The contribution of genetic and environmental effects to Bergmann's rule and Allen's rule in house mice

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Abstract

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 describe larger body sizes and shortened extremities in colder climates, respectively. Although numerous studies have found inter- and intraspecific evidence for both ecogeographic patterns, we still have a poor understanding of the extent to which these patterns are driven by genetics, environment, or both. Here, we measured the genetic and environmental contributions to Bergmann's rule and Allen's rule across introduced populations of house mice (*Mus musculus domesticus*) in the Americas. First, we documented clines for body mass, tail length, and ear length in natural populations, and found that these conform to both Bergmann's rule and Allen's rule. We then raised descendants of wild-caught mice in the lab and showed that these differences persisted in a common environment and are heritable, indicating that they have a genetic basis. Finally, using a full-sib design, we reared mice under warm and cold conditions. We found very little plasticity associated with body size, suggesting that Bergmann's rule has been shaped by strong directional selection in house mice. However, extremities showed considerable plasticity, as both tails and ears grew shorter in cold environments. These results indicate that adaptive phenotypic plasticity as well as genetic changes underlie major patterns of clinal variation in house mice and likely facilitated their rapid expansion into new environments across the Americas.

Keywords: body size, extremity length, adaptive plasticity, heritability, *Mus*

Introduction

Clines in phenotypes have historically been attributed to natural selection, reflecting adaptation to local environments (Huxley 1939; Endler 1977). Two of the best described clinal patterns in animals are Allen's rule and Bergmann's rule. Allen's rule is the observation that extremities, such as limb length and tail length, are shorter in colder climates compared to warmer regions, resulting in latitudinal clines (Allen 1877). Bergmann's rule is the observation that body sizes are larger in colder climates, resulting in 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 thus 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; Ruff 2002; Foster and Collard 2013; Betti et al. 2015). Moreover, various meta-analyses have supported the generality of these rules (Ashton et al. 2000; Ashton 2002; Freckleton et al. 2003; Meiri and Dayan 2003; Blackburn and Hawkins 2004; Millien et al. 2006; Nudds and Oswald 2007; Olson et al. 2009; Symonds and Tattersall 2010; Alhajeri et al. 2020). On the other hand, several meta-analyses have questioned the ubiquity of these patterns arguing that statistical support is weak (Geist 1987; Gohli and Voje 2016; Riemer et al. 2018) or that phenotypic differences are more likely to be driven by resource abundance or other factors rather than by considerations of temperature (Scholander 1955; McNab 1971; Geist 1987; Alhajeri and Steppan 2016; Alroy 2019). 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 measurements. Moreover, virtually all studies to date are based on observations of individuals sampled in natural populations in which factors such as age, reproductive condition, social status, pathogen and parasite loads, and overall health are not easily controlled. Thus, 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. Environmentally influenced traits are phenotypically plastic traits, and these traits may also harbor genetic variation for plasticity (i.e., genotype-by-environment interactions) (Des Marais et al. 2013). Most traits associated with Bergmann's rule and Allen's rule are complex, meaning they are both polygenic and strongly influenced by the environment (Falconer and Mackay 1996; Lynch et al. 1998; Yang et al. 2010; Harpak and Przeworski 2021). In fact, in his original description of clinal patterns, Allen (1877) emphasized the role of the environment in directly modulating phenotypes. Disentangling

genetic from non-genetic effects in natural populations is difficult when using phenotypic data collected from wild-caught animals. Genetic contributions to trait values may be masked by environmental effects (plasticity) and genotype-by-environment interactions (Conover and Schultz 1995; Alho et al. 2011). Phenotypic plasticity may also generate clinal patterns, giving a false impression of adaptive clines (James 1983). In fact, many temporal changes in body size 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 of 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 (i.e., 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 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*) provide a tractable system for disentangling the genetic and environmental contributions to 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 to Alaska. Across this broad latitudinal range, mice are exposed to various environmental gradients, including both temperature and aridity (Phifer-Rixey and Nachman 2015). Despite residing in these novel environments for only a few hundred generations, there is evidence for clinal variation across latitudes. Specifically, mice in eastern North America follow Bergmann's rule (Lynch 1992; Phifer-Rixey et al. 2018), with larger mice in more northern populations. These body size differences persist in a common environment over several generations, indicating that they have a genetic basis (Lynch 1992; Phifer-Rixey et al. 2018). Selection on house mice over ten generations in the laboratory recapitulates these clinal patterns: mice bred at lower temperatures become larger and undergo genetic divergence in body size (Barnett and Dickson 1984). Furthermore, previous work has revealed an environmental influence on tail length when exposed to cold temperatures. Specifically, house mice reared in a cold environment grew significantly shorter tails than mice reared at warm temperatures, consistent with Allen's rule (Sumner 1909, 1915; Barnett 1965). However, these earlier studies investigated only a single population of mice or used classical inbred laboratory strains of mice, making it difficult to place the results in an explicit evolutionary framework. We still have little understanding of the phenotypic variation of house mice across their entire

latitudinal distribution, and even less understanding of the contributions of genetics and environment to 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 house mice from North and South America. First, we determined if house mice conform to both Bergmann's rule and Allen's rule across North and South America by analyzing phenotypic data from wild-caught individuals. Second, we collected individuals from 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 lab environment and identified a heritable basis to both 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 lab populations of mice using a full-sib design in a cold and warm environment and measured the effects on body size and extremity length. Measuring developmental plasticity within and between populations allowed us to assess the influence of temperature on complex traits and to understand the evolutionary mechanisms underlying these clinal patterns. Specifically, we found that unlike body size, tail and ear length are highly plastic and this plastic response goes in the same direction as the evolved response of New York mice, highlighting an example of adaptive phenotypic plasticity.

Materials and Methods

Phenotypic data from wild-caught mice

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: *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 omitted individuals explicitly listed as pregnant, juvenile, subadult, or immature, and included individuals listed as adult, mature, or with no age class or reproductive condition specified. 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

137 (greater than 3.5 standard deviations from the mean) and were removed from downstream
138 analyses. Sample information for the final VertNet dataset (n = 3,018) is provided in Data S1.

139 We assessed the overall relationship between body mass, extremity length, and latitude
140 by fitting linear models in R (v. 4.1.1), including sex as a covariate (Table S1). We tested for
141 clinal patterns of body mass and extremity length across latitude in wild-caught house mice
142 using Spearman correlations for each sex separately. To test if mice from colder regions conform
143 to Bergmann's rule and Allen's rule, we extracted mean annual temperature data for all VertNet
144 sampling locations at 1 km (30-seconds) spatial resolution from WorldClim 2.1 (Fick and
145 Hijmans 2017). We tested for clinal patterns of body mass and extremity length across mean
146 annual temperature (°C) using Spearman correlations for each sex separately.

147

148 *Laboratory-reared mice - common garden experiment 1*

149 To disentangle genetic effects from environmental effects, we collected live animals from
150 two locations that represent the ends of a latitudinal transect: Manaus, Amazonas, Brazil
151 (MAN), located near the equator at 3°S latitude, and Saratoga Springs, New York, USA (SAR),
152 located at 43°N latitude. Details of this common garden experiment are given in (Phifer-Rixey et
153 al. 2018) and (Suzuki et al. 2020). Briefly, live mice from both Brazil (n = 38) and New York (n =
154 30) were brought back to the lab at the University of California, Berkeley. Within each
155 population, unrelated pairs of wild-caught mice were mated to produce first generation (N1)
156 lab-reared mice. Mice were then paired in sib-sib matings to generate inbred lines. These inbred
157 lines (New York: n = 10 lines; Brazil: n = 12 lines) have been maintained through sib-sib matings
158 for over 10 generations. Wild-caught mice and their descendants were housed in a standard
159 laboratory environment at 21°C with a 12-hr dark and 12-hr light cycle. Water and commercial
160 rodent chow (Teklad Global, 18% protein, 6% fat) were provided ad libitium. Standard museum
161 measurements (total length, tail length, hind foot length, ear length, and body mass) were taken
162 for all wild-caught and inbred mice from each population (see Data S2). We removed outliers
163 for tail length (< 50mm (n = 2)) and ear length (< 8mm (n = 1)) from downstream analyses.

