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Source: *The American Naturalist*, Vol. 183, No. 5 (May 2014), pp. 711-727
Published by: The University of Chicago Press for The American Society of Naturalists
Stable URL: http://www.jstor.org/stable/10.1086/675497
Accessed: 17/04/2014 15:34

Symposium

Genome-Wide Association Mapping of Phenotypic Traits Subject to a Range of Intensities of Natural Selection in *Timema cristinae*⋆

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Dryad data: http://dx.doi.org/10.5061/dryad.ck2cm.

abstract: The genetic architecture of adaptive traits can reflect the evolutionary history of populations and also shape divergence among populations. Despite this central role in evolution, relatively little is known regarding the genetic architecture of adaptive traits in nature, particularly for traits subject to known selection intensities. Here we quantitatively describe the genetic architecture of traits that are subject to known intensities of differential selection between host plant species in *Timema cristinae* stick insects. Specifically, we used phenotypic measurements of 10 traits and 211,004 single-nucleotide polymorphisms (SNPs) to conduct multilocus genome-wide association mapping. We identified a modest number of SNPs that were associated with traits and sometimes explained a large proportion of trait variation. These SNPs varied in their strength of association with traits, and both major and minor effect loci were discovered. However, we found no relationship between variation in levels of divergence among traits in nature and variation in parameters describing the genetic architecture of those same traits. Our results provide a first step toward identifying loci underlying adaptation in *T. cristinae*. Future studies will examine the genomic location, population differentiation, and response to selection of the trait-associated SNPs described here.

Keywords: adaptation, genetics, natural selection, genome-wide association mapping studies (GWAS), stick insect.

Introduction

Since Darwin described natural selection as a fundamental mechanism of evolution (Darwin 1859), numerous examples of phenotypes subject to selection have accumulated (e.g., Grant and Grant 1995; Nagel and Schluter 1998; Reimchen and Nosil 2002; Benkman et al. 2003; Lowry et al. 2008; Mullen and Hoekstra 2008; Vignieri et al. 2010; reviewed by Kingsolver et al. 2001). The interplay between selection acting at the phenotypic level and the genetic variation underlying adaptive phenotypes has also long been a major focus in evolutionary genetics (Fisher 1930) because it affects the evolutionary response to selection (Lande 1979). Adaptive phenotypic evolution can also influence ecological and community-level processes (Urban et al. 2008; Farkas et al. 2013). Thus, determining links between phenotype and genotype can increase our understanding of evolution (e.g., Hancock et al. 2011) as well as provide a more holistic understanding of the link between evolutionary and ecological processes. Compared to a long history of measuring selection at the phenotypic level, it is only recently that we have begun to measure selection at both the genetic and phenotypic level (Bradshaw and Schemske 2003; Weinig et al. 2003; Barrett et al. 2008; Lowry and Willis 2010; Fournier-Level et al. 2011; Hancock et al. 2011; Mojica et al. 2012). As an illustrative example, of the 51 study systems reviewed by Kingsolver et al. (2001) for the direction and strength of phenotypic selection, only six have information, beyond estimates of heritability, regarding the genetic architecture of the traits (determined via Web of Science searches; see app. A for details).

Knowledge of the genetic architectures for diverse traits involved in adaptation can help address outstanding questions such as, how many loci are involved in adaptation, and what is the relative contribution of major and minor effect alleles to adaptive evolution (Stinchcombe and Hoekstra 2008)? For example, theory predicts that major effect loci are more likely to play a role in adaptation toward a distant phenotypic optimum than when adaptation is to-

⋆ This paper was originally presented at the Vice Presidential Symposium at the American Society of Naturalists annual meetings in Ottawa, Ontario, in July 2012.

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Am. Nat. 2014. Vol. 183, pp. 711–727.
DOI: 10.1086/675497

ward a closer phenotypic optimum (Orr 1998; Uecker and Hermisson 2011), and there is some evidence for this in natural populations (Rogers et al. 2012). Another theoretical prediction is that when adaptation occurs from standing genetic variation, fixation of loci with minor phenotypic effects is more likely than when adaptation relies on de novo mutations (Hermisson and Pennings 2005; Barrett and Schluter 2008; Pritchard and Di Rienzo 2010).

The geographic context of population differentiation can also affect patterns of genetic variation (Felsenstein 1976). When populations adapting to different environments experience gene flow, divergence can most readily occur if per-trait or per-locus selection coefficients are greater than rates of gene flow (Slatkin 1987; Storfer et al. 1999; Hendry et al. 2002; Moore et al. 2007). Thus, all else being equal (e.g., population size, total strength of selection acting on a trait, etc.), it is easier for individual traits to diverge if they are controlled by a few loci with large phenotypic effects than many loci with small effects, because in this case, per-locus selection coefficients will be larger (Nosil et al. 2009; Yeaman and Whitlock 2011). The interplay between selection and gene flow during divergence can also lead to the replacement of small-effect alleles by those with larger effects (Griswold 2006; Holt and Barfield 2011; Yeaman and Whitlock 2011).

