Fundamental Evolutionary Limits in Ecological Traits Drive *Drosophila* Species Distributions

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Species that are habitat specialists make up much of biodiversity, but the evolutionary factors that limit their distributions have rarely been considered. We show that in *Drosophila*, narrow and wide ranges of desiccation and cold resistance are closely associated with the distributions of specialist and generalist species, respectively. Furthermore, our data show that narrowly distributed tropical species consistently have low means and low genetic variation for these traits as compared with those of widely distributed species after phylogenetic correction. These results are unrelated to levels of neutral variation. Thus, specialist species may simply lack genetic variation in key traits, limiting their ability to adapt to conditions beyond their current range. We predict that such species are likely to be constrained in their evolutionary responses to future climate changes.

Specialist species within particular climatic zones and/or dependent on particular hosts make up much of biodiversity (*1*), but generally investigations have not considered whether evolutionary factors may limit the distributions of these species (*2*, *3*). Models predicting climate change suggest that it is crucial to determine how species will evolve and contract or expand from their current distributions, particularly in the case of threatened climate specialists. Distributional patterns may be explained by the fact that species simply lack the appropriate genetic variation in key traits necessary to adapt to conditions beyond their distributions and thus are constrained by a fundamental genetic limit (*4*). Such a lack of genetic variation contradicts the theory that traits are highly variable (*5*), which originated from quantitative genetic theory that shows mutational variance to be large enough to maintain genetic variation within traits (*6*).

In particular, the idea that genetic variance might limit evolutionary responses was supported by the fact that certain plant species exhibited phenotypic variation for resistance to soil contamination and could successfully colonize mine tailings contaminated with heavy metals (*7*). Species or populations unable to evolve higher levels of metal resistance were limited by low levels of genetic variation for this trait. Few studies had gone on to further test this prediction until it was suggested that a lack of appropriate variation for desiccation resistance might be linked to the distribution of two rain forest *Drosophila* species (*3*, *8*). These desiccation-sensitive species did not respond to persistent selection for increased desiccation resistance, whereas cross-generation comparisons suggested that this was due to very low genetic variation for this trait (*8*, *9*) in contrast to other widespread *Drosophila* species (*10*).

Environmental variables are tightly associated with the distribution of *Drosophila* and insects more generally (*11*). It is well documented that species distributions become narrower toward the tropics (Rapaport's rule) (*12*). One hypothesis to explain this pattern and which has empirical support is the climatic variability hypothesis (*12*), which proposes that species with greater physiological tolerance to climatic variables will be able to extend their distribution to higher latitudes. This leaves species that are unable to increase their tolerance restricted to the tropics.

We extend the climatic variability hypothesis and suggest that levels of genetic variation in key ecological traits are driving differences in physiological tolerances between tropical and widespread *Drosophila* species. To examine this, we initially compared patterns of mean resistance to two stresses, desiccation and cold, for 30 species (*13*). Both resistance traits have been closely linked to differences in species distributions [cold resistance in (*14*–*16*) and desiccation resistance in (*15*, *17*)]. We found that species restricted to the tropics showed low levels of resistance to both desiccation and cold in comparison with more widespread temperate species (Fig. 1). Consequently, there was a strong association between levels of resistance and tropical versus widespread distribution, with the exception of *Drosophila funebris*, which is adapted to cold environments (Fig. 1). These data are in accord with the climatic variability hypothesis and with studies that also show that levels of cold resistance in species match expectations on the basis of climatic data (*18*, *19*). Furthermore, these results confirm that cold and desiccation are important variables influencing distributional patterns in *Drosophila* species. Non-drosophilid tropical insects also have a much narrower range of thermal optima (*12*).

To establish whether low genetic variation in resistance traits influences tropical and widespread distributions, we estimated genetic variation (mostly as h_n^2, the narrow sense heritability) in five tropically restricted and five widespread *Drosophila* species. For most traits, pedigree information was analyzed with statistical animal models (*13*, *20*) to generate small SEs around heritability estimates. Patterns of genetic variation in the traits of cold and desiccation resistance were consistent with our hypothesis; low levels of genetic variation