164

165 *Estimating heritability*

166 To estimate heritability for body mass and extremity length in house mice, we
167 performed midparent-offspring regression on N2 (parents) and N3 (offspring) laboratory-born
168 mice. Generations N2 and N3 were chosen to eliminate any residual environmental and

169 maternal effects that could influence heritability estimates. Midparent values were calculated as
170 the mean trait value between mother and father, and heritabilities were calculated as the
171 regression coefficients (slopes) of offspring values against midparent values (Lynch et al. 1998).
172 We performed midparent-offspring regressions on 13 Brazil families (representing 10 different
173 inbred lines) and 13 New York families (representing 10 different inbred lines). We also
174 calculated regression coefficients of offspring values against maternal and paternal values
175 separately for all three traits. Heritabilities from these regressions were estimated as twice the
176 slope of the regression of offspring values against maternal or paternal values (Lynch et al.
177 1998). We assessed the significance of regression coefficients for each heritability estimate using
178 ANOVAs, implemented in the car package (v. 3.0.11) (Fox and Weisberg 2019).

179

180 *Developmental phenotypic plasticity - common garden experiment 2*

181 To determine the influence of phenotypic plasticity on body mass and extremity length,
182 we performed a second common garden experiment by rearing laboratory-born mice from both
183 populations in a cold and warm environment. We used temperature as the environmental
184 variable because temperature is highly correlated with latitude (Millien et al. 2006) and
185 phenotypic variation in wild house mice across North and South America is explained most by
186 temperature-related variables (Suzuki et al. 2020). Specifically, we used two wild-derived
187 inbred lines each from Brazil (MANA, MANB) and New York (SARA, SARB). Each line has
188 been inbred for more than 10 generations, and thus mice within a line harbor reduced levels of
189 genetic variation. Roughly equal numbers of males and females were produced for each within-
190 line comparison (New York: $n = 40$; Brazil: $n = 40$; see Data S3 and S4). These population-
191 specific sample sizes align with previous experimental studies in house mice (e.g., Phifer-Rixey
192 et al. 2018). Full-sibs were born at room temperature (21°C) and singly-housed at weaning (~21
193 days old). After a brief acclimation period, we randomly assigned 3.5-week-old mice into size-
194 matched groups based on sex-specific body mass, and then housed mice at either 5°C or
195 remained at 21°C for the duration of the experiment (~50 days total). We measured initial body
196 mass and tail length and recorded subsequent body mass and tail lengths once a week for each
197 mouse. At the end of the experiment, we euthanized mice at 75 ± 3 days of age, and recorded
198 final body mass and tail length, in addition to standard museum measurements. Two final ear
199 lengths were not included in downstream analyses due to ear damage. We deposited skulls and
200 skeletons of all mice in the Museum of Vertebrate Zoology, University of California, Berkeley
201 (catalog numbers are given in Data S4). All experimental procedures were in accordance with
202 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. 4.1.1). Within R, we used the tidyverse (v. 1.3.1) (Wickham et al. 2019), performance (v. 0.8.0) (Lüdecke et al. 2021), cowplot (v. 1.1.1), here (v. 1.0.1), and rmarkdown (v. 2.11) (Allaire et al. 2021) packages, along with R base library. Relative tail length and relative ear length were calculated by dividing tail or ear length by body mass for each individual. We also performed all analyses using tail length residuals and ear length residuals (by regressing length from body mass across individuals) and obtained similar results.

For common garden experiments 1 and 2, we fitted linear mixed models in the lme4 package (v. 1.1.27.1) (Bates et al. 2015) to determine if morphology varied between sex, population, generation, or environment. In each model, we included: 1) the morphological trait as the response variable; 2) sex, population, generation (experiment 1) or environment (experiment 2), and their interaction as fixed effects; and 3) inbred lines as a random effect. The significance of interactions was evaluated using ANOVA based on type III (partial) sums of squares, implemented in the car package (v. 3.0.11) (Fox and Weisberg 2019). We performed *post hoc* comparisons on significant two-way interactions using Tukey's HSD tests ($P < 0.05$). Lastly, we calculated the effect size ($\omega 2$) of predictors using the effectsize package (v. 0.5) (Ben-Shachar et al. 2020) to evaluate the relative influence of sex, population, and generation or environment on body mass and extremity length (Olejnik and Algina 2003) (Table 1; Table S2).

Results

Evidence for Bergmann's rule and Allen's rule in wild 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 found little 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 house mice from the Americas, with both 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). In each of these comparisons, however, latitude explained less than 1% of the phenotypic variation (Table S1). Similar patterns of Bergmann's rule and Allen's rule were seen

using mean annual temperature (°C) as a predictor (Figure S1). Specifically, body mass declined and extremity length increased with increasing mean annual temperature (Figure S1A,C,E).

The lack of evidence for Bergmann's rule in Figure 1A and S1A may be due to the influence of uncontrolled factors (e.g., age, diet, health), environmental effects, or phenotypic plasticity. Although we minimized variation in age and reproductive status by removing explicitly labeled 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 further filtered the VertNet dataset to only include explicitly labeled adult males (Figure 1B, 1D, 1F; $n = 445$). We focused on males since females show more variation in body mass than males (Levene's test; $F = 17.89$; $P = 0.005$), likely due to reproductive condition. In this more controlled set of adult males, we see strong evidence for both Bergmann's rule (Figures 1B, S1B) and Allen's rule (Figures 1D, 1F, S1D, S1F). The comparison between the larger dataset and the more curated dataset highlights how uncontrolled variation in collated museum metadata may obscure broad ecogeographic patterns.

Differences in body mass and extremity length persist in a common environment and are heritable

The phenotypic clines observed across wild house mice could represent genetic differences, phenotypic plasticity, or both. To disentangle genetics from plasticity, we collected live mice from near the equator (Manaus, Amazonas, Brazil) and from 43°N latitude (Saratoga Springs, New York, USA) and brought them into a common laboratory environment. Population-specific differences in body mass and extremity length in wild-caught mice (N0) persisted across generations of laboratory-reared mice (Figure S2). Specifically, mice from New York were larger than mice from Brazil (ANOVA, $P < 0.001$, $\omega^2 = 0.56$) (Figure S2A; Table S2). New York mice also had shorter tails (ANOVA, $P < 0.001$, $\omega^2 = 0.65$) (Figure S2B; Table S2) and shorter ears (ANOVA, $P < 0.001$, $\omega^2 = 0.58$) (Figure S2C; Table S2) compared to mice from Brazil. The maintenance of body mass and extremity length differences in a common environment and across generations suggests that these traits are heritable in house mice.

To estimate the heritability (h^2) of body mass and extremity length in New York and Brazil house mice, we performed midparent-offspring regressions on N2 and N3 mice (Figure 2). Both Brazil and New York mice yielded similar, high heritability values for body mass and extremity length. Specifically, Brazil mice showed significant and very similar heritabilities for all three traits (ANOVA, $P < 0.05$; Figure 2). These heritability estimates are in general agreement with previous estimates of body mass and tail length in house mice (Rutledge et al.

268 1973). Furthermore, using single-parent offspring regressions, both New York and Brazil mice
269 showed similar heritabilities for all three traits (Figure S3). Maternal- and paternal-offspring
270 regressions suggest that body mass and extremity length in New York and Brazil mice are not
271 determined by inheritance of just one sex, since heritability estimates are in rough agreement
272 with midparent-offspring regression estimates. Overall, these results suggest that body mass
273 and extremity length are heritable and under strong genetic control in house mice.