Empirical studies have now documented both major and minor effect loci underlying adaptive phenotypes. Loci with major effects on phenotypes are frequently identified when trait variation segregates into discrete categories (e.g., Jiggins and McMillan 1997; Peichel 2001; Bradshaw and Schemske 2003; Colosimo et al. 2004; Hoekstra et al. 2006; Joron et al. 2006; Steiner et al. 2007; Linnen et al. 2009; Chan et al. 2010; Rosenblum et al. 2010), and indeed, genetic variants of large effect may be necessary to produce such phenotypic distributions (Weiss 2008; Rockman 2012). However, adaptive traits that show more continuous patterns of variation are frequently controlled by a more complex and polygenic genetic architecture, as exemplified by studies of *Arabidopsis thaliana* (Brachi et al. 2010; Chan et al. 2011), *Zea mays* (Buckler et al. 2009; Tian et al. 2011; Hung et al. 2012), *Mus* (Valdar et al. 2006; Huang et al. 2009), and *Drosophila* (reviewed by Flint and Mackay 2009).

Estimating the genetic architecture of traits is frequently accomplished by scanning recombinant mapping families created from genetic crosses for genetic regions that covary with phenotypic variation (i.e., quantitative trait loci [QTL]; Orr 2001; Barton and Keightley 2002; Slate 2005). Although QTL studies have advantages, they require either a detailed population pedigree or controlled crosses (Slate 2005), often lack sufficient recombination for fine-scale mapping (Gupta et al. 2005; Buerkle and Lexer 2008), and characterize genetic variation that is not necessarily representative of that found in natural populations (Rockman 2012).

Genome-wide association mapping studies (GWAS) differ from QTL studies in that they utilize numerous molecular markers (e.g., single-nucleotide polymorphisms [SNPs]) to search for statistical associations between genotypes at specific loci and phenotypic variation in natural populations (Hirschhorn and Daly 2005). GWAS take advantage of lower levels of linkage disequilibrium (LD) due to longer histories of recombination existing within natural populations than in controlled crosses and natural levels of genetic variation to identify genetic markers associated with phenotypes, often to a finer scale than QTL approaches (e.g., Cho et al. 2009; Brachi et al. 2010; Chan et al. 2011; Fournier-Level et al. 2011). GWAS have primarily been carried out in model genetic systems; however, recent advances in sequencing technologies now allow for genome-wide coverage of molecular variation to be achieved in a large number of individuals (e.g., Gompert et al. 2010; Hohenlohe et al. 2010; Elshire et al. 2011; Andolfatto et al. 2011), facilitating GWAS in a wider range of organisms (e.g., Parchman et al. 2012; Gompert et al. 2012).

Here we used genome-wide SNP data to test for associations with traits involved in host plant adaptation in a phenotypically variable population of *Timema cristinae*. We used multilocus Bayesian variable selection regression (BVSR) to describe the genetic architecture for 10 traits that exhibit varying degrees of population differentiation, are subject to variable selection intensities, and whose divergence is constrained by gene flow (Nosil and Crespi 2006; Nosil 2007 for review). Multilocus BVSR provides significant statistical advantages over the single-SNP approaches frequently applied to GWAS (Wu et al. 2009; Valdar et al. 2009; Guan and Stephens 2011). For example, BVSR places priors on parameters and hyperparameters that quantitatively describe aspects of genetic architecture, thereby allowing their values to be inferred from the data while accounting for uncertainty in the specific SNPs underlying trait variation (Guan and Stephens 2011).

Specifically, BVSR provides estimates of the number of SNPs underlying a given trait (N_{SNP}), the proportion of variance explained by the SNPs included in the model (PVE), the average effect of SNPs on a given trait (σ_{AVE}), the conditional prior probability of a SNP being in the model (p_{SNP}), the posterior inclusion probability (PIP) for individual SNPs, and the magnitude of the relationship between individual SNPs and the phenotype of interest (β). In biological terms, β represents the shift in trait value (in standard deviations) expected by a single allelic substitution at a locus. Our goal here is to provide the first estimates of the genetic architecture of traits involved in host plant adaptation in *T. cristinae* and test whether these estimates are associated with levels of trait divergence between natural populations on different hosts. Future work

could focus on identifying the specific functional variants underlying trait variation.

Study System

Timema cristinae (Phasmatodea: Timematodea) is a flightless herbivorous insect endemic to the Santa Ynez Mountains near Santa Barbara, California (Vickery 1993). Populations are found primarily on one of two species of host plant (*Ceanothus spinosus*: Rhamnaceae and *Adenostoma fasciculatum*: Rosaceae; fig. 1*a*). Divergent natural selection between the two host species has generated two phenotypically distinct ecotypes of *T. cristinae* (Sandoval 1994*a*, 1994*b*; Nosil and Crespi 2006). Seven of the 10 traits we focus on here are involved in adaptive color and pattern differences between populations of *T. cristinae* living on either of the two species of host plant: the ecotype that lives on *C. spinosus* is a light green color and generally lacks any distinct patterning (fig. 1) while the ecotype found on *A. fasciculatum* is a darker shade of green and often possesses a light green to white colored dorsal stripe running along the length of the midsagittal body line (fig. 1). Manipulative experiments and observational field studies have shown that each ecotype has reduced fitness on their nonnative host as a result of increased rates of visual predation (Sandoval 1994*a*, 1994*b*; Nosil 2004, 2007; Nosil and Crespi 2006).

