

# cis-regulatory changes are environmentally robust and underlie rapid climatic adaptation

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1 **Gene regulation plays an important role in adaptive evolution, yet  
2 little is known how the environment, tissue-type, or sex modulates  
3 gene regulatory evolution. Here, using wild-derived inbred lines of  
4 house mice collected from temperate and tropical environments, we  
5 determine the role of gene regulatory evolution in rapid, environmental  
6 adaptation.**

gene regulation | rapid adaptation | *Mus* | cis-regulation | genotype-by-environment

1 Understanding how organisms adapt to new environments  
2 is a major goal of evolutionary biology. Gene regulation has  
3 been shown to play an important role in adaptation to new  
4 environments (or something about rapid adaptation). In fact,  
5 cis-regulated genes tend to be drivers of adaptation. However,  
6 many of these studies have only characterized cis/trans under  
7 one environmental context, or in one tissue, or just one sex.  
8 Gene regulation is highly context-dependent, and in order  
9 to fully understand the role of gene regulation in adaptation,  
10 need to investigate gene regulation in a complex manner. Gene  
11 regulation plays an important role in environmental adaptation  
12 (Emerson and Li 2010; Signor and Nuzhdin 2018), with both  
13 cis- and trans-regulatory changes underlying evolved gene  
14 expression differences. While the evolutionary significance of  
15 gene regulation is well recognized, we have little understanding  
16 of how the environment, tissue-type, or sex influences the  
17 regulatory architecture of adaptive evolution. It has been  
18 well demonstrated that gene expression is highly dependent  
19 on the environment (Gibson 2008; Aubin-Horth and Renn  
20 2009; Hodgins-Davis and Townsend 2009; Grishkevich and  
21 Yanai 2013), and that expression plasticity can facilitate or  
22 constrain adaptation to new environments (Ghalambor et  
23 al. 2007, 2015). Moreover, population genetic variation for  
24 plasticity (i.e., genotype-by-environment interactions, GxE)  
25 may constitute a large proportion of gene expression variation  
26 (Hodgins-Davis and Townsend 2009; Grishkevich and Yanai  
27 2013), highlighting the important role the environment plays  
28 in the evolution of gene regulation. Yet, we still have little  
29 understanding of how plasticity in gene expression is regulated  
30 at the molecular level, leaving the question regarding the role  
31 of gene regulation in environmental adaptation unresolved.

32 patterns of gene regulation (intraspecific) between pop-  
33ulations that have been diverged for awhile. Our study is  
34 of house mice that have only been diverged recently (rapid  
35 adaptation) very few studies have looked at how environment  
36 mediates regulatory patterns, especially in endotherms Few  
37 studies have characterized the relative contributions of cis- and  
38 trans-regulation to environment-dependent gene expression  
39 (Signor and Nuzhdin 2018). In yeast and *C. elegans*, cis-acting  
40 changes in gene expression tend to be robust and insensitive to  
the environment (Li et al. 2006; Smith and Kruglyak 2008; Li  
41 and Fay 2017)), suggesting that most cis-regulatory divergence

43 is not associated with expression plasticity. Conversely, trans  
44 effects tend to exhibit greater sensitivity to environmental  
45 change (Smith and Kruglyak 2008; Tirosh et al. 2009; Cubillos  
46 et al. 2014), and thus seem to play larger roles in regulat-  
47 ing gene expression plasticity. Moreover, genetic variation  
48 for plasticity (GxE) tends to be modulated by trans effects  
49 (Grishkevich and Yanai 2013), likely due to the large muta-  
50 tional target of transcription factors (Landry et al. 2006, 2007;  
51 Grishkevich and Yanai 2013; Hill et al. 2020). Overall, investi-  
52 gations into these patterns have been taxonomically limited,  
53 with most studies conducted in yeast, *C. elegans*, and plants.  
54 Very few studies have investigated the gene regulatory basis  
55 of plasticity in natural populations (but see (Verta and Jones  
56 2019; Tangwancharoen et al. 2020)). Thus, relatively little is  
57 known about the regulatory architecture of plasticity and the  
58 contributions of cis and trans changes in expression plasticity  
59 underlying environmental adaptation.

60 House mice, *Mus musculus domesticus*, provide a unique  
61 opportunity to assess the environmental influence on gene  
62 regulation. House mice have rapidly adapted to various envi-  
63 ronments across the Americas, and gene regulation has been  
64 shown to play an important role in this process. For example,  
65 cis-expression quantitative trait loci (cis-eQTLs) associated  
66 with adaptive body size variation have been identified in pop-  
67ulations of house mice along the east coast of North America  
68 (Mack et al. 2018; Phifer-Rixey et al. 2018). Moreover, house  
69 mice collected from desert and temperate environments show  
70 varying levels of gene expression plasticity, with differences  
71 in plasticity reflecting local adaptation (Bittner et al. 2021).  
72 These studies demonstrate the important role that gene reg-  
73 ulation has played in the rapid colonization of house mice  
74 to various environments. However, it remains unclear how  
75 the environment influences the gene regulatory architecture  
76 of house mice and whether the relative contributions of cis  
77 and trans effects underly patterns of adaptive evolution in  
78 house mice. House mice are perfect since they've recently