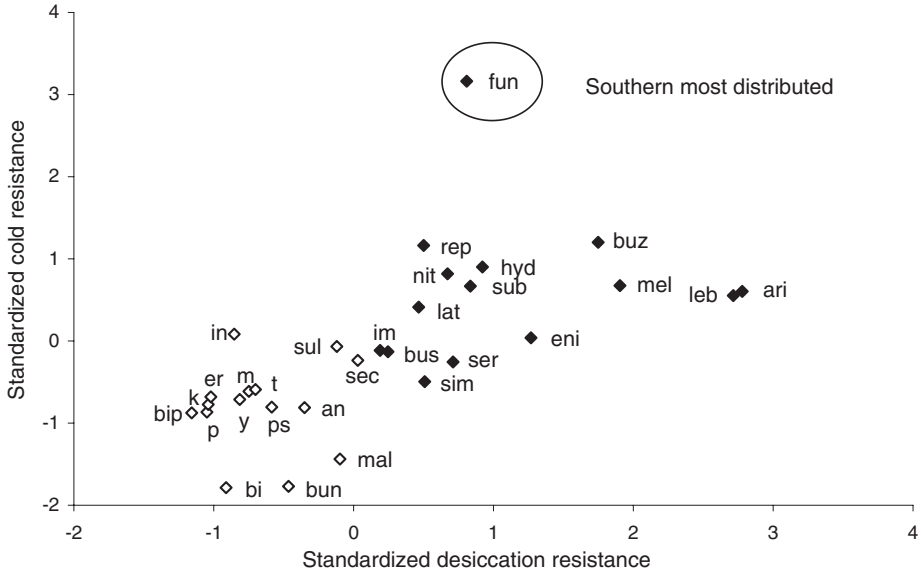

Fig. 1. Standardized cold resistance plotted against standardized desiccation resistance for *Drosophila* species. ◆ indicates species with a widespread distribution; ◇ indicates species with a restricted/tropical distribution. an, *ananassae*; ari, *arizonensis*; bip, *bipectinata*; bi, *birchii*; bus, *busckii*; bun, *bunnanda*; buz, *buzzatii*; er, *erecta*; eni, *enigma*; fun, *funebris* (cold adapted); hyd, *hydei*; im, *immigrans*; in, *inornata*; k, *kikkawai*; lat, *lativittata*; leb, *lebanonensis*; mal, *malerkotliana*; m, *mauritiana*; mel, *melanogaster*; nit, *nitidithorax*; p, *paulistorum*; ps, *pseudoananassae*; rep, *repleta*; sec, *sechellia*; ser, *serrata*; sim, *simulans*; sub, *subobscura*; t, *teisseri*; y, *yakuba*; and sul, *sulfurigaster*. The outlier noted in the text is circled.

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were found in all tropical species, as indicated by low heritability estimates (Fig. 2). Those cases in which we observed only small and nonsignificant estimates of h_n^2 were unlikely to reflect a lack of power because we obtained low SEs for most comparisons. In contrast, we observed significant levels of variation in all widespread species, and the difference in h_n^2 between tropical and widespread species overall was greater than twofold for both desiccation and cold resistance (table S1). Patterns of additive genetic variance (V_A) also matched that of h_n^2, highlighting that specialization to tropical environments may reflect low genetic variation in key ecological traits. Fundamental limits in genetic variation therefore appear to be driving distribution limits in these *Drosophila* species.

The most commonly invoked explanation for such observed low levels of genetic variation in traits is that they are subject to directional selection that causes the fixation of favored alleles (*21*). However, if this is true then low genetic variation should be coupled with high (directionally selected) trait means if high resistance levels are favored. Instead, if genetic variation is directly involved in limiting species distributions, we would expect low genetic variation to be coupled with low trait means, whereas species with high levels of genetic variation should not be constrained in this way (*22*). Under these predictions, the mean expression of a distribution-limiting trait and levels of genetic variation for that trait should be correlated, as was observed in this study (Fig. 2). If experimental error was swamping estimates of h_n^2, particularly in the restricted species, an association between environmental variance and trait mean would be expected; this was not the case for either desiccation ($r^2 = 0.05$, $P = 0.51$) or cold ($r^2 = 0.26$, $P = 0.14$) resistance.

To ensure that phylogenetic history was not responsible for the observed patterns in trait mean, h_n^2, and species distribution, we generated phylogenetic independent contrasts (PICs) (*13*, *23*). PICs generate statistical independence within a data set by standardizing trait values by the branch length in two sister species. Each comparison results in an independent contrast, and consequently the number of contrasts equals $n - 1$. We used a highly resolved phylogenetic tree (fig. S1), created on the basis of two mitochondrial (*COII* and *ND5*) and two nuclear (*ADH* and *hb*) genes, to phylogenetically correct our estimates of h_n^2, V_A, and trait mean. The strength of the relationships decreased slightly after correction for phylogeny but were still significant (Table 1). When PICs for cold and desiccation resistance were plotted, a strong positive and linear association remained. Thus, trait mean was closely associated with levels of genetic variation (Fig. 2 and table S1), emphasizing that levels of genetic variation for cold and desiccation resistance, rather than phylogeny, are driving species distributions.