The degree of population differentiation between the ecotypes of *T. cristinae* varies strongly with two factors. First, for all traits considered here, mean phenotypic divergence between populations on different hosts is inversely related to rates of between-host gene flow (Sandoval 1994*b*; Nosil 2007). Second, the degree of trait-specific divergence is strongly related to the strength of differential selection on a trait (fig. 1*c*).

In contrast to the detailed natural history knowledge of phenotypes involved in host-plant adaptation in *T. cristinae*, we know little about their genetic basis. Here we take advantage of a phenotypically variable population of *T. cristinae* to conduct GWAS for the adaptive phenotypes described in Nosil and Crespi (2006).

Methods

Sampling

We collected *Timema cristinae* from a population living on *Adenostoma fasciculatum* in an approximately 1-km^2 area of Los Padres National Forest, California (population code FHA; N34°30.958′, W119°48.050′). We chose this population because it satisfied the conditions necessary for conducting GWAS. Specifically, we were able to collect a large number of individuals who showed variation in the traits of interest (details below). Phenotypic variation within this population is likely in part the result of gene flow between populations on different hosts as both host species occur in the general FHA area, a situation where between-host gene flow has been detected whenever tested for (Nosil et al. 2012*a*, 2012*b* for details).

We collected *T. cristinae* with sweep nets and maintained them in 500-mL plastic containers in the lab. Individuals were fed ad lib. on a mixture of fresh *A. fasciculatum* and *Ceanothus spinosus* clippings until adulthood. For each of 264 adults, we removed three legs along the left side of the body as tissue samples (stored in 100% ethanol at −20°C). Each individual was then immediately euthanized using ethyl acetate and photographed on grid paper (2 × 2-mm grid size) that included a standard color chip. Photographs were recorded using a Canon PowerShot SX100 IS digital camera mounted on a tripod under standardized conditions with a shutter speed of 1/20 s and an aperture of *f*/4.

A subset ($n = 49$) of these pictures were taken under different lighting conditions and were excluded from color measurements (see below). Six individuals that had a "red" phenotype with markedly different color and pattern than green-colored individuals were also excluded from both the color and pattern measurements. To estimate repeatability, we collected five photographs each from a subset of 10 individuals. All color measurements described below were then recorded for each of these 50 photographs and repeatability was estimated as described by Whitlock and Schluter (2011, pp. 416, 420). All traits were significantly repeatable (see apps. A and B).

Phenotypic Variation

We collected the same six color measurements as Nosil and Crespi (2006; n male = 132, n female = 77) using the eyedropper tool in Photoshop, version 12.0.4 (Adobe Systems) set to average over a 5 × 5-pixel area. We then calculated the mean of each color measurement for either the dorsal (average of two measurements; fig. 1*b*) or lateral (average of three measurements; fig. 1*b*) body regions to generate six mean color measurements: dorsal hue, dorsal saturation, dorsal brightness, lateral hue, lateral saturation, and lateral brightness. We used ANCOVA and extracted residuals that controlled for slight variation in the ambient lighting conditions among photographs taken on different days and differences in color between the sexes (app. A).

To describe variation in dorsal pattern, we estimated the proportion of the dorsal body surface that was striped by dividing the area of the dorsal stripe by total dorsal body area (fig. 1*b*). Area measurements were obtained using the polygon selection tool in the software ImageJ, version 1.46 (Abràmoff et al. 2004). We corrected for a difference in