## Significance Statement

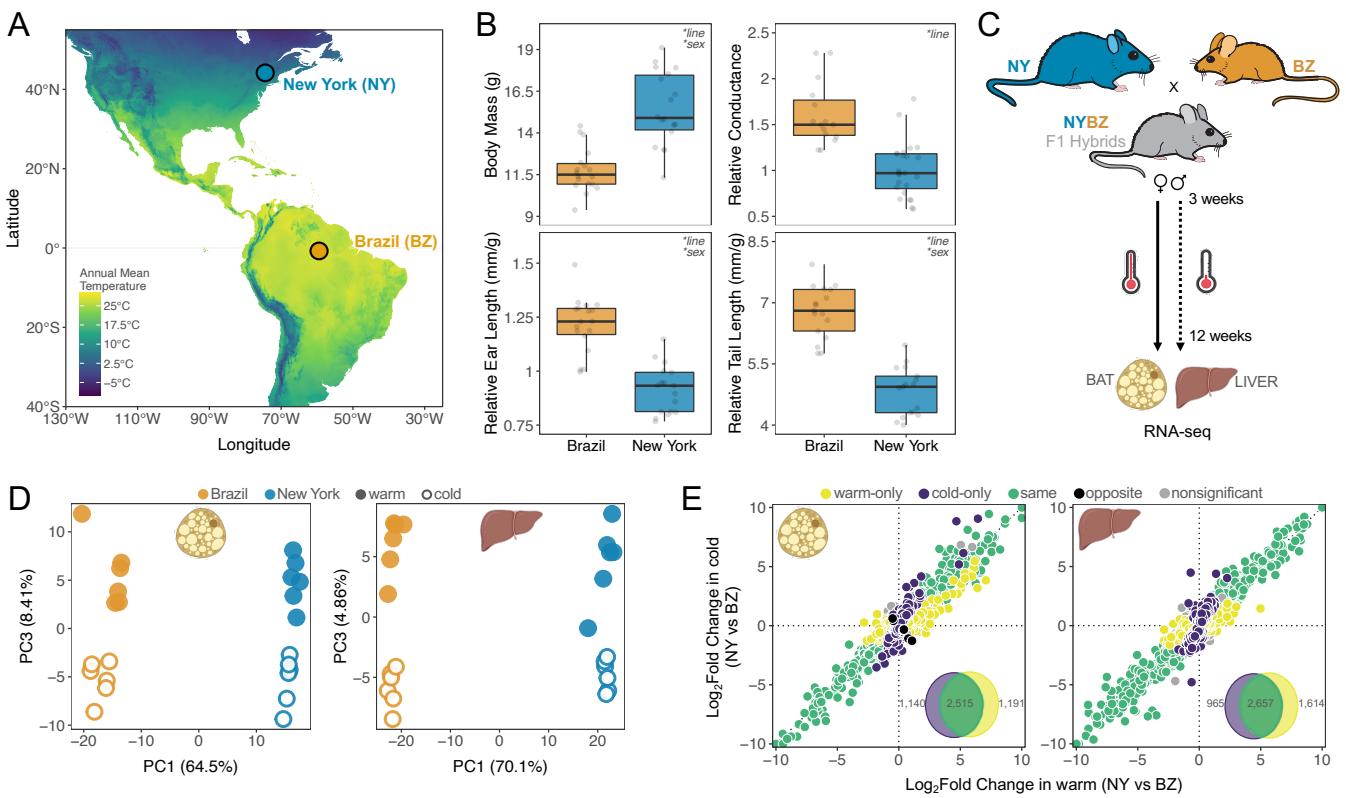
Organisms rapidly adapt to new environments through changes in gene regulation. In particular, cis-regulatory changes have been shown to play major roles; however, understanding how the environment, tissue-type, or even sex modulates gene regulatory evolution is lacking.

M.A.B. and M.W.N. designed research. M.A.B., S.M.D., and E.A.R. performed research. M.A.B. and K.L.M. analyzed data. M.A.B., K.L.M., and M.W.N. wrote the paper.

The authors declare no conflict of interest.

<sup>1</sup> M.A.B. and K.L.M. contributed equally to this work.

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**Fig. 1.** This is Figure 1, which introduces the awesome system of house mice we have. (A) Mice are found throughout North and South America, and throughout this invasive range, temperature is major climatic variable. We are using wild-derived inbred lines of house mice collected from upstate New York (brrrr) and equatorial Brazil (it's gettin' hot in here). (B) Mice collected from these localities show differences in thermoregulatory phenotypes after many generations in the lab, indicating a genetic basis. (C) For this study, specifically, we took an allele-specific approach across two tissues, two sexes, and two environments ( $2 \times 2 \times 2$ ) to identify the gene regulatory signature of rapid adaptation.

adapted to varying thermal regimes. And gene regulation has been shown to play an important role in this adaptation. However, fully understanding how the environment modulates gene regulation, and the influence of tissue-type or sex is not well understood.

Here, we use two wild-derived inbred lines of house mice collected from climatically distinct environments to assess the influence of plasticity on gene regulation. Specifically, we use house mice collected from upstate New York and equatorial Brazil as they have rapidly adapted to climatically divergent environments. New York mice are larger with shorter extremities than Brazil mice, presumably reflecting adaptations to a cold environment (Ballinger and Nachman 2022; Figure 1). As phenotypic adaptation often involves changes in gene regulation OR To determine the regulatory mechanisms underlying these patterns of adaptation, we first characterize expression differences between New York and Brazil lines across two tissues, two temperatures, and both sexes. We next examine allele-specific expression (ASE) in F1 hybrids to characterize regulatory divergence between lines and identify the contributions of *cis* and *trans* changes to adaptive expression differences. We then explore the degree of plasticity in gene regulation by comparing *cis*- and *trans*-effects across temperatures. Lastly, we identify *cis*- and *trans*-by-environment interactions underlying genes that show patterns of adaptive plasticity. Overall, our findings highlight the role the environment plays in adaptive gene regulation in house mice.