Population processes such as inbreeding or drift could alter levels of genetic variation and explain the observed patterns. However, if this was true we would expect low levels of genetic variation in all traits. To test this, we estimated h_n^2 for wing size (*13*). In contrast to the stress resistance traits, we saw no consistent association between h_n^2 or V_A and trait mean between restricted and widespread species. All estimates of both h_n^2 and V_A differed significantly from zero in all species (table S1). We observed no relationship between mean wing size and h_n^2 ($r^2 = 0.001$, $P = 0.92$). We also tested for a relationship between wing size and mean resistance because body size could influence trait resistance (*24*); however, we did not detect a relationship between any of the traits (for desiccation, $r^2 = 0.29$, $P = 0.10$; for cold, $r^2 = 0.10$, $P = 0.36$). Furthermore, in some species microsatellite repeat variation has been characterized. For these species, it was high and comparable for species that are widespread and those that are climatically specialized (*25*).

It is unclear what mechanisms produce these patterns. Hypotheses involving asymmetrical gene flow that might account for evolutionary stasis do not predict that low levels of genetic variation will limit evolutionary responses (*22*). The rate of mutation seems unlikely to limit variation in our tropical species because we would expect equivalent levels of variation to arise through mutation if desiccation resistance is influenced by similar genes across species. Our results may be caused by a loss of genetic variance through DNA decay (*22*); if selection on these traits is weak or essentially neutral, theory suggests that mutations will accumulate that could potentially result in loss of function of genes, and hence traits (*26*). The hypothesis that the loss of underlying