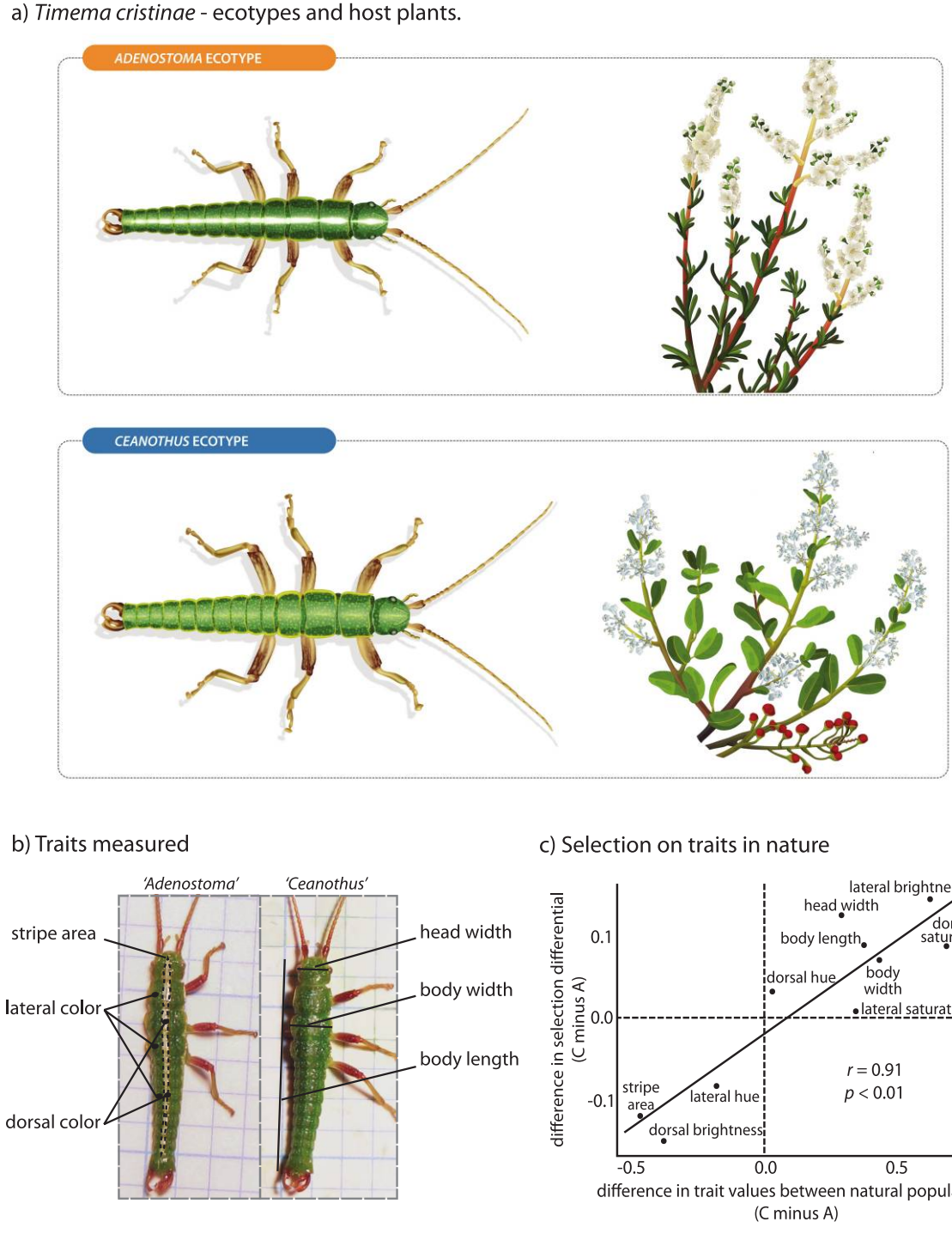

Figure 1: Ecotypes of *Timema cristinae*, their respective host plants, phenotypic traits measured in this study, and evidence for selection on those same phenotypic traits. *a*, Typical phenotypes of the two *T. cristinae* ecotypes are illustrated (*left*) with their respective host plants (*right*). The "*Adenostoma*" ecotype is found on the host plant *Adenostoma fasciculatum*, while the "*Ceanothus*" ecotype is found on *Ceanothus spinosus*. Illustrations courtesy of R. Ribas. *b*, Photographs of male *T. cristinae* indicating the phenotypes focused on in this study. Each phenotypic trait was measured for >200 individuals (see text for details). *c*, Relationship between adaptive divergence (*X*-axis) and the strength of differential selection (*Y*-axis) acting on the same traits as in *b* ($C - A = A$. *facisculatum* population value subtracted from *C. spinosus* population). This strong relationship between levels of divergence and the strength of selection is found in the presence but not the absence of avian predators. Modified from Nosil and Crespi (2006) with permission from the National Academy of Sciences of the USA.

area between the sexes by extracting residuals from linear models (app. A).

To describe variation in size and shape we measured body length, body width (widest area of thoracic segment two), and head width (distance between the eyes) for all individuals (fig. 1*b*). These measurements were taken to the nearest thousandth of a centimeter using the line tool in Photoshop. We removed the effect of sex on overall size by extracting the residuals from linear models, while we accounted for the effects of size and sex on head width and body width by extracting residuals from ANCOVAs (app. A). We tested for correlations between phenotypic traits using Pearson's correlation coefficients. All statistical analyses were carried out in R, version 2.15.0 (R Development Core Team 2012).

To assess how phenotypic variation in the population we sampled compared to phenotypic divergence between the two host ecotypes of *T. cristinae*, we also measured the same 10 traits described above for individuals collected from two additional populations: one living on the host plant *A. fasciculatum* (HVA) and another living on *C. spinosus* (VPC; app. A). These populations were chosen to represent maximal levels of trait divergence (Nosil and Crespi 2004, 2006) and therefore provide information on the total amount of phenotypic variation in these traits observed among natural populations. All raw phenotypic measurements are deposited in the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.ck2cm (Comeault et al. 2014).

Genetic Variation

To generate genome-wide SNP data we sequenced reduced complexity genomic libraries for 223 of the individuals that were scored for phenotypic traits. We used the library preparation protocol of Parchman et al. (2012) that is designed for Illumina sequencing chemistry and that we have previously applied to *T. cristinae* (Nosil et al. 2012). Briefly, to generate these libraries we first isolated genomic DNA from each individual using DNeasy Blood and Tissue Kits (Qiagen). Genomic DNA was then digested with the restriction endonucleases *Mse*I and *Eco*RI (New England Biolabs). We incubated the restriction digests with T4 DNA ligase (New England Biolabs) and oligonucleotides containing the first Illumina adaptor sequence followed by eight, nine, or 10 bases of barcode sequence and the *Eco*R1 cut site and oligonucleotides containing the second Illumina adaptor and the *Mse*I cut site. Fragments were then amplified via polymerase chain reaction (PCR; 35 total cycles) using standard Illumina primers, pooled to facilitate multiplexed sequencing, and size selected and purified from agarose gels using the QiaQuick Gel Extraction Kit (Qiagen; fragment sizes between 300 and 500 bp). We pooled 192 of our uniquely barcoded individual libraries into one pooled library and pooled the remaining 31 uniquely labeled individuals with 161 individuals that were sequenced as part of another study in a second library. Concentrations of these two pooled libraries were evaluated on an Agilent BioAnalyzer, and the concentration of restriction fragments containing the Illumina sequencing adaptors was verified using qPCR. Sequencing of the pooled libraries was accomplished on two lanes each of the Illumina HiSeq 2000 platform with V3 reagents, generating 100-bp reads in each of these four sequencing lanes.

