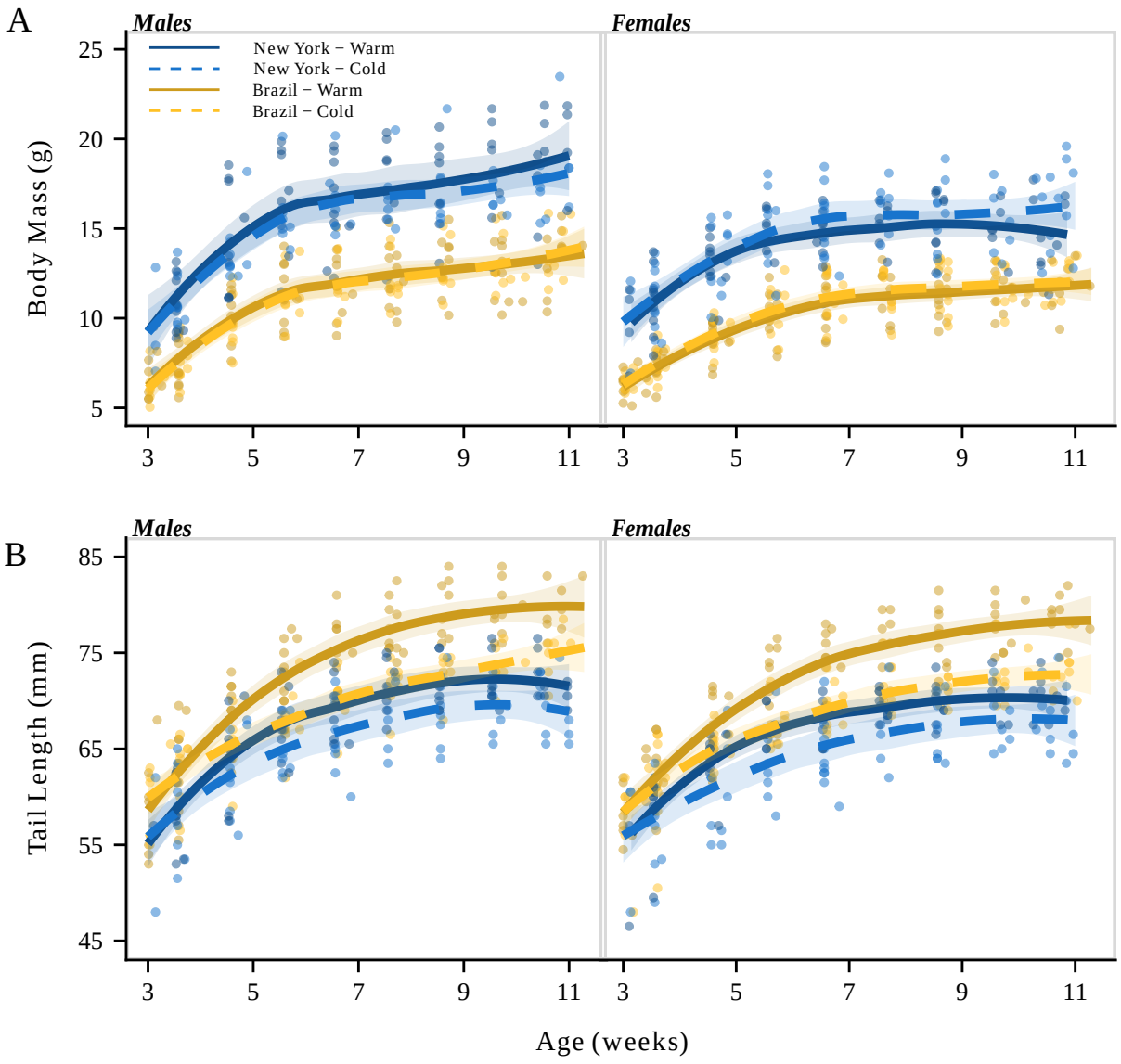


Figure 3. Body mass and tail length growth trajectories across environments. Cold temperatures have very little influence on overall body mass in New York and Brazil house mice (A). Tail length is highly influenced by cold temperatures, with cold-housed mice growing shorter tails compared to warm-housed mice (B). Both New York and Brazil house mice show plasticity in tail length across development. Individuals are represented as individual points (n = 80), with population means depicted as smoothed regression fits, with standard error shading.