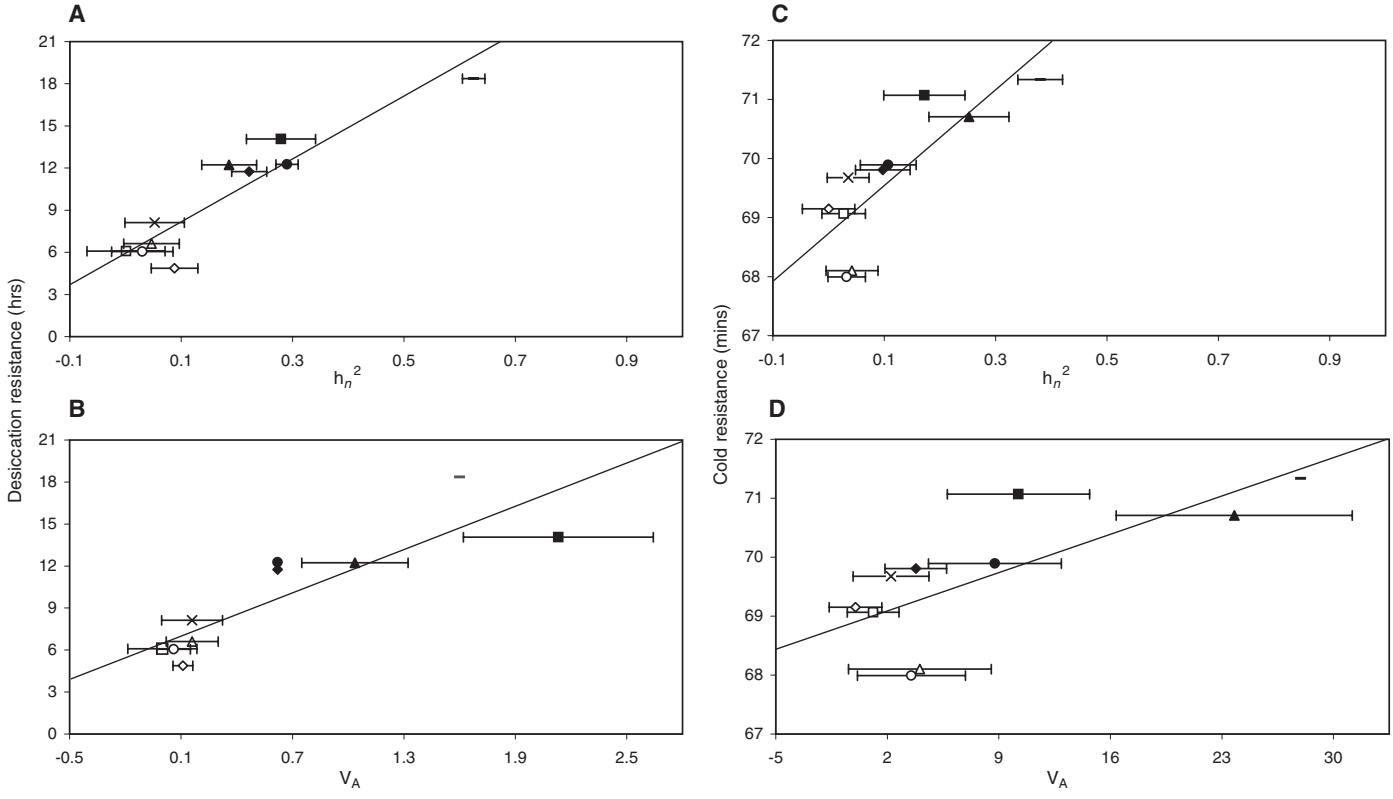

Fig. 2. Species comparison with trait mean and genetic variance estimates. –, *D. melanogaster*; ●, *D. simulans*; ▲, *D. repleta*; ■, *D. hydei*; ♦, *D. serrata*; ○, *D. birchii*; △, *D. bunnanda*; □, *D. pseudoananassae*; ×, *D. sulfurigaster*; and ◇, *D. bipectinata*. (**A**) h_n^2 versus desiccation resistance. (**B**) V_A versus desiccation resistance. (**C**) h_n^2 versus cold resistance. (**D**) V_A versus cold resistance. Error bars reflect one SE.

Table 1. Regression analyses for trait means onto genetic variance estimates for desiccation and cold resistance. Regressions were computed on both the original unstandardized data set and the PICs.

	Desiccation		Cold	
h_n^2				
	Original	PIC	Original	PIC
Slope ± SE	21.69 ± 0.92	17.97 ± 1.23	7.76 ± 0.60	6.13 ± 0.54
r^2	0.87	0.75	0.67	0.64
P	<0.001	0.03	0.01	0.01
V_A				
Slope ± SE	5.21 ± 0.33	4.20 ± 0.43	0.09 ± 0.01	0.07 ± 0.01
r^2	0.76	0.56	0.55	0.48
P	<0.001	0.04	0.01	0.03

genetic variability through DNA decay leads to ecological specialization has been proposed to occur in some bacterial systems (*27*). The tropical species may be evolutionarily derived, or else the more widespread species are derived after activation of resistance, perhaps after gene duplication or the development of novel resistance pathways. Tradeoffs might also be involved if they produce strong directional selection for sensitivity to cold and desiccation in tropically restricted species.

Our analysis supports the hypothesis that specialization to tropical environments in *Drosophila* species reflects fundamental genetic limits in ecologically important traits. We show that genetic variation in tropical *Drosophila* species for both desiccation and cold resistance is consistently low, which is in contrast to widely distributed species. Whether other tropically restricted species share a similar evolutionary fate remains to be determined. However, evidence suggests that an absence of genetic variation may be limiting distributions in other systems (*2*). Further work examining the genetic architecture of traits that may be linked to species distributions will be important for the assessment of the future evolutionary potential of species. If species distributions are commonly driven by genetic limits, the consequences of climate change on biodiversity may be greater than previously assumed. It is worth considering heat resistance as well as cold and desiccation resistance within this context, particularly because recent research has highlighted the pending threat of increases in temperature on the persistence of tropical species (*12*, *28*, *29*).

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Supporting Online Material

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Materials and Methods
Fig. S1
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Common Regulatory Variation Impacts Gene Expression in a Cell Type–Dependent Manner

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Studies correlating genetic variation to gene expression facilitate the interpretation of common human phenotypes and disease. As functional variants may be operating in a tissue-dependent manner, we performed gene expression profiling and association with genetic variants (single-nucleotide polymorphisms) on three cell types of 75 individuals. We detected cell type–specific genetic effects, with 69 to 80% of regulatory variants operating in a cell type–specific manner, and identified multiple expressive quantitative trait loci (eQTLs) per gene, unique or shared among cell types and positively correlated with the number of transcripts per gene. Cell type–specific eQTLs were found at larger distances from genes and at lower effect size, similar to known enhancers. These data suggest that the complete regulatory variant repertoire can only be uncovered in the context of cell-type specificity.

Variation influencing gene expression can manifest itself as gene expression differences among populations, among individuals in a population, among tissues, and in response to environmental factors. The genetic basis of the first two types of gene expression variation has been investigated, with the quantification of mRNA in one tissue and the identification of genetic variants correlated with the variation of expression quantitative trait loci (eQTLs) in a single or multiple populations (*1*–*7*). The complexity in higher eukaryotes, however, results in a vast set of highly specialized cell types and tissues. Some genes exhibit ubiquitous patterns of expression; others display tissue-specific activity (*8*–*10*). Although some studies have identified eQTLs in human (*11*–*13*) and mammalian tissues (*14*, *15*), we know of no systematic study that has compared eQTLs across different cell types while controlling for confounding associations (population samples, differences in technology, or statistical methodology). Documenting tissue-specific genetic control of gene expression variation may connect cellular activities in health and disease (*11*, *13*, *16*). Efforts to use