274

275 *Extremity length, but not body size, is greatly influenced by temperature*

276 The results presented above identified phenotypic divergence in body mass and tail and
277 ear length in house mice, with New York mice having shorter tails and ears and larger body
278 sizes than mice from Brazil, consistent with Allen's rule and Bergmann's rule, respectively. To
279 determine the influence of phenotypic plasticity on these traits, we reared laboratory-born mice
280 from both populations in a cold and warm environment. Genetic differences in body mass
281 between inbred lines of New York and Brazil mice were evident at weaning (Figure 3A), with
282 New York mice larger than Brazil mice. These body mass differences between populations
283 persisted across developmental stages from 3 to 11 weeks. In all cases, males were larger than
284 females. Full-sibs (of the same sex) reared at different temperatures showed no differences in
285 body mass (Figure 3A). At the end of the experiment, body size differences recapitulated
286 patterns seen across generations, with New York mice larger than Brazil mice (Table 1; Figure
287 3B) and males larger than females (Table 1; Figure 3B). The lack of plasticity in body mass was
288 not a result of differences in fat accumulation, as body mass index (BMI) did not differ between
289 populations (ANOVA, χ^2=0.50, P>0.05) or environments (ANOVA, χ^2=1.28, P>0.05) (Figure
290 S4). These results suggest that phenotypic plasticity does not play a significant role in body size
291 evolution of house mice.

292 In contrast to body mass, tail length was greatly influenced by developmental
293 temperature, with the first few weeks post-weaning having the greatest influence on absolute
294 tail length (Figure 4). Specifically, inbred mice reared in a cold environment grew shorter tails
295 than inbred mice reared in a warm environment (Table 1; Figures 4, 5A). The magnitude of this
296 effect was striking in Brazil mice, corresponding to 5.40 mm, on average, or 7% of the total adult
297 tail length. Despite developmental temperature playing a significant role in tail length, genetic
298 differences in tail length were evident at the end of the experiment, with Brazil mice growing
299 longer tails than New York mice in both environments (Table 1; Figures 4, 5A). Thus, tail length
300 exhibits both genetic divergence and phenotypic plasticity. Similarly, cold-reared mice grew
301 shorter ears than warm-reared mice (Table 1; Figure 5B), and the magnitude of the plastic

302 response corresponded to 5% of the total adult ear length in both populations. These
303 temperature-growth responses of the extremities were not a simple consequence of body size
304 differences, as body mass did not differ between treatments (Figure 3). Overall, unlike body
305 size, extremity length showed significant plasticity in response to temperature, with mice
306 growing shorter extremities in a cold environment, consistent with patterns of Allen's rule.

307

308 *Adaptive phenotypic plasticity in extremity length*

309 Differences between warm- and cold-reared mice revealed a strong plastic response to
310 temperature in extremity length. Because plasticity is considered adaptive when a phenotype is
311 altered in the same direction as natural selection (Ghalambor et al. 2007), we next asked
312 whether phenotypic plasticity of Brazil mice goes in the same or opposite direction as the
313 evolved response of New York mice. For absolute tail length, the mean trait value of Brazil
314 house mice reared in the cold nearly recapitulates the tail length of New York mice reared in a
315 warm environment (Figure 4), highlighting an example of adaptive phenotypic plasticity.
316 Interestingly, the plastic response of tail length for New York mice (i.e., difference between
317 warm New York tail length and cold New York tail length) was attenuated in comparison to the
318 plastic response of tail length for Brazil mice (Figures 4, 5A), suggesting that New York house
319 mice may be closer to the phenotypic optimum or that there is a developmental constraint on
320 minimum tail length. Lastly, plasticity in ear length of Brazil house mice went in the same
321 direction as the evolved response of New York house mice reared in a warm environment
322 (Figure 5B), further illustrating adaptive phenotypic plasticity. The overall degree and direction
323 of plasticity in extremities mirror patterns associated with Allen's rule. In fact, the plastic
324 response of both tail length and ear length in Brazil mice (i.e., difference between warm Brazil
325 trait value and cold Brazil trait value) explains roughly 40% of the mean phenotypic differences
326 we observe in wild mice (i.e., between N0 Brazil mice and N0 New York mice).

327

328 **Discussion**

329 Previous studies have provided conflicting assessments of the generality of Bergmann's
330 and Allen's rules and have rarely combined field and laboratory studies to identify the
331 contribution of genetic and non-genetic effects to phenotypic variation. Here, we focused on one
332 species that has recently expanded its range across many degrees of latitude, and we studied
333 phenotypic variation in the wild and in the lab. Moreover, by rearing inbred mice at different

334 temperatures we were able to assess the contribution of phenotypic plasticity to patterns seen in
335 nature.

336 First, we found that wild house mice across North and South America conform to
337 Bergmann's rule and Allen's rule, as house mice are larger in size with shortened extremities
338 farther from the equator and at colder temperatures. Second, heritable differences in body mass
339 and tail and ear length in a common environment indicated a genetic basis to Bergmann's rule
340 and Allen's rule, presumably reflecting thermoregulatory adaptations. Finally, we measured the
341 contributions of phenotypic plasticity to these traits and found that tail and ear length are
342 highly plastic in response to cold temperature, while body size is not. The plastic response in
343 extremity length to cold temperatures appears adaptive, matching the direction of change of
344 extremity length seen in wild, temperate house mice. Adaptive plasticity associated with Allen's
345 rule, in conjunction with strong selection for body size, likely promoted the rapid expansion of
346 house mice into new environments across the Americas.

347

348 *Genetic contributions to ecogeographic rules*

349 Parallel phenotypic clines across multiple transects provide strong evidence for natural
350 selection (Endler 1977). Body mass in house mice increases as latitude increases (Figure 1A-B),
351 resembling patterns seen in eastern North America (Lynch 1992; Phifer-Rixey et al. 2018), South
352 America (Suzuki et al. 2020), and Australia (Tomlinson and Withers 2009). These clinal patterns
353 are consistent with Bergmann's rule. Notably, this pattern is much clearer when looking at a
354 dataset that includes only adult males (Figure 1B) compared to a dataset with all animals
355 (Figure 1A). This difference may help to explain the discrepancies between previous meta-
356 analyses based on museum collections (e.g., Ashton et al. 2000; Meiri and Dayan 2003; Riemer et
357 al. 2018). Moreover, the substantial trait variation in Figure 1 among individuals, even when
358 sampled at the same latitude, provides strong motivation for studying these traits in a common
359 laboratory environment.

360 Phenotypic measurements of lab-reared mice from temperate and tropical populations
361 revealed a heritable basis for the difference in body mass, with mice from a colder climate
362 significantly larger than mice from a tropical environment (Figure 2A). This pattern was seen
363 across generations (Figure S2) and heritability estimates for body mass were relatively high
364 across both populations (Figure 2). These results agree with previous studies that also found a
365 genetic basis for body size differences in house mice from eastern North America (Lynch 1992;
366 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 leading to latitudinal clines for body size has also been shown for other non-native species, such as in the genus *Drosophila*. Specifically, body size clines in introduced species of *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). Latitudinal clines for body size have also arisen rapidly in introduced populations of house sparrows (Johnston and Selander 1964, 1971) and European starlings (Cardilini et al. 2016). Together, these results suggest that introduced species may undergo strong selection while colonizing new environments, allowing patterns conforming to Bergmann's rule to become quickly established.

Phenotypic measurements of wild mice revealed clines for relative tail length and relative ear length, with shorter extremities seen in mice collected farther from the equator and at colder temperatures, consistent with Allen's rule (Figures 1, S1). As with body size, a clearer picture of clinal variation emerged when only considering adult males (Figures 1D, 1F, S1D, S1F) compared to all animals. Measurements of lab mice showed that these differences are heritable (Figures 2, S2). Shorter tails in northern populations of house mice may minimize heat loss and thus be an 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.

Contributions of phenotypic plasticity to ecogeographic rules

Body size in house mice shows very little plasticity in response to cold temperature (Figure 3), 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. The environmental influence of temperature may need to occur pre-weaning or prenatal to elicit a plastic response

401 (e.g., Weaver and Ingram 1969; Burness et al. 2013; Andrew et al. 2017). Exposure to high
402 temperatures instead of low temperatures may elicit a plastic response in body size, as seen
403 previously in some endotherms (Ashoub 1958; Gordon 2012; Burness et al. 2013; Andrew et al.
404 2017).