After removing reads containing contaminant sequences (Nosil et al. 2012*a* for details), we retained 410,750,977 reads for analysis. We used an artificial reference sequence constructed from a de novo assembly of similar data (Nosil et al. 2012*a*), which consisted of 142,997 contigs (continuously sequenced 100-bp fragments of DNA). We then assembled the full set of 410,750,977 sequences onto this artificial reference using SeqMan NGen, version 1.0.3.3 (DNASTAR). All other details of the reference-based assembly are as described in Nosil et al. (2012*a*). This assembly placed 178,506,384 reads onto the reference, for an average coverage depth of 1,248 times per sequenced region (6.4 times per individual per sequenced region).

We used custom Perl scripts in combination with samtools and bcftools (Li et al. 2009) to identify variable sites and count the number of reads supporting each alternative nucleotide state for each individual at each locus. We used the full prior in bcftools to filter variant sites retained for estimating genotypes (see below). We then discarded genetic regions where individuals appeared to have more than two haplotypes and where the observed allele counts from apparently heterozygous individuals were unlikely given a binomial distribution with $P = .5$. We also discarded loci where reads were present for fewer than 30% of the individuals and where the probability of the data under the assumption that all individuals were homozygous at the reference allele for each SNP was greater than 0.05. We disregarded insertions and deletions and recorded counts for the number of reads representing each SNP state for each locus in each individual. A final set of 211,004 SNPs distributed over 114,898 contigs was retained for further analyses. Given that *T. cristinae* has a genome size of approximately 1.3 Gb, our sequencing strategy sequences approximately 8% of the genome.

Although the genomic location of these SNPs is yet to be determined, levels of linkage disequilibrium within the population used in this study (population code *FHA*) are generally very low (Burrow's composite measure of Hardy-Weinberg and linkage disequilibrium (Δ): mean $\Delta = 0.004$ for SNPs on different contigs, 0.007 for SNPs on the same contig; Gompert et al. 2014). Data from other populations of *T. cristinae* also show that LD among loci generated by the molecular technique used here is typically low (Nosil

et al. 2012*a*, 2012*b*). However, despite mean LD typically being low for most pairs of SNPs, some pairs of SNPs have higher LD (maximum estimate of Δ, averaged across all SNPs, of $\Delta = 0.035$, 99% upper quantile $= 0.088$; Gompert et al. 2014). Thus, although the SNPs we analyze here represent largely independent markers, moderate statistical associations among some physically linked or unlinked genomic regions exist. Future linkage mapping and whole genome sequencing studies will greatly increase our understanding of the genomic distribution of the SNPs we analyze here and their linkage relationships with potential causative variants.

We used a Bayesian model described in Gompert et al. (2012) to estimate population allele frequencies and genotypes for each SNP. We treated the genotype at a locus and the population allele frequency as unknown model parameters and estimated genotype probabilities and allele frequencies from the SNP count data obtained from samtools and bcftools (Li et al. 2009). We obtained posterior probabilities for parameters using Markov chain Monte Carlo. Each analysis consisted of a single chain iterated for 20,000 steps where samples were recorded at every fourth step. We calculated the expected genotypic value for each individual at each locus as: (the homozygous reference allele genotype probability $\times$ 2) plus (the heterozygote genotype probability $\times$ 1). For example, an individual with estimated genotype probabilities of 0.01 (homozygous for the reference allele), 0.60 (heterozygous), and 0.39 (homozygous for the nonreference allele) would receive a genotypic value of 0.62 (i.e., $0.01 \times 2 + 0.60 \times 1 + 0.30 \times 0$). The point estimate of the genotypic value therefore spans $[0, 2]$ and represents the dosage of the arbitrary first allele within a genotype and incorporates uncertainty in the specific genotype of an individual at a given locus. Files containing SNP count information and expected genotypic values are deposited in the Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.ck2cm.

Genetic Architecture of Adaptive Phenotypes

We carried out multilocus BVSR analyses as implemented in the program piMASS, version 0.9 (Guan and Stephens 2011). BVSR implements a linear model:

$$\mathbf{y}|\boldsymbol{\gamma}, \boldsymbol{\mu}, \tau, \boldsymbol{\beta}, X \sim N_n(\boldsymbol{\mu} + X_{\boldsymbol{\gamma}}\boldsymbol{\beta}_{\boldsymbol{\gamma}}, \tau^{-1}I_n),$$

where $\mathbf{y}$ and X are the observed data, with $\mathbf{y}$ denoting the phenotypic scores of individuals and X containing the genotypes of individuals at all loci. The vector $\boldsymbol{\gamma}$ is a set of binary variables that indicate which SNPs have nonzero values for $\boldsymbol{\beta}$; $\boldsymbol{\mu}$ is the intercept, and τ the precision (inverse of the variance) of the residual errors.