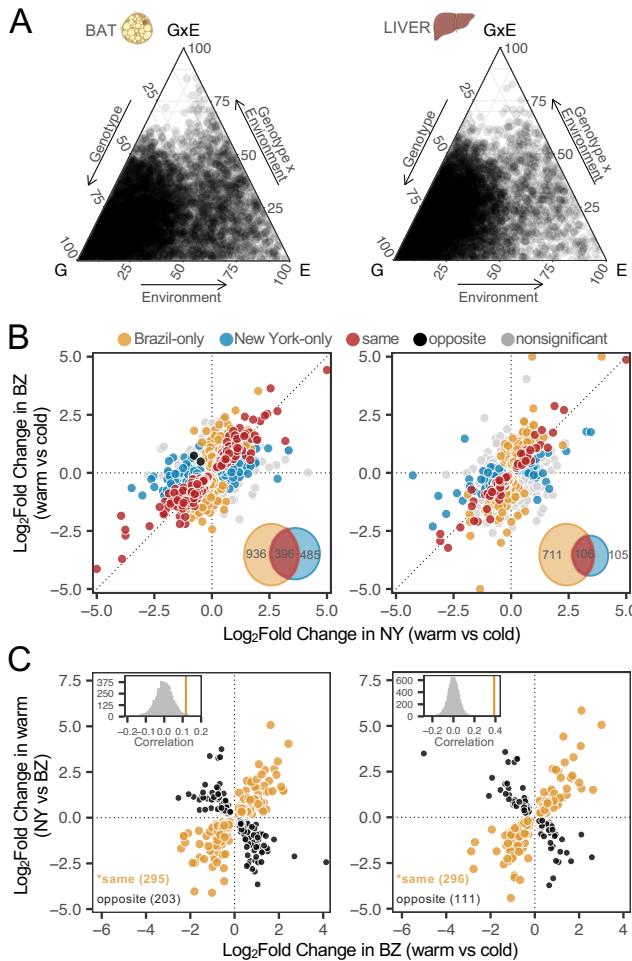
While both liver and BAT both play essential roles in

homeostasis and metabolism, these tissues have distinct functional properties that may lend differentially to their role in environmental adaptation.

We determine the role of gene regulation in rapid adaptation by characterizing gene expression patterns in wild-derived inbred lines of house mice that were collected from cold and warm environments. We expose these warm- and cold-adapted lines to warm and cold temperatures and determine the relative contributions of *cis/trans* across environments, tissues, and sexes. (Given that both New York and Brazil mice have adapted to divergent thermal environments, we reasoned that temperature-dependent gene regulation may play a role in local adaptation. We therefore asked how the environment modulates gene regulatory evolution). We also determine if *cis*-regulatory variations are under positive selection in wild mice collected from the warm and cold populations. We find that *cis*-regulatory changes are the predominant gene regulatory signature, highlighting the rapid evolution of *cis*. We also show that these *cis*-genes are robust to temperature and sex, and both tissues show the same pattern. Finally, we overlap these *cis*-regulated genes with PBSn1 outliers and identify regions of the genome that are associated with environmental adaptation.

## Results

**Extensive divergence in gene expression between New York and Brazil mice.** As phenotypic variation is often associated with gene expression divergence, we first explored patterns of



**Fig. 2.** Gene expression patterns of New York and Brazil male house mice. Female-specific patterns are presented in Figure SX.

tissues exhibited strong patterns of divergence, regardless of the environment (effect size of G » effect size of E; Fig 2B; Figure SX (divergence expression)). (BUT) BAT showed greater proportion of E genes compared to liver (diff effect sizes), highlighting the highly plastic nature of BAT (fig Sx - BAT plasticity) and overlal tissue-specific differences in gene expression plasticity.

Despite this strong signal of divergence, of genes (XXX) and (XXX) showed genotype x environment interactions (GxE) in BAT and liver, respectively (Figures 2C and SX(females)). (AND) Across both tissues, Brazil mice harbored more DEG than New York mice (Figure 3), with New York mice showing canalization of plasticity between environments (Figure 2). - also include volcano plot of DEG for each tissue? (AND) Given the large number of plastic genes in Brazil, we next asked whether the plastic response of these genes correlated with expression divergence between New York and Brazil lines. Specifically, we asked if the logFC of Brazil warm and Brazil cold was positively correlated with the logFC of New York warm and Brazil warm. (AND) Across both tissues, plasticity in Brazil was positively correlated with divergence, suggesting that overall patterns of expression plasticity in response to cold temperatures are adaptive. consistent with previous studies in house mice (Bittner et al. 2021). (THEREFORE) Together, these results demonstrate that, while the majority of differentially expressed genes show strong patterns of divergence (expression differences are concordant across environments) across environments, a subset of genes show genotype-specific regulation in response to temperature (i.e., genetic variation for plasticity (GxE)).

.1. **Genotype-by-Sex.** Although males and females showed similar patterns of gene expression across tissues and environments, we did identify genotype x sex interactions for each tissue and environment separately (see Methods). Specifically, males harbored more DEG than females and RESULTS Given the strong influences of both tissue and sex (see Figure SX), we performed downstream analyses for each tissue and sex separately. These results again demonstrate that divergence is playing a significant role, while tissue, sex, and environment specific effects play less roles.

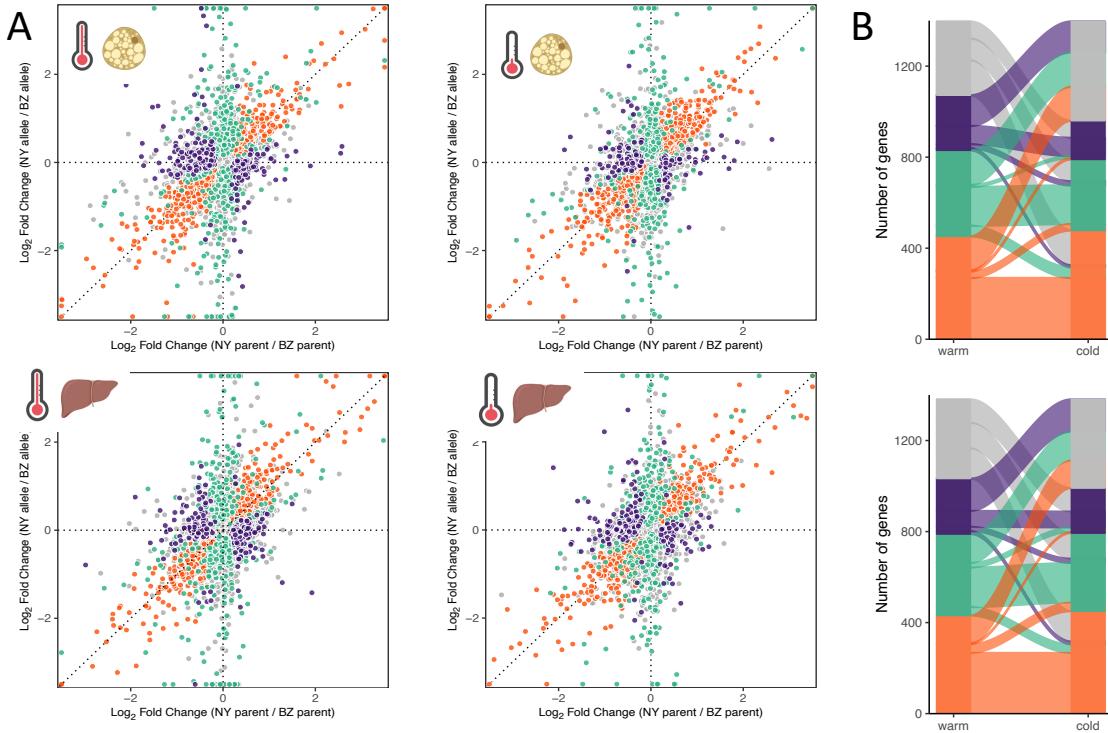
**Expression divergence is predominantly due to *cis*-regulatory changes, which are robust to environmental temperature.** To investigate the gene regulatory mechanisms underlying expression differences between New York and Brazil mice, we generated BAT and liver RNA-seq from New York (dam) x Brazil (sire) F1 hybrids that were also reared in warm or cold environments. Dominance of gene expression