405 Unlike Bergmann's rule, Allen's rule can be generated via developmental phenotypic
406 plasticity, as extremity length is highly sensitive to ambient temperature in both mammals and
407 birds (Serrat 2014; Tattersall et al. 2017). Our results in house mice agree with previous studies
408 in mammals, with mice growing shorter tails and ears in a cold environment (Figure 5) (Ogle
409 and Mills 1933; Harland 1960; Chevillard et al. 1963; Weaver and Ingram 1969). In laboratory
410 mice, temperature directly affects the growth of cartilage in both tails and ears, influencing
411 extremity length (Serrat et al. 2008). Furthermore, the widespread patterns of tail length
412 plasticity in response to cold are also recapitulated at the skeletal level, with both the length and
413 number of caudal vertebrae decreasing in response to cold temperatures in mice (Barnett 1965;
414 Noel and Wright 1970; Thorington Jr 1970; Al-Hilli and Wright 1983). Although we did not
415 measure skeletal differences between New York and Brazil mice, it seems likely that the tail
416 length plasticity we observed is a result of plasticity in both number and length of individual
417 caudal vertebrae. Moreover, ear length shows the greatest plasticity in both populations, with
418 both New York and Brazil mice growing shorter ears in the cold. The pronounced plastic
419 response of ears compared to tails may indicate that smaller appendages consisting entirely of
420 cartilage are less developmentally canalized. Less constraint associated with extremities may
421 also underly the highly plastic nature of Allen's rule compared to Bergmann's rule. This is
422 illustrated by tail length and ear length plasticity accounting for roughly 40% of the observed
423 differences among wild New York and Brazil house mice.

424

425 *Adaptive phenotypic plasticity and Allen's rule*

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

There are two ways by which adaptive phenotypic plasticity can facilitate the colonization of new environments. 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). Alternatively, 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). We find evidence for the first scenario for both tail and ear length in house mice. Specifically, both genetic and plastic contributions generate shorter tails and ears in colder environments. Despite the plastic response of extremity length in Brazil mice explaining roughly 40% of the mean phenotypic differences observed in wild mice, we see clear evidence of genetic differences in tail length and ear length between New York and Brazil house mice. This suggests that phenotypic plasticity moves extremity length close to the local optimum but does not shield it from subsequent selection. Overall, adaptive phenotypic plasticity 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 thank Michael Sheehan and Felipe Martins for collecting wild mice, and we thank Kathleen Ferris, Gabriela Heyer, Dana Lin, Felipe Martins, Megan Phifer-Rixey, Michael Sheehan, and Taichi Suzuki for help with mouse husbandry. We also thank Jesse Alston, Libby Beckman, Sylvia Durkin, Emilie Richards, Michelle St. John, Molly Womack, Daniel Bolnick, David Lowry, and two anonymous reviewers for their constructive feedback that improved the manuscript. Funding and support for this work was provided by the National Institutes of Health (R01 GM074245 and R01 GM127468), an American Society of Mammalogists Grant-in-Aid of Research, graduate student research funds from the Museum of Vertebrate Zoology and Department of Integrative Biology, a National Science Foundation Graduate Research Fellowship (DGE-1106400), Junea W. Kelly Museum of Vertebrate Zoology Graduate Fellowship, and a UC Berkeley Philomathia Graduate Fellowship.

Statement of Authorship

Both authors conceived and designed the study and obtained funding. M.A.B. performed data collection, analysis, and visualization with contributions and guidance from M.W.N. The manuscript was written by M.A.B. and edited by M.W.N.

Data and Code Availability

The code to perform analyses for this study is available as a git-based version control repository on GitHub (https://github.com/malballinger/Ballinger_allenbergmann_AmNat_2021). The analysis can be reproduced using a GNU Make-based workflow with built-in bash tools (v. 3.81) and R (v. 4.1.1). Supporting data files and scripts have been deposited on Zenodo (https://zenodo.org/record/5823597).

Tables

Table 1. Results of linear mixed models investigating the effects of sex, population, environment, and their interaction on body mass and extremity length in house mice.

Trait	Factor	χ^2	DF	*P*-value	Effect size (ω^2)
Body Mass (g)					
	Sex	42.15	1	< 0.001	0.36
	Population	4.00	1	0.045	0.43
	Environment	1.72	1	0.19	<0.01
Relative Tail Length (mm/g)					
	Sex	42.44	1	< 0.001	0.37
	Population	6.54	1	0.011	0.58
	Environment	25.59	1	< 0.001	0.25
	Population:Environment	3.71	1	0.054	0.04
Relative Ear Length (mm/g)					
	Sex	45.62	1	< 0.001	0.39
	Population	3.72	1	0.054	0.40
	Environment	20.07	1	< 0.001	0.21