As suggested by Guan and Stephens (2011) we normal quantile–transformed all our phenotypic variables before analyses. This transformation standardizes all phenotypic traits to have a mean of 0 and variance of 1 and facilitates comparisons of the phenotypic effect of SNPs across traits. For each trait we ran four independent chains in piMASS, each with a burn-in of 1,000,000 steps followed by 8,000,000 steps with sampling every four-hundredth step. For results presented here, we specified priors that restricted the hyperparameters h to $[0.01, 0.9]$ and p [1, 1,000] (the hyperparameter h is used to estimate the proportion of variance explained by the model while p is the conditional prior probability and defines the sparsity of the model) and set the minimum and maximum number of SNPs in the model to 1 and 100. Varying the prior options among runs resulted in qualitatively similar results; however, analyses conducted using the prior options described above resulted in the best convergence to the stationary distribution as determined by visually inspecting posterior probability traces of parameters and hyperparameters.

For each trait, we report means as point estimates and 95% credible intervals (95% equal-tail probability intervals) for N_{SNP}, PVE, σ_{AVE}, and p_{SNP}). We also calculate the number of SNPs with PIPs greater than 0.01 ($\text{PIP}_{0.01}$ SNPs) and identify the three SNPs with the highest PIPs for each trait. Although a PIP of 0.01 seems relatively low, we note that a SNP with a PIP of 0.01 is approximately 1,000 times more likely to be included in the model than a randomly selected SNP from our data set. For all $\text{PIP}_{0.01}$ SNPs, we report both their PIP and the estimated magnitude of their phenotypic effect (β) as the Rao-Blackwell estimates. Finally, we calculated associations between the genetic architecture of different traits by calculating the proportion of shared $\text{PIP}_{0.01}$ SNPs between all pairwise comparisons as the number of shared $\text{PIP}_{0.01}$ SNPs divided by the total number of unique $\text{PIP}_{0.01}$ SNPs between the two traits.

Selection, Genetic Architecture, and Population Divergence

For a given number of loci and strength of total selection on a trait, traits that are affected by a few large effect loci should be able to diverge in the face of gene flow more readily than those affected by many loci of small effect (Yeaman and Whitlock 2011). We tested whether aspects of genetic architecture predict levels of trait-specific phenotypic differentiation between host ecotypes in the wild using multiple regression analyses (where phenotypic differentiation is the dependent variable and was estimated using the size-corrected difference in mean trait values between *Ceanothus* and *Adenostoma*; data from table S2 of Nosil and Crespi 2006). Because past work already demonstrated that the difference in selection between hosts (selection differential on *Ceanothus* minus selection differential on *Adenostoma*, from table S3 of Nosil and Crespi

2006) was associated with phenotypic differentiation, we included this as an independent variable in the model. We also included the estimates of PVE, N_{SNP}, σ_{AVE}, highest PIP for a SNP associated with a trait, and highest β estimated for a SNP as additional independent variables describing the genetic architecture of traits. Because this model included numerous correlated independent variables, we estimated a reduced regression model using backward elimination. We also conducted univariate pairwise correlations between trait divergence and the estimates of selection and genetic architecture described above.

The results of the multiple regression analysis should be interpreted with caution for several reasons. First, correlations between traits (e.g., which affect the independent variable) violate the assumptions of linear regression. However, we note that previous work deriving uncorrelated principal component axes from trait values showed that correlations between traits had little effect on the ability for selection to predict trait divergence (Nosil and Crespi 2006). Second, there is error in our independent variable, violating another assumption of regression analysis. Third, although selection and genetic architecture can affect trait divergence, reverse causality is also possible.

Results

Phenotypic Variation

All phenotypic traits we measured were variable (fig. 2). The standard error (SE) of the corrected residuals describing dorsal color traits ranged from 0.314 to 0.596, while the SE of residual lateral color measurements ranged from 0.231 to 0.305. Body length had a SD of 0.210. Corrected residuals describing body width had SE = 0.003, and those describing head width had SE = 0.001. Sex-corrected residuals of dorsal striped area, a measurement we used to describe dorsal patterning, had a SE of 0.003. A number of statistically significant correlations were observed between traits we measured (table 1). The strongest correlations were between dorsal traits, with weaker correlations between lateral color, size, and shape traits. The distribution of trait values we measured within our focal study population spanned the mean values observed in other populations of *Timema cristinae* living on both host plant species (fig. 2, vertical lines; see app. A for details).

Genetic Architecture of Adaptive Phenotypes: Multilocus Results

We modeled trait variation as a function of 211,004 SNPs, using BVSR. The proportion of variance explained varied among traits. For dorsal color and pattern traits, BVSR models explained a large amount of phenotypic variation (point estimates of PVE = 0.435–0.586; table 2). For lateral color, body size, and body shape traits, our SNP data explained a smaller but still moderate proportion of variation (lateral color traits PVE = 0.136–0.237; body length PVE = 0.112; body width PVE = 0.201; head width = 0.220).