Measuring gene expression in F1 hybrids allows us to discern if parental gene expression differences are due to *cis*- and/or trans-acting changes by assessing patterns of allele-specific expression (ASE): differences in expression between alleles are indicative of *cis*-regulatory divergence, while differences observed between parental lines but not in F1 hybrids are due to trans-acting variation.

Overall, we detected a total of 5,898 genes with ASE based on the presence of fixed differences between parental Brazil and New York lines (see Methods), with both liver and BAT harboring similar numbers of gene underlying regulatory divergence (Figure 4). These results demonstrate that *cis*-regulatory evolution underlies divergence between wild mouse populations,

gene expression divergence by rearing New York (SARA) and Brazil (MANA) mice under two temperatures and sequenced <brown adipose tissue (BAT) and liver transcriptomes of 48 individuals (6 x line x sex x environment; Figure 1C). Principal component analysis (PCA) revealed tissue-type as the largest source of variance in transcriptional profiles (PC1 ~97% of variance explained; Figure S1), while sex explained the second-most variation (PC2 ~1.5%; Figure S1). Within each tissue and sex, mice separated by genotype along PC1 (>60% of variance explained), while PC3 largely separated warm- and cold-reared mice (Figures 1D and SX(males PC2 and females PC1-3)). Within a temperature regime, roughly 35% of the genes were differentially expressed between BZ and NY, with greater expression divergence in liver (X# of DEG or 34% of all expressed genes) compared to BAT (xK DEG or 37% of all expressed genes). Both temperature regimes within a tissue showed similar expression divergence. (expression differences are concordant across environments)

The strong patterns of divergence were also apparent when we categorized differentially expressed genes as those showing genetic variation (G), environmental variation [i.e., plasticity (E)], or genetic variation for plasticity (i.e., G x E) (Figure 2B; Figure SX), genotype explained the most variation, as the vast majority of differentially expressed genes in both



**Fig. 3.** This is Figure 1, which introduces the awesome system of house mice we have. (A) Mice are found throughout North and South America, and throughout this invasive range, temperature is major climatic variable. We are using wild-derived inbred lines of house mice collected from upstate New York (brrrr) and equatorial Brazil (it's gettin' hot in here). (B) Mice collected from these localities show differences in thermoregulatory phenotypes after many generations in the lab, indicating a genetic basis. (C) For this study, specifically, we took an allele-specific approach across two tissues, two sexes, and two environments (2x2x2) to identify the gene regulatory signature of rapid adaptation.

219 consistent with previous studies in house mice (refs.).

220 Given that both New York and Brazil mice have  
 221 adapted to divergent thermal environments, we reasoned that  
 222 temperature-dependent gene regulation may play a role in  
 223 local adaptation. We therefore asked how the environment  
 224 modulates gene regulatory evolution by comparing patterns  
 225 of cis- and trans-regulatory differences across environments.  
 226 Similar to differential expression patterns in the parents, the  
 227 majority of genes showed the same gene regulatory patterns  
 228 between environments (88%), suggesting that cis- and trans-  
 229 effects play larger roles in expression divergence than that of  
 230 plasticity. Moreover, when we compared the difference in mag-  
 231 nitude of cis- and trans-differences between environments, we  
 232 found that cis-regulatory changes were robust to environmen-  
 233 tal temperature while trans-differences were greater between  
 234 environments for both tissues (Wilcoxon signed-rank test,  $P >$   
 235 0.05; Figure 4B, 4D - effect sizes). We also found that the pro-  
 236 portion of genes with only cis-divergence was the same across  
 237 environmental temperatures (Chi-square tests: BAT,  $p=0.51$ ;  
 238 liver  $p=0.66$ ), while the cold environment harbored a lower  
 239 proportion of genes with trans-divergence (Chi-square tests:  
 240 BAT,  $p=0.0003$ ; liver,  $p=0.02$ ). These results suggest that  
 241 trans-effects are more sensitive to environmental condition,  
 242 consistent with previous studies (refs.).

243 Although most gene regulatory patterns are robust to environ-  
 244 ment, genes showing GxE patterns may be controlled by  
 245 environment-specific regulation. To robustly identify genes for  
 246 which there was a significant effect of temperature on regu-  
 247 latory divergence, we next asked if either the *cis* component  
 248 and/or *trans* component showed a significant interaction with

temperature.