Figures

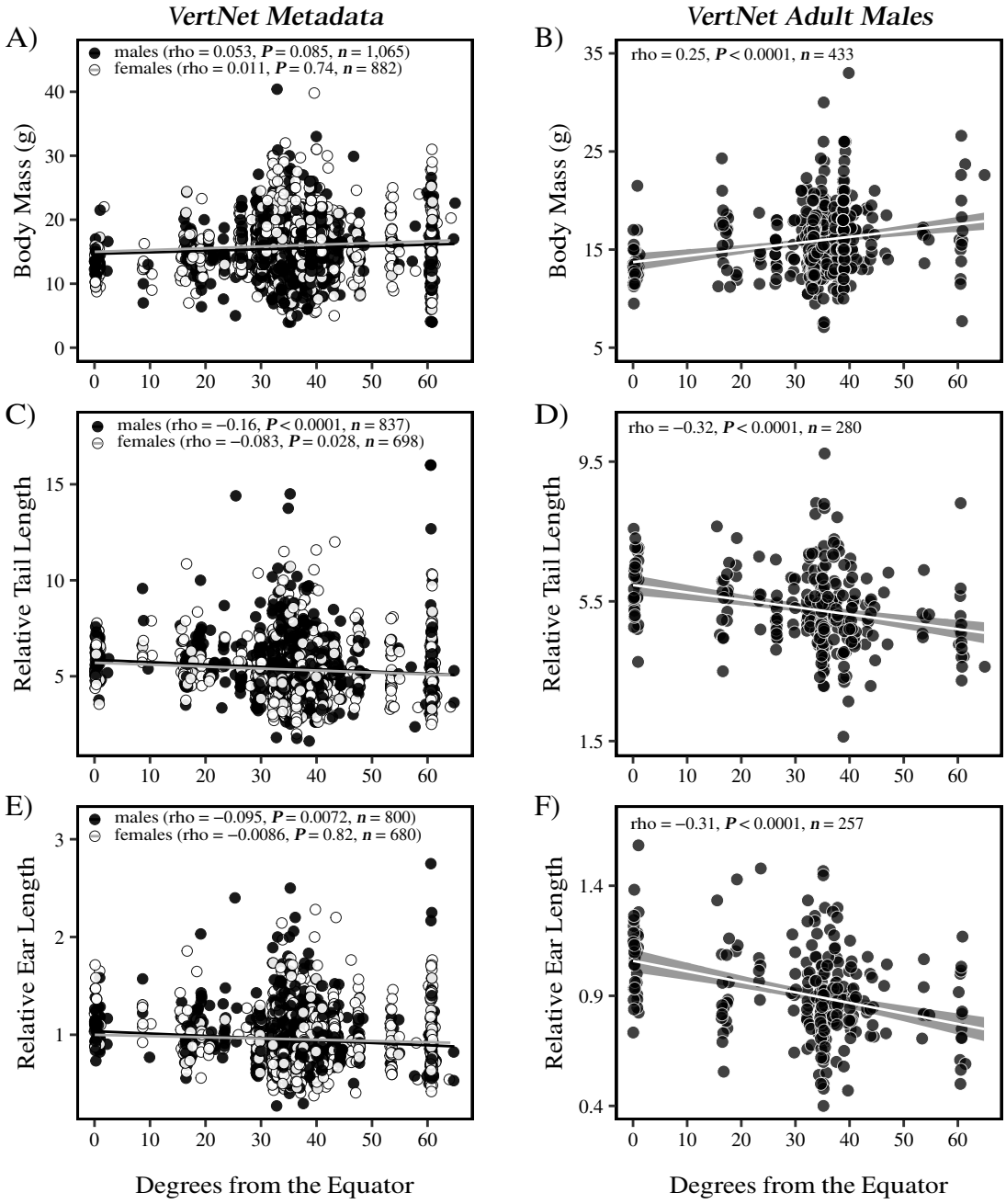

Figure 1. Bergmann's rule and Allen's rule in house mice from North and South America. Associations between body mass (A-B), tail length (C-D), ear length (E-F), and absolute latitude across wild-caught North and South American house mice. Tail length and ear length are plotted relative to body mass for each individual. Individuals are represented as individual points, with males depicted in black and females depicted in white. Results from Spearman correlations are presented in each plot, along with sample sizes. For clarity, standard error shading is omitted from linear regression lines associated with the VertNet Metadata (panels A, C, and E).

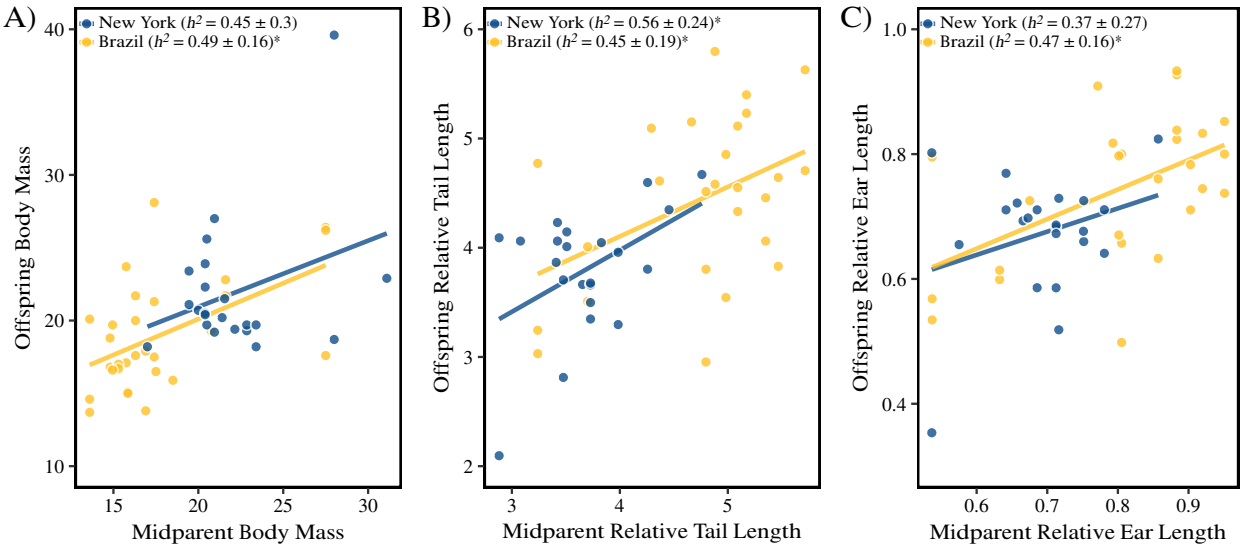

Figure 2. Heritability (h^2) estimates for body mass and extremity length in New York and Brazil house mice. Midparent-offspring regressions were performed on body mass (A), relative tail length (B), and relative ear length (C). Midparent values were calculated as the mean trait value between mothers and fathers for 13 families in each Brazil and New York populations. Heritabilities (h^2 ± standard error) were estimated as slopes of midparent-offspring regressions. Significance of each regression was assessed with ANOVAs and is represented with an asterisk. Sample sizes: New York: n = 22; Brazil: n = 26.

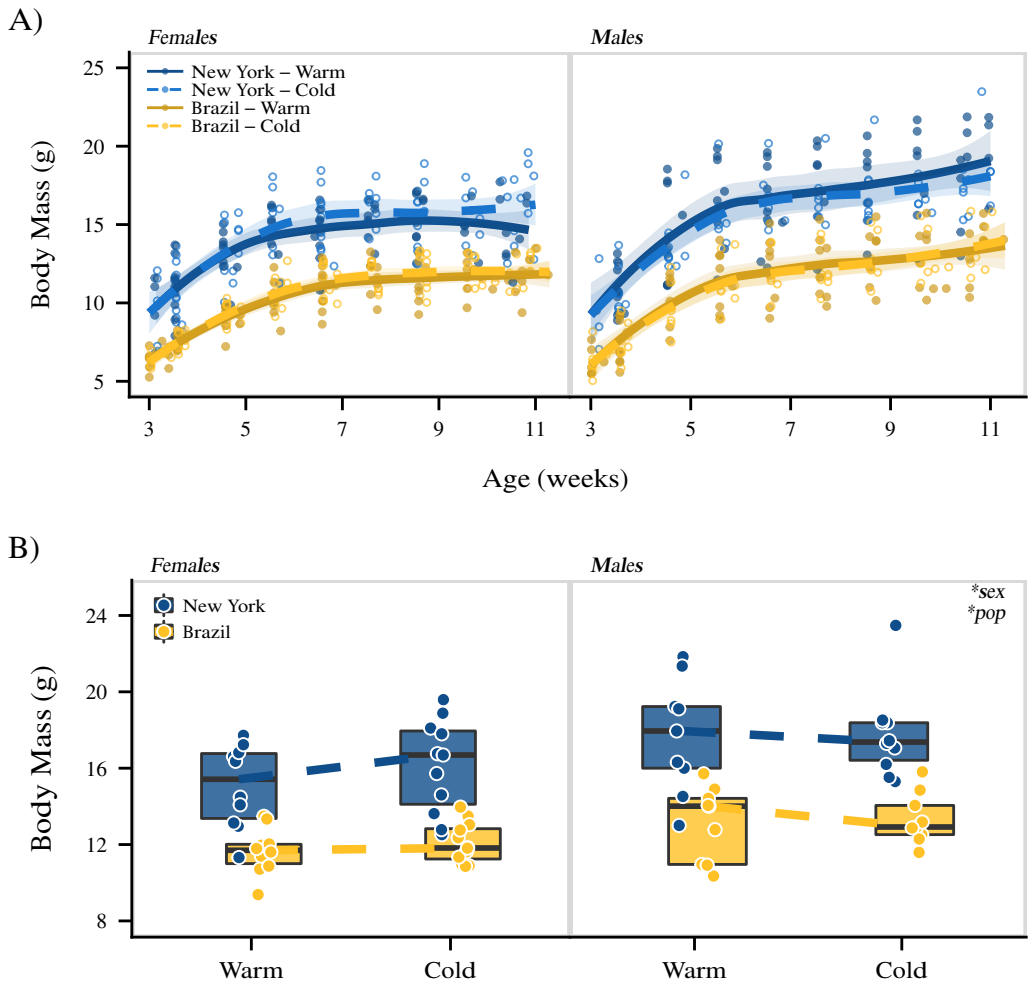

Figure 3. Genetic differences and very little plasticity in body mass among New York and Brazil house mice. A) Body mass growth trajectories across environments and 11 weeks of development in New York (blue) and Brazil (gold) house mice. Individuals are plotted as individual points, with cold-reared mice denoted as open circles and warm-reared mice denoted as filled circles (New York: $n = 20$ per treatment; Brazil: $n = 20$ per treatment). Population means are depicted as smoothed regression fits, with cold mice denoted as dashed lines and warm mice denoted as solid lines. Each line is accompanied with standard error shading. B) Individuals at 11 weeks of age are represented as individual points (New York: $n = 20$ per treatment; Brazil: $n = 20$ per treatment), and boxplots indicate the 25th, median, and 75th quartiles. Dashed lines connect median values of each genotype across warm and cold environments. Results from linear mixed models are presented in the upper right corner (*P<0.05; Table 1).