Estimates of the number of SNPs (N_{SNP}) underlying traits ranged from 4 for both dorsal hue and dorsal brightness to 34 for head width (table 2); however, 95% credible intervals (CIs) surrounding these point estimates were typically large (see table 2). Notably, 95% CIs of N_{SNP} for dorsal color and pattern traits were much smaller than for lateral color, body size, and body shape traits. For example, 95% CIs for the number of SNPs underlying dorsal color traits had a lower limit of 1 and an upper limit of 38 SNPs, while for lateral color traits, 95% CIs for N_{SNP} ranged from 1 to 95 SNPs. The average effect of associated SNPs (σ_{AVE}) varied similarly among traits as PVE: dorsal color and pattern traits had higher estimates of σ_{AVE} (1.239–1.414) than lateral color traits (0.334–0.663). Estimates of σ_{AVE} for body size and shape traits were similar to estimates for lateral color traits (range: 0.346–0.365).

Genetic Architecture of Adaptive Phenotypes: Results concerning Individual SNPs

Estimates of the strength of association between genotypic variation at individual SNPs and phenotypic variation (i.e., $|\beta|$) followed a similar trend among traits as estimates of σ_{AVE}; we recovered individual SNPs with larger phenotypic effects for dorsal color and pattern traits than for SNPs associated with all other traits (fig. 3). For example, when considering all SNPs with PIP > 0.01, SNPs associated with dorsal color and pattern traits had larger estimates of $|\beta|$ (dorsal hue, $|\beta|$ = 0.006–1.057; dorsal saturation, $|\beta|$ = 0.007–0.989; dorsal brightness, $|\beta|$ = 0.009–1.648; dorsal stripe area, $|\beta|$ = 0.007–1.181; fig. 3*b*, 3*e*) than SNPs associated with variation in lateral color ($|\beta|$ = 0.004–0.6243; fig. 3*a*). Aside from a single SNP associated with lateral hue, all SNPs with estimates of $|\beta| > 0.5$ were associated with variation in dorsal color and pattern (six unique SNPs total). When considering only the three SNPs recovered with the largest PIP for each trait, estimates of $|\beta|$ were larger for SNPs associated with dorsal color and pattern traits than for all other traits (table 3).

SNPs that associated with multiple traits also tended to have large effects on at least one of the traits they associated with. For example, SNP 23472 associated with all dorsal color and pattern traits and had the highest PIP and estimate of $|\beta|$ for dorsal saturation and stripe area (see tables 3 and 4 for values). SNP 122741 was also associated with all dorsal color and pattern traits and had the third highest PIP and $|\beta|$ for the stripe area trait but was less