249 *Trans*-by-treatment identified for 19 genes in BAT and liver  
 250 (Table XX)(FDR<0.1). *Cis*-by-treatment/temperature effects  
 251 were identified for 11 genes in BAT (gstt1, wars2, hsd11b1,  
 252 itih5, dst, tmed2, plbd1, cdh13, scd1, tmem45b, s100a13)  
 253 and 4 in the liver (elovl3, hmgs2, wars2, ebpl) (FDR<0.1).  
 254 Scd1 is a central lipogenic enzyme catalyzing the synthesis  
 255 of monounsaturated fatty acids and plays an important role  
 256 in basal and cold-induced thermogenesis. Scd1 is required  
 257 for maintaining body temperature under cold temperatures,  
 258 and mice from New York show higher average expression of  
 259 this under warm conditions in BAT than mice from Manaus.  
 260 Elovl3 expression is also involved in the response to exposure  
 261 to cold stress and mutants show defects in lipid recruitment  
 262 in response to cold exposure. Where most of these are cases  
 263 where allele-specific expression differs in magnitude between  
 264 temperature treatments, we also observed cases where allelic-  
 265 specific expression was induced by one temperature treatment  
 266 (i.e., wars2, tmed2, cdh13, s100a13, ebpl, hmgs2).

267  
**Cis-regulatory changes are largely tissue-biased and are re-**  
**268**  
**lated to body size and metabolism.** While both liver and BAT  
 269 both play essential roles in homeostasis and metabolism, these  
 270 tissues have distinct functional properties that may lend differ-  
 271 entially to their role in environmental adaptation. Comparing  
 272 gene expression evolution in BAT and liver, we found regula-  
 273 tory divergence to be largely tissue-biased. The majority of  
 274 genes (80%) with evidence for regulatory divergence in at least  
 275 one of the tissues were not categorized as having the same un-  
 276 derlying regulatory divergence in the other tissue (2954/3672  
 277

278 genes). In particular, we found that *trans*- divergence was more  
279 likely to be restricted to one tissue (with expression conserved  
280 between populations in the other), compared to *cis*- changes  
281 which were more often shared (>2-fold more)(Chi-square test  
282 p<0.0001; Tables SX). This may reflect the general observation  
283 of increased tissue-specificity of trans-effects relative to  
284 cis-effects [15].

285 Contrasting allele-specific expression measurements in BAT  
286 and liver for paired hybrid samples, we identified 338 genes  
287 with evidence for differential allele-specific expression between  
288 tissues (Figure 3A). While the majority of these genes (77%)  
289 showed significant allele-specific expression in just one tissue,  
290 we also identified cases where allele-specific was present in both  
291 tissues but with differences in the magnitude or directionality  
292 (23%). Forty-three genes of these genes were found to have  
293 discordant allele-specific expression between tissues, where  
294 the opposite parental allele was up-regulated between tissues.  
295 Genes with tissue-biased ASE were enriched for metabolic  
296 phenotypes (e.g., abnormal lipid homeostasis, q=0.00027; in-  
297 creased food intake, q=0.036) and tissue specific functions and  
298 physiology (e.g., abnormal adipose tissue physiology, q=0.007;  
299 and abnormal liver morphology (q=0.00097). <reference  
300 petrov or pritchard, in which they're excited about cis-genes  
301 that are found in one tissue only>

302 Given that most regulatory divergence between New York  
303 and Brazil house mice is governed in *cis*, and because *cis*-  
304 regulatory variations are often drives of local adaptation (refs),  
305 we reasoned that genes with evidence for allele-specific expres-  
306 sion between lines should be enriched for similar functional  
307 roles. In both the liver and BAT, genes with evidence for *cis*-  
308 divergence were enriched for GO terms related to metabolic  
309 processes, as well as the Reactome pathway for metabolism  
310 (Liver q=6.55 x10-8, BAT q=1.49 x 10-8) compared to the  
311 background set of genes that were tested for allele-specific ex-  
312 pression. Genes with *cis*- regulatory changes in the liver were  
313 also enriched for several mutant phenotype annotations for  
314 homeostasis and metabolism, including abnormal lipid home-  
315 ostasis (q= 6.248 x 10-5), abnormal cholesterol level (q=0.003),  
316 and abnormal energy expenditure (q=0.001), abnormal triglyc-  
317 eride level (q=0.008). Additionally, genes with *cis*- changes  
318 in the liver also showed a greater than 2-fold enrichment of  
319 genes with mutant phenotypes for abnormal susceptibility to  
320 weight gain (q=0.014), and were also nominally significantly  
321 enriched for several other phenotypes related to body weight,  
322 size, and composition (e.g., increased body size and weight,  
323 increased food intake, abnormal percent body fat/body weight,  
324 decreased susceptibility to diet-induced obesity) (Table SX).  
325 Among these are genes (i.e., bcat2, adam17) for which expres-  
326 sion levels in the liver and *cis*-eQTL were previously associated  
327 with body mass variation in wild populations of North Ameri-  
328 can house mice [14] as well as genes with well-documented roles  
329 in body weight variation other species (e.g., lepr, fads2, bbs1,  
330 prkar2b). These results further support the strong divergence  
331 in body size we see between NY and BZ mice, as body size is  
332 canalized and shows no plasticity (Figure SX).

333 **Selection on genes with *cis*-regulatory divergence in wild**  
334 **house mouse populations.** To understand regulatory diver-  
335 gence between these lines in the context of genetic divergence  
336 between populations from these localities, we analyzed popu-  
337 lation genomic data from wild-caught individuals. We com-  
338 pared complete exomes sequenced at moderate coverage for

339 18 individuals captured in New Hampshire/Vermont (10 indi-  
340 viduals)(NH/VT)(cite megan) and Manaus (8 individuals).

341 Genetic PCA clearly distinguished populations based on geo-  
342 graphy. Mice from the Americas were compared to previously  
343 published population genomic data from Eurasian populations  
344 of *M. domesticus* (cite Harr)(Figure 4). LD-pruned genotype  
345 data clustered all individuals by subspecies and *M. m. domes-*  
346 *ticus* by population-of-origin (Figure SX). Consistent with the  
347 suggestion that mice from eastern North America are most  
348 closely related to populations in northern Europe, mice from  
349 NH/VT clustered most closely with mice from Germany (cite  
350 Tichy et al. 1994, Morgan et al. BioRxiv paper).