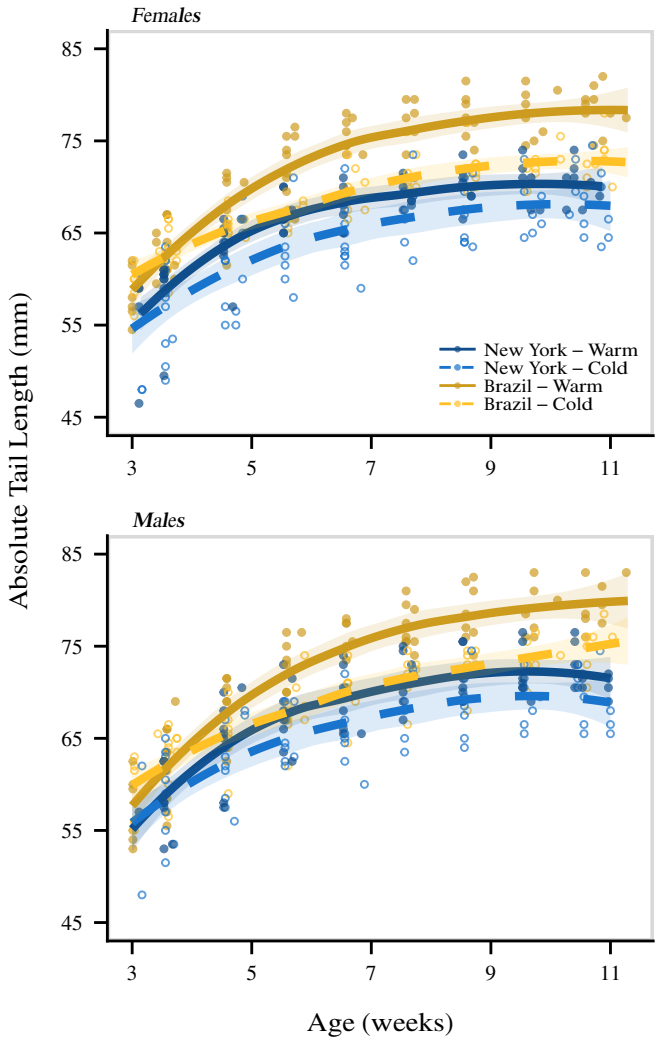

Figure 4. Tail length is highly influenced by cold temperature across development. Absolute tail length growth trajectories across environments in New York (blue) and Brazil (gold) house mice. Individuals are plotted as individual points, with cold-reared mice denoted as open circles and warm-reared mice denoted as filled circles (New York: $n = 20$ per treatment; Brazil: $n = 20$ per treatment). Population means are depicted as smoothed regression fits, with cold mice denoted as dashed lines and warm mice are denoted as solid lines. Each line is accompanied with standard error shading. The same individuals depicted here are also depicted in Figure 5.

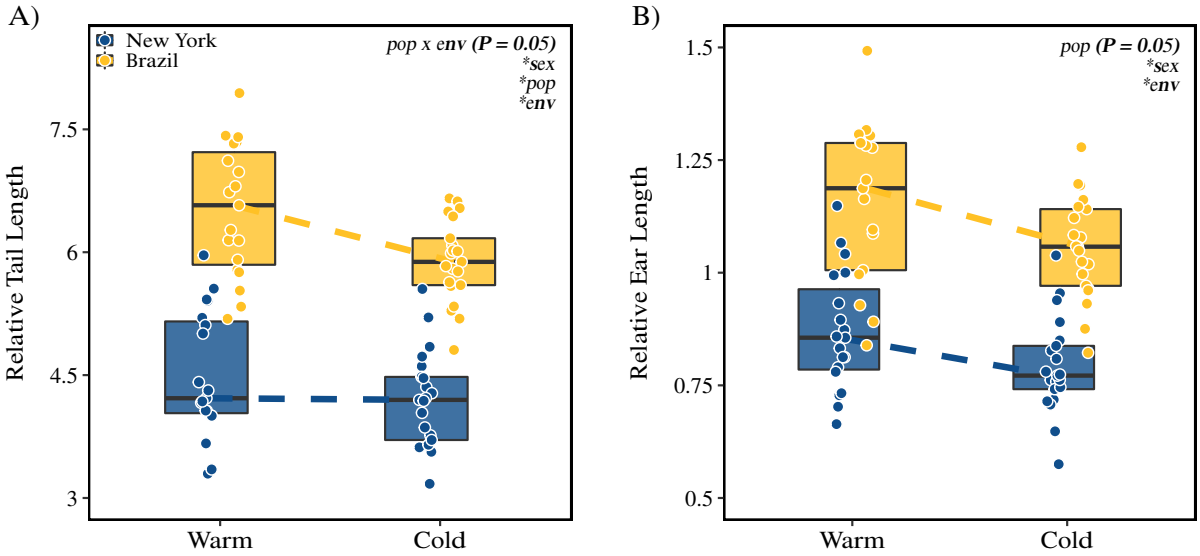

Figure 5. Adaptive phenotypic plasticity in extremity length among New York and Brazil house mice. Tail length (A) and ear length (B) are plotted relative to body mass for each individual. Individuals are represented as individual points (A) New York: $n = 20$ per treatment; Brazil: $n = 20$ per treatment; B) New York: $n = 20$ per treatment; Brazil: $n = 19$ per treatment). Boxplots indicate the 25th, median, and 75th quartiles. Dashed lines connect median values of each genotype across warm and cold environments. Both sexes were combined for simplicity. Results from linear mixed models are presented in the upper right corner (*P<0.05; Table 1). The same individuals depicted here are also depicted in Figure 4.

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757 **Supplemental Tables**

758

759 **Table S1.** Results of linear models investigating the relationship between body mass, extremity
760 length, and absolute latitude. Sex was included as a covariate in each model.

Trait	Adj. R^2	Factor	F-value	DF	*P*-value	Effect size (ω^2)
Body Mass (g)	0.008					
		Absolute Latitude	11.53	1	< 0.001	<0.001
		Sex	4.74	1	0.03	<0.001
Relative Tail Length (mm/g)	0.009					
		Absolute Latitude	14.62	1	<0.001	<0.001
		Sex	0.89	1	0.35	<0.001
Relative Ear Length (mm/g)	0.005					
		Absolute Latitude	9.21	1	0.002	<0.001
		Sex	0.08	1	0.78	<0.001

761

762 **Table S2.** Results of linear mixed models investigating the effects of sex, population, generation,
763 and their interaction on body mass and extremity length in house mice. Results of analysis are
764 plotted in Figure S2.