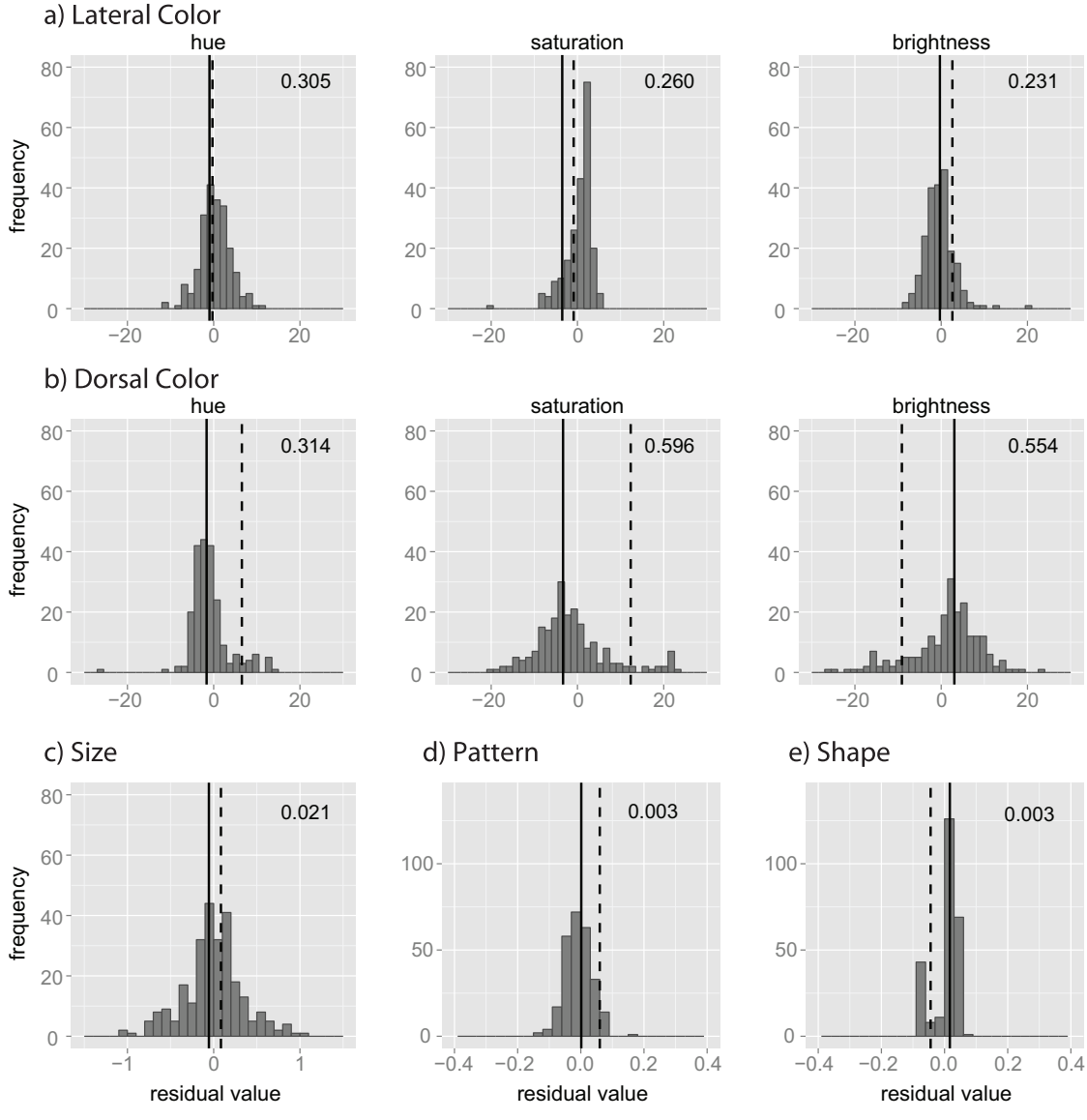

Figure 2: Levels of trait variation within our study population and for two populations adapted to alternate host plants in nature. Histograms showing variation in traits describing lateral color (*a*), dorsal color (*b*), size (i.e., body length; *c*), shape (body width; similar results were obtained for head width [see text]; *d*), and stripe area (*e*) are shown, along with standard errors (top right of each panel). Vertical lines represent population mean trait values for populations living on the two host plant species in nature (dashed line, *Ceanothus* host; solid line, *Adenostoma* host).

strongly associated with dorsal color traits (PIP range for dorsal color traits = 0.012–0.050; $|\beta|$ range for dorsal color traits = 0.014–0.071). SNP 171787 was associated with all dorsal color traits and had the second highest PIP and $|\beta|$ for dorsal saturation. SNP 155501 had the highest PIP and estimate of $|\beta|$ for both dorsal hue and dorsal brightness (tables 3, 4, and B2), and SNP 169259 had a weaker but still relatively large effect on these two traits. Other SNPs that associated with two traits tended to have variable and more modest effects on those traits.

Selection, Genetic Architecture, and Population Divergence

We found no significant relationship between parameter estimates describing the genetic architecture of traits and levels of trait divergence in nature ($P > .1$ for all genetic architecture parameters, e.g., PVE, σ_{AVE}, etc.). All pairwise univariate correlations between mean trait divergence and the strength of differential selection or parameters describing genetic architecture were also nonsignificant (all $P > .1$).

Table 1: Correlations between phenotypic traits (above diagonal) and the proportion of shared $PIP_{0.01}$ single-nucleotide polymorphisms between the same traits (below diagonal)

	D1	D2	D3	L1	L2	L3	SA	BL	BW	HW
D1	...	**.650**	**−.780**	**.470**	**.150**	.100	**−.670**	.000	.010	**.150**
D2	.063	...	**−.700**	−.030	.070	**.180**	**−.680**	.040	.050	**.160**
D3	.161	.098	...	−.130	.090	.040	**.740**	.040	−.030	**−.150**
L1	.000	.000	.000	...	.130	**−.210**	.030	−.010	−.010	.040
L2	.000	.000	.000	.000	...	.090	.040	**−.160**	.050	.100
L3	.000	.000	.000	.000	.000	...	**−.230**	−.080	−.010	.100
SA	.040	.052	.070	.000	.000	.022	...	**.160**	−.010	−.010
BL	.000	.000	.000	.000	.000	.000	.000	...	.000	.000
BW	.000	.000	.000	.000	.000	.000	.000	.000	...	**.500**
HW	.000	.000	.000	.000	.000	.000	.000	.000	.016	...

Note: Statistically significant correlations between traits ($P < .05$) are shown in bold. D1 = dorsal hue, D2 = dorsal saturation, D3 = dorsal brightness, L1 = lateral hue, L2 = lateral saturation, L3 = lateral brightness, SA = stripe area, BL = body length, BW = body width, and HW = head width. PIP = posterior inclusion probability.

Discussion

We estimated the genetic architecture of traits involved in adaptive divergence between ecotypes of *Timema cristinae* using a Bayesian approach to genome-wide association mapping. This approach provides us with quantitative estimates of the genetic architecture of these traits while accounting for uncertainty in the specific SNPs that underlie trait variation (Guan and Stephens 2011). However, the level of confidence with which we were able to estimate parameters describing the genetic architecture of traits varied among traits. For example, 95% credible intervals tended to span nearly the entire prior range when estimating the number of SNPs underlying nondorsal traits, indicating that there was little information in the data to support inference of the number of SNPs controlling variation in these traits. In contrast to nondorsal traits, confidence intervals surrounding estimates of the number of SNPs underlying dorsal color and pattern traits were much smaller. This discrepancy between trait types could be due to a number of factors, including low sample size, variation in heritability among the traits, variation in the true genetic architecture of the traits, or a combination of these factors. Below we focus on interpreting our results while considering the uncertainty in parameter estimates we observed.

Major Effect Loci