351 To identify genetic signatures of adaptation in house mice  
352 from the Americas, we performed a scan for regions of genetic  
353 differentiation consistent with selection using a normalized  
354 version of the population branch statistic (PBSn1). PBSn1  
355 captures loci where allele frequencies are especially differen-  
356 tiated in a focal population when compared with two other  
357 populations (Yi et al. 2010; Crawford et al. 2017). We used  
358 this test to identify highly differentiated loci in our focal pop-  
359ulations in the Americas, Manaus and New York, relative to  
360 Eurasian populations (see Methods). In total, 83,538 and  
361 84,420 non-overlapping five-SNP windows were analyzed for  
362 Manaus and NH/VT, respectively. We considered windows as  
363 outliers if they fell within the top 1% of windows for PBSn1  
364 score. Outlier windows in NH/VT and Manaus overlapped  
365 538 and 530 genes, respectively (File SX).

366 <Justification for looking at *cis*- overlap.> Next, we over-  
367 lapped candidate regions for selection based on PBSn1 out-  
368 lier windows with genes for which we identified evidence for  
369 *cis*- regulatory changes in BAT or the liver. In NH/VT, we  
370 identified 99 outlier windows that overlapped 64 genes with  
371 evidence for *cis*- regulatory divergence (Figure 4). These  
372 genes were enriched for mutant phenotypes related body size  
373 and growth relative to other genes with *cis*- divergence be-  
374 tween populations: (1) growth/size/body region phenotype  
375 (q=0.008; 1.74-fold enrichment, 30 genes), (2) abnormal post-  
376 natal growth/weight/body size (q=0.026; 1.97-fold enrichment,  
377 23 genes), and (3) abnormal body composition (q=0.05; 2-fold  
378 enrichment, 18 genes). This set included genes whose expres-  
379 sion in the liver was previously associated with body mass  
380 variation in natural populations of North American house mice  
381 (bcat2, col6a1, col5a2, col3a1). Additionally, this set included  
382 genes implicated in obesity and metabolic phenotypes in hu-  
383 mans (e.g., wrn, plaat3, prkar2b, sulf2, smoc1) . In Manaus,  
384 we identified an overlap of 68 outlier windows overlapping 37  
385 genes with evidence for *cis*- regulatory divergence (Figure SX).  
386 These genes were enriched for homeostasis/metabolism pheno-  
387 types (q=0.04, 1.62-fold enrichment, 20 genes) and as well as  
388 several non-metabolic phenotypes (e.g., integument phenotype,  
389 abnormal locomotor activation) relative to a background of  
390 genes with evidence for *cis*- divergence.

391 For the NH/VT comparison, we found that the overlap  
392 between genes with *cis*- regulatory divergence and outlier  
393 windows was greater than expected by chance (hypergeometric  
394 test, p=0.0017, 1.45-fold enrichment), where we do not observe  
395 this enrichment for Manaus (p=0.16). Additionally, genes with  
396 evidence for *cis*- regulatory divergence showed higher average  
397 PBSn1 than other genes when NH/VT is a focal population  
398 (Permutation test, p<0.00005), but not when Manaus was a  
399 focal population (p=0.91).

## 400 Discussion

401 Understanding how both genetic and environmental variation  
402 influence gene regulation is essential to understanding adaptive  
403 evolution. Here, we utilized allele-specific expression to charac-  
404 terize cis and trans changes underlying divergence and plastic-  
405 ity in temperate and tropical house mice. We found that the  
406 majority of gene expression differences showed concordant pat-  
407 terns of regulatory divergence across environments, suggesting  
408 that genetic effects are more pervasive than environmental ef-  
409 fects. In particular, we found that most regulatory divergence  
410 was underlined by cis-regulatory variation, and that these  
411 cis-effects were highly independent of temperature. These re-  
412 sults are consistent with previous studies in yeast (Smith and  
413 Kruglyak 2008; Tirosh et al. 2009; Naranjo et al. 2015); (Li et  
414 al. 2006); (Chen et al. 2015); (Cubillos et al. 2014) in which  
415 most concordant expression differences across environments  
416 are controlled by conserved cis effects. Moreover, the universal  
417 patterns in the stability of cis-regulatory variation further  
418 illustrates the significance of cis-acting factors in adaptive evo-  
419 lution (Wittkopp et al. 2008; Emerson et al. 2010; Massouras  
420 et al. 2012; Tung et al. 2015; Verta and Jones 2019). Overall,  
421 our results demonstrate that cis-regulatory variation plays a  
422 significant role in the divergence of North and South American  
423 house mice.

### 424 A. Rapid divergence of introduced populations of house mice.

425 These results highlight that divergence is a major axis of  
426 expression variation in house mice. Divergence between sub-  
427 species (Mack et al. 2016) resulted in about 9K fixed SNPs.  
428 Here, almost 6K fixed SNPs.

429 Changes in the environment preferentially impacted trans-  
430 regulation profiles in house mice compared to cis-regulation  
431 (Tables 1 and 2; Figure 2), suggesting that trans-effects play a  
432 more pronounced role in gene expression plasticity. Greater  
433 sensitivity of trans-effects to the environment is in strong  
434 agreement with previous studies in yeast (Smith and Kruglyak  
435 2008; Tirosh et al. 2009; Naranjo et al. 2015), nematodes (Li  
436 et al. 2006), flies (Chen et al. 2015), and plants (Cubillos et  
437 al. 2014). The generality of trans-effects being more sensitive  
438 across environmental conditions may be due to the role trans-  
439 acting factors play in signaling pathways that become activated  
440 in response to environmental change (Ehrenreich and Pfennig  
441 2016). Moreovoer, the large mutational target space of trans-  
442 regulatory variants make them much more pleiotropic than cis  
443 effects (Denver et al. 2005; Landry et al. 2007), allowing them  
444 to impact the regulation of many genes that are contingent  
445 upon an environmental stimulus (Promislow 2005). Indeed,  
446 we found that the effect sizes of trans were greater than that  
447 of cis across both tissues in house mice (Figure S4), suggesting  
448 that much of expression plasticity we observed is governed by  
449 changes in trans.