Trait	Factor	χ^2	DF	*P*-value	Effect size (ω^2)
Body Mass (g)					
	Sex	28.81	1	< 0.001	0.06
	Population	38.66	1	< 0.001	0.56
	Generation	9.87	3	0.020	0.01
	Sex:Generation	23.47	3	< 0.001	0.04
	Population:Generation	8.67	3	0.034	0.01
Relative Tail Length (mm/g)					
	Sex	17.46	1	<0.001	0.03
	Population	52.15	1	<0.001	0.65
	Generation	28.39	3	< 0.001	0.05
	Sex:Generation	32.22	3	<0.001	0.06
	Population:Generation	26.26	3	< 0.001	0.05
Relative Ear Length (mm/g)					
	Sex	28.95	1	< 0.001	0.06
	Population	37.04	1	< 0.001	0.58
	Generation	39.58	3	< 0.001	0.07
	Sex:Population	4.05	1	0.044	<0.01
	Sex:Generation	27.36	3	< 0.001	0.05
	Population:Generation	10.98	3	0.012	0.02

765

Supplemental Figures

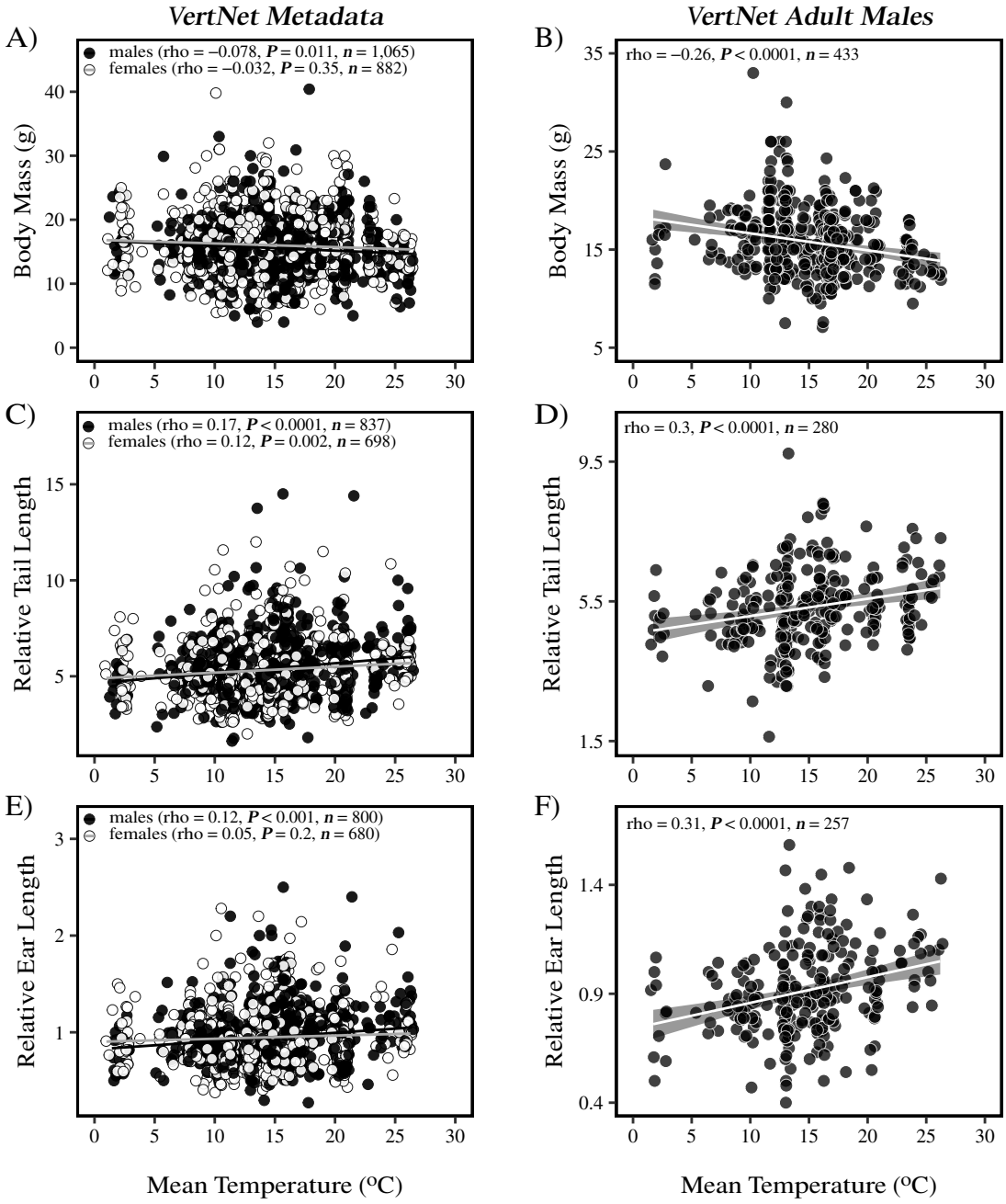

Figure S1. Bergmann's rule and Allen's rule in house mice from North and South America. Associations between body mass (A-B), tail length (C-D), ear length (E-F), and mean annual temperature (°C) across wild-caught North and South American house mice. Tail length and ear length are plotted relative to body mass for each individual. Individuals are represented as individual points, with males depicted in black and females depicted in white. Results from Spearman correlations are presented in each plot, along with sample sizes. For clarity, standard error shading is omitted from linear regression lines associated with the VertNet Metadata (panels A, C, and E).

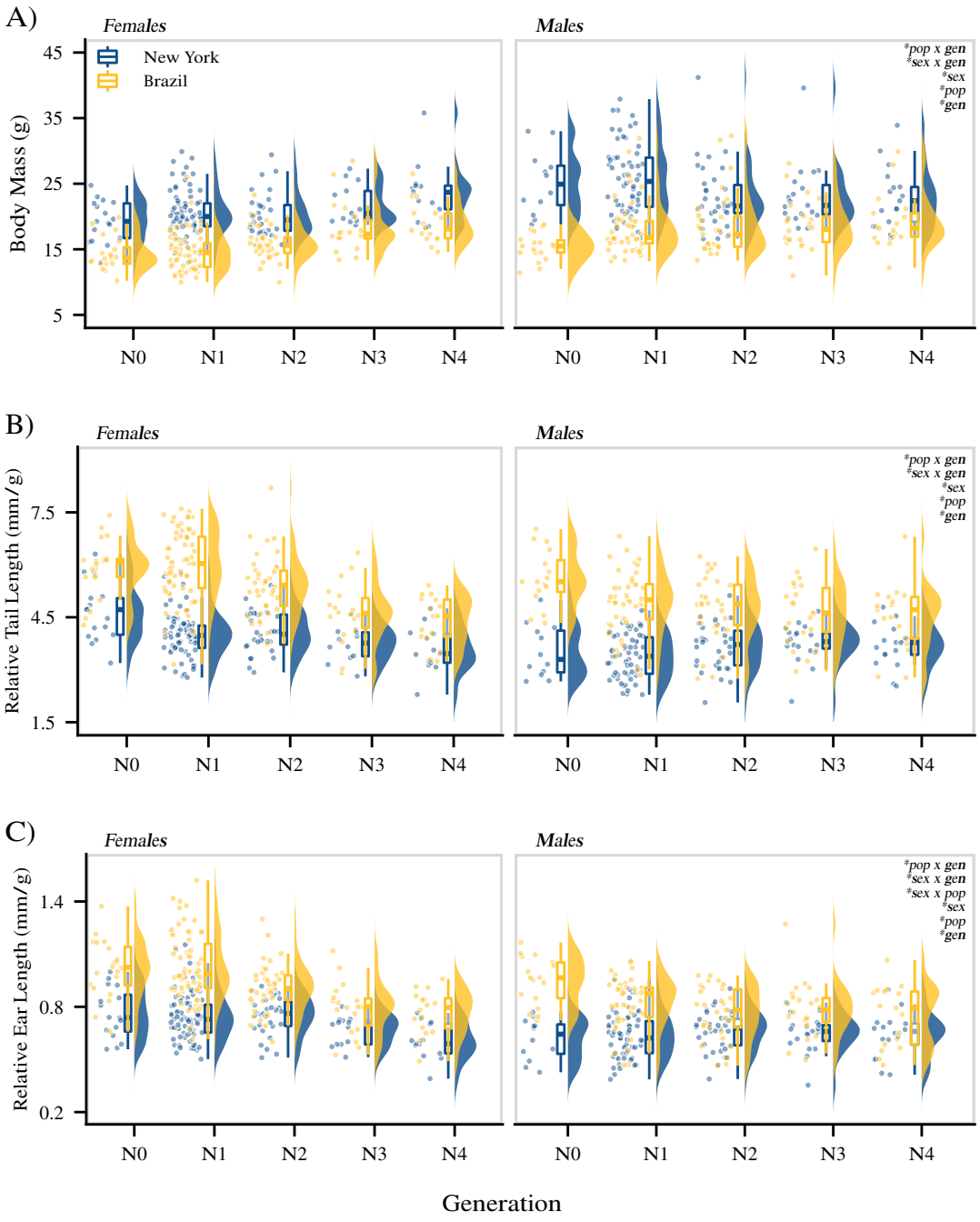

Figure S2. Body mass and extremity length differences among populations persist over generations in a common lab environment. Differences in body mass (A), tail length (B), and ear length (C) between New York mice (blue) and Brazil mice (gold) across generations. Tail length and ear length are plotted relative to body mass for each individual. Population-level data are depicted 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 models are presented in the upper right corner of each panel (*P<0.05; Table S2). Abbreviations: N0 = generation 0 (wild mice); N1 = generation 1; N2-N4 = generations 2-4. Sample sizes: (A) Females (New York: n = 146; Brazil: n = 157); Males (New York: n = 140; Brazil: n = 133); (B) Females

(New York: $n = 145$; Brazil: $n = 156$); Males (New York: $n = 140$; Brazil: $n = 125$); (C) Females (New York: $n = 145$; Brazil: $n = 156$); Males (New York: $n = 139$; Brazil: $n = 128$).