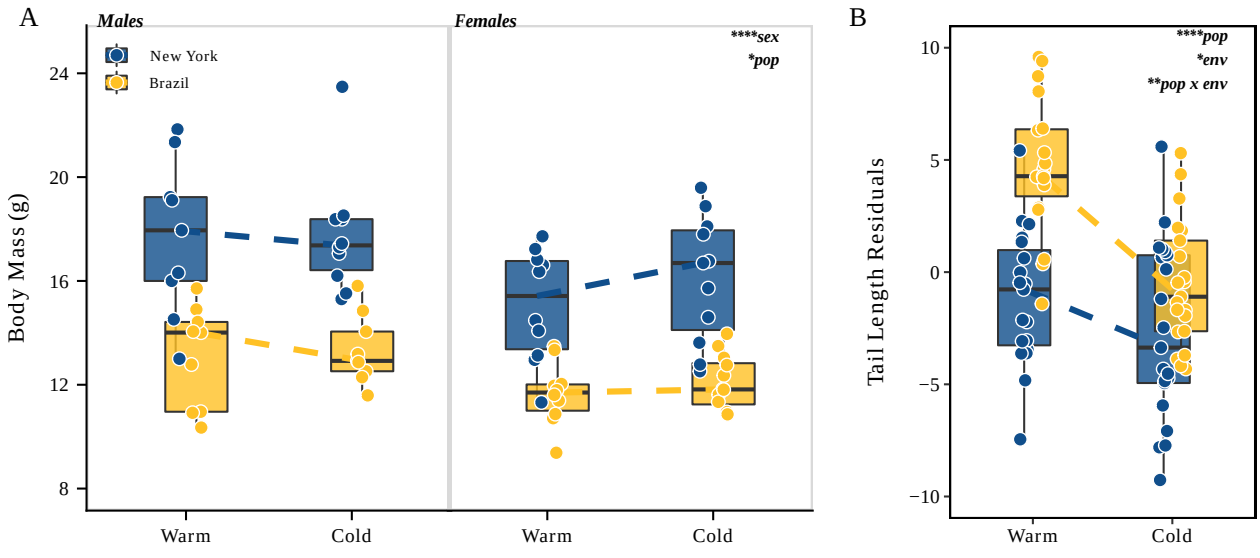
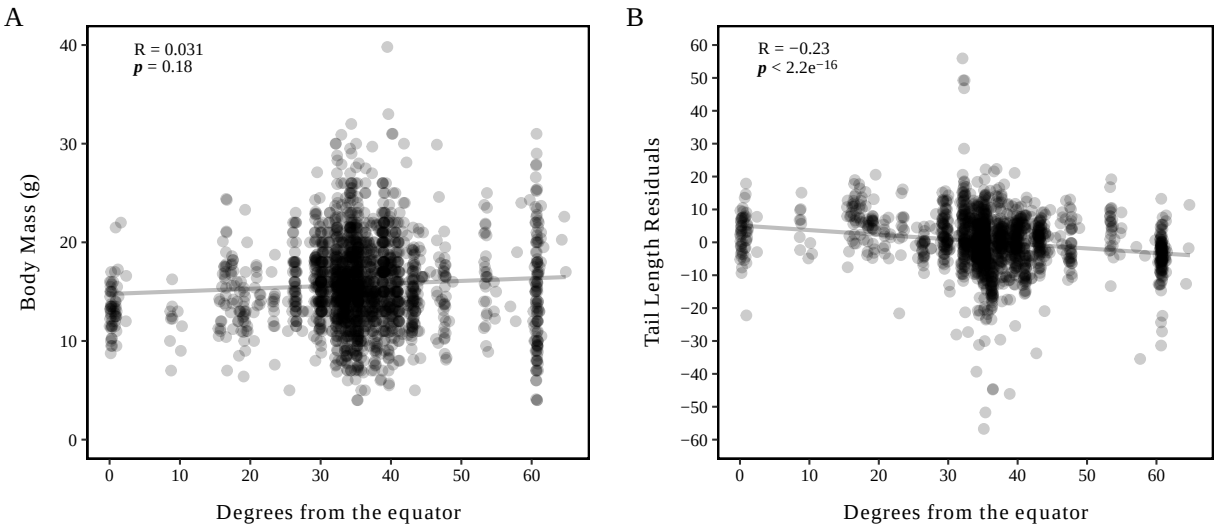


Figure 4. Evolved and plastic phenotypic variation among New York and Brazil house mice. Very little plasticity in body mass in New York and Brazil house mice (A). Tail length is highly plastic in both populations, with tails growing shorter in the cold (B). Brazil house mice show adaptive plasticity in tail length. Tail length residuals were calculated by regressing tail length from body mass. Vertical lines on boxplots denote 1.5x the interquartile range. Individuals are represented as individual points (n = 80). Results from linear mixed models are presented in each plot, with the following significance levels: *P<0.05, **P <0.01, ***P <0.001, ****P <0.0001



211

212 **Figure S1. Bergmann's rule and Allen's rule using VertNet metadata.**

Acknowledgements

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