450 Gene regulation is not only dependent on environmental  
451 conditions but it is also tissue specific (GTEx). Although  
452 both liver and BAT exhibited similar levels of cis- and trans-  
453 regulatory variation across enviornments (Table 1), we did  
454 observe more cis- and trans-by-temperature effects in BAT  
455 compared to liver (Table S1). These patterns may reflect  
456 the developmentally plastic nature of BAT in response to  
457 cold temperatures (Cannon and Nedergaard 2004). Alterna-  
458 tively, these patterns may reflect differences in pleiotropy or  
459 tissue-specificity. For example, threespine sticklebacks show a

460 preponderance of both cis (Verta and Jones 2019) and trans  
461 (Hart et al. 2018) regulation underlying parallel environmen-  
462 tal adaptation. The discrepancies between the two studies  
463 are likely a result of differences in tissues analyzed, as the  
464 genetic architectures of simple and complex tissues differ from  
465 the effect of heterogeneity (Hart et al. 2018; Verta and Jones  
466 2019). Similar influences may be contributing to differences  
467 seen between BAT and liver. For example, BAT is a more  
468 heterogeneous tissue than the liver, with varying levels of  
469 lipid droplets and mitochondria (Ikeda et al. 2018; Oguri and  
470 Kajimura 2020). Moreover, the greater proportion of trans-  
471 by-temperature effects in BAT compared to the liver may be  
472 indicative of BAT being more specialized and pleiotropic, es-  
473 pecially in terms of plasticity. Overall, these results highlight  
474 the importance of investigating the effects of gene regulation  
475 across multiple tissues.

476 Finally, the role of expression plasticity in facilitating or  
477 constraining adaptation has received considerable attention  
478 in the last few years (Ghalambor et al. 2015; Velotta et  
479 al. 2018; Campbell-Staton et al. 2021; Josephs et al. 2021).  
480 Although numerous studies have identified roles for both adap-  
481 tive and non-adaptive expression plasticity, very few stud-  
482 ies have characterized the underlying regulatory architecture  
483 of such patterns (He et al. 2021). We overlapped cis- and  
484 trans-by-temperature candidates with patterns of adaptive  
485 plasticity previously identified (Ballinger and Nachman, in  
486 prep) and found that genes exhibiting adaptive plasticity are  
487 underlined by both cis-by-treatment and trans-by-treatment  
488 effects (Figure 3). Interestingly, most genes exhibiting adap-  
489 tive plasticity are constitutively expressed across warm and  
490 cold environments in New York mice. Although we do not  
491 have direct evidence for genetic assimilation (Waddington  
492 1952, 1953; Crispo 2007), these candidates illustrate the po-  
493 tential regulatory mechanisms underlying genetic assimilation.  
494 For example, cis regulatory variants could rapidly canalize  
495 expression through the loss or gain of specific binding sites for  
496 conditionally expressed transcription factors, thereby decou-  
497 pling a gene's expression from the environment (Ehrenreich  
498 and Pfennig 2016). Alternatively, trans effects could activate  
499 environmentally-sensitive signaling pathways in the absence  
500 of an environmental cue, causing a gene's expression to be  
501 constitutively expressed (Yvert et al. 2003; Ehrenreich and  
502 Pfennig 2016). However, these hypotheses do not consider  
503 the effects of gene regulatory networks nor identify causal  
504 mutations of candidate genes. Thus, future work is needed  
505 to identify causal mutations underlying adaptive plasticity  
506 and genetic assimilation (Corl et al. 2018; van der Burg et  
507 al. 2020). Regardless, these are exciting candidates for further  
508 investigation into the molecular and regulatory underpinnings  
509 of adaptive plasticity in house mice.

## 510 Methods

511 **Animals and experimental design.** We used two wild-derived in-  
512 bred lines of house mice collected from upstate New York  
513 (SARA) and equatorial Brazil (MANA). The establishment of  
514 these lines and the experimental design implemented in this  
515 study have been described previously (Ballinger and Nachman,  
516 2022). Briefly, we generated F1 hybrids by crossing a New York  
517 female with a Brazil male. All experimental animals were born  
518 at room temperature (20oC). We weaned and singly housed  
519 SARA, MANA, and F1 hybrids at ~3 weeks of age. We split

520 3.5-week-old full-sibs and F1 hybrids into size-matched experimental groups across cold (5oC) and warm (21oC) treatments.  
521 Mice were kept in their respective experimental environment up until ~12 weeks of age, at which point individuals were  
522 euthanized via cervical dislocation. We took standard museum measurements and then rapidly dissected and preserved liver  
523 and brown adipose tissue in RNAlater at 4oC overnight and moved to -80oC until RNA extraction. Skins were removed  
524 and dried for subsequent thermal conductance measurements (ref; see SI methods). All experimental procedures were in  
525 accordance with the UC Berkeley Institutional Animal Care and Use Committee (AUP-2017-08-10248). Food and water  
526 were provided *ad libitum*.

527 **RNA extraction, library preparation, and sequencing.** We extracted total RNA from both liver and BAT from each sample (n = ~6 per genotype/sex/treatment/tissue) using the RNeasy PowerLyzer Kit (QIAGEN). We generated Illumina cDNA libraries from 1 ug of purified RNA using KAPA Stranded mRNA-Seq Kit (Illumina), and uniquely indexed libraries using unique dual indexes (Illumina). Libraries were pooled in equal molar concentration and sequenced on one lane each of 150 bp paired-end NovaSeq S1 and NovaSeq S4 at the Vincent J. Coates Genomics Sequencing Center at UC Berkeley. We filtered raw reads below a Phred quality score of 15 and trimmed adapter sequences using fastp (Chen et al. 2018).