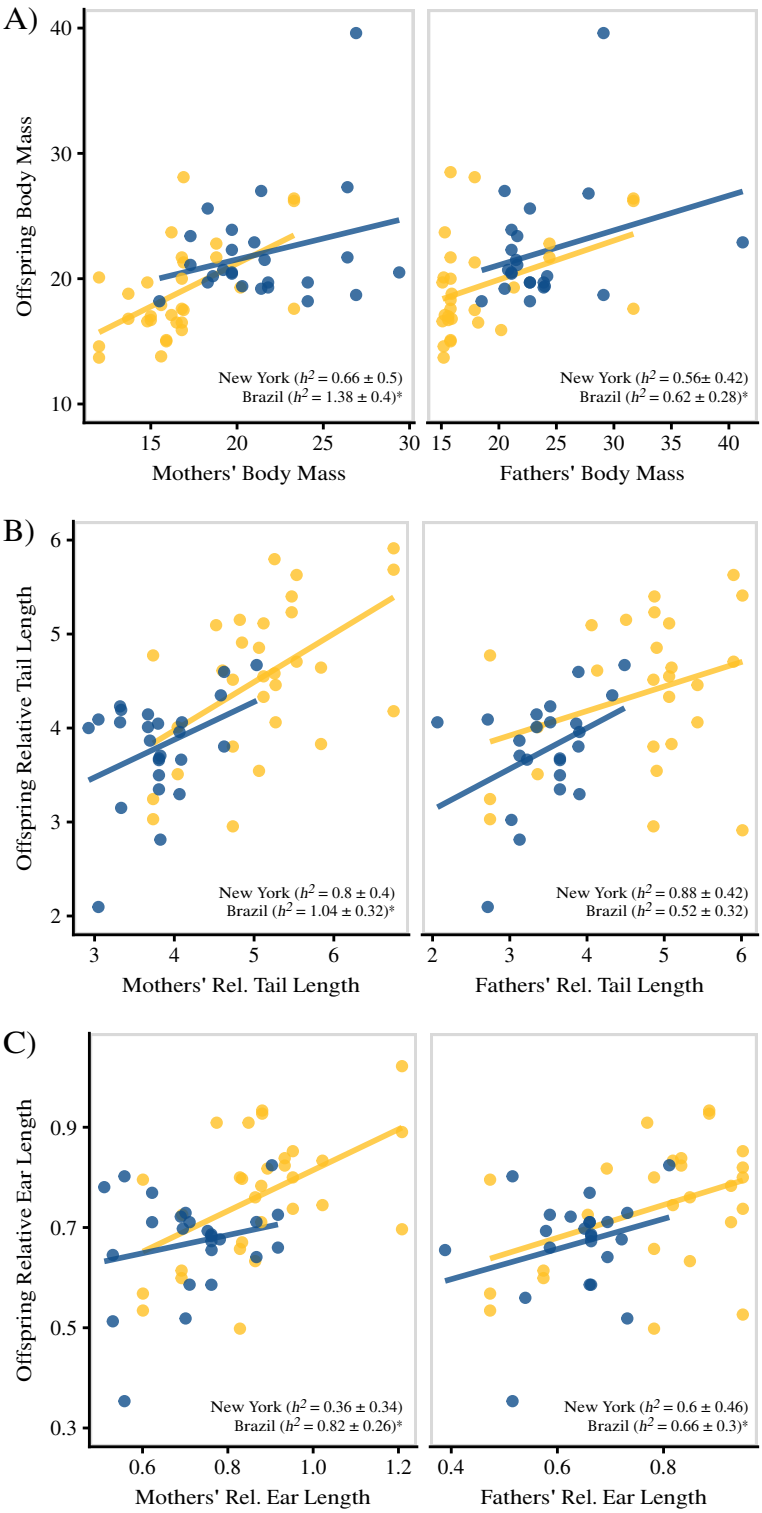

Figure S3. Maternal- and paternal-offspring heritability (h^2) estimates for body mass and extremity length in New York and Brazil house mice. Maternal-offspring and paternal-offspring regressions were performed on body mass (A), relative tail length (B), and relative ear length (C). Heritabilities (h^2 ± standard error) were estimated as twice the slope and standard error of

single-parent offspring regressions. Heritability values higher than one are the result of doubling statistics with large standard errors. Significance of each regression were assessed with ANOVAs and are represented with an asterisk. Sample sizes: New York: $n = 25$; Brazil: $n = 30$.

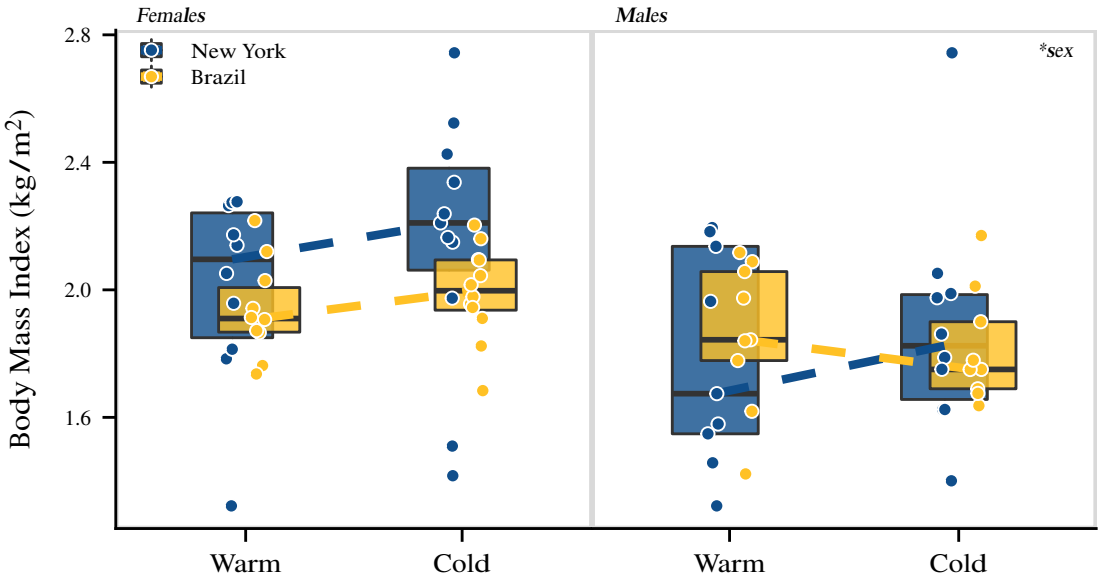

Figure S4. No genetic differences or plasticity in body mass index (BMI) among New York mice and Brazil mice. Individuals are represented as individual points (New York: $n = 20$ per treatment; Brazil: $n = 20$ per treatment), and boxplots indicate the 25th, median, and 75th quartiles. Dashed lines connect median values of each genotype across warm and cold environments. The same individuals depicted here are also depicted in Figure 3.