528 **Parental gene expression analyses.** After cleaning and trimming parental sequences of MANA and SARA, we mapped reads to the Mus musculus reference genome (GRCm38/mm10) using STAR (Dobin et al. 2013). We counted reads overlapping exons using HTSeq (Anders et al. 2015) based on the Ensembl GRCm38.98 annotation. After pre-processing and filtering, we retained >14K genes for downstream analyses. We detected ~14K expressed genes that were shared across all samples (XXX for warm-reared mice, XXX for cold-reared mice; normalized counts – 10 reads per gene across all samples in each tissue separately). We imported raw count data into R (v.4.1) and used DESeq2 (Love et al. 2014) to quantify expression patterns by fitting a generalized linear model following a negative binomial distribution. First, we determined effects of genotype, environment, and genotype-by-environment on expression patterns between lines for each tissue separately. We then computed differential expression between lines with the model population + environment + population\*environment. We removed genes with a mean fewer than 10 reads across samples per tissue. Lastly, we used a Benjamini-Hochberg multiple test correction (Benjamini and Hochberg 1995) on the resulting P-values and considered genes with a false discovery rate (FDR) smaller than 0.05 to be significantly differentially expressed.

530 **Identifying variants between parental lines.** To identify differences between lines for allele-specific read assignment, SNP calling was performed on whole genome sequence data from one female each of MANA and SARA. Genomic reads were mapped with Bowtie2 (Langmead and Salzberg 2012) to the mm10 reference genome (setting: –very-sensitive) obtained from Ensembl. Duplicates were marked with the Picard tool MarkDuplicates and then the GATK tools HaplotypeCaller and GenotypeGVCFs were used for joint genotyping across genomic samples. We filtered for low quality SNP calls with VariantFiltration (QD < 2.0; QUAL < 30.0; FS > 200; ReadPosRankSum < -20.0).

To reduce the influence of genotyping error on allele-specific expression, we mapped RNAseq reads from all individuals and then counted allele-specific reads aligned to each site we genotyped with the GATK tool ASEReadCounter. Sites for which we did not have coverage of at least 5 reads from each population-specific allele were excluded. These SNPs were then used for identifying allele-specific reads.

532 **Mapping allele-specific reads.** For allele-specific expression analyses, reads from hybrid individuals were mapped to the mouse reference genome (GRCm38/mm10) using STAR. We used WASP (van de Geijn et al. 2015) to reduce the potential for reference mapping bias. Reads that overlapped a population-specific variant and that passed WASP filtering were retained for our allele-specific expression analysis. Reads overlapping informative variants were separated into allele-specific pools (NY, BR) based on genotype for quantification. We used HTSeq to count the number of reads associated with each gene per population based on the overlap of reads and annotated exonic regions based on the Ensembl GRCm38.98 annotation. We examined per site allelic reads with ASEReadCounter to quantify read mapping bias. Proportions of reads overlapping the references vs. alternative allele (REF allele / (ALT allele + REF allele)) show a median 0.5 across samples (Figure S5). Consequently, we do not find evidence for reference mapping bias. Overall, a total of 5,861 genes were tested for allele-specific expression based on the presence of fixed differences between Brazil (MANA) and New York (SARA) lines.

534 **Identifying cis- and trans- divergence.** To characterize regulatory divergence in warm and cold temperature treatments, we compared expression in Brazil and New York lines (F0 generation) with differences in expression in alleles in a hybrid (F1 generation). Differential expression between alleles in hybrids is evidence for cis- regulatory divergence, where differences in the ratio of expression between alleles in a hybrid and between genes in the F0 generation are evidence for trans divergence. Differential expression in the F0 generation (parents) was inferred by analyzing raw counts using the DESeq2 package (see above). To identify genes with evidence of allelic imbalance in hybrid individuals, we took reads that mapped preferentially to either New York or Brazil alleles and fit these to a model with allele (NY vs. BZ), sample (individual), and tissue (BAT, liver) for hybrid male samples (~temperature + individual:temperature + allele:temperature) in DESeq2. The trans component was assessed through a Fisher's Exact Test on reads mapping to each parental allele in the hybrid versus parental read counts, summed over all replicates. We randomly down-sampled to account for library size differences between parental and F1 replicates. P-values for each test were corrected for false-discovery rate with the Benjamini-Hochberg method. Tests with FDR < 0.05 were considered significant. Genes with differences in expression between alleles in the hybrid and expression in the F0 generation and without a difference between these ratios (via the Fisher's Exact Test) were considered divergent in cis alone. Genes without allele-specific expression in hybrids but differential expression in the F0 generation and a significant difference in these ratios were considered divergent in trans alone. Cases where we identified a significant trans component and divergence in either alleles in the hybrid or expression in the F0 generation were indicative of cis and trans divergence together.

640 **Population genomic analyses.**

641 **A.1. Data availability (#data-availability .unnumbered).**

642 **Supporting Information (SI).** Authors are limited to no more  
643 than 10 SI files, not including movie files. Authors who place  
644 detailed materials and methods in SI must provide sufficient  
645 detail in the main text methods to enable a reader to follow  
646 the logic of the procedures and results and also must reference  
647 the online methods.

648 **SI Text.** Supply Word, RTF, or LaTeX files (LaTeX files must  
649 be accompanied by a PDF with the same file name for visual  
650 reference).

651 **SI Figures.** Provide a brief legend for each supporting figure  
652 after the supporting text. Provide figure images in TIFF, EPS,  
653 high-resolution PDF, JPEG, or GIF format; figures may not  
654 be embedded in manuscript text. When saving TIFF files, use  
655 only LZW compression; do not use JPEG compression. Do  
656 not save figure numbers, legends, or author names as part of  
657 the image. Composite figures must be pre-assembled.

658 **SI Tables.** Supply Word, RTF, or LaTeX files (LaTeX files must  
659 be accompanied by a PDF with the same file name for visual  
660 reference); include only one table per file. Do not use tabs or  
661 spaces to separate columns in Word tables.

662 **SI Datasets.** Supply Excel (.xls), RTF, or PDF files. This file  
663 type will be published in raw format and will not be edited or  
664 composed.

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676 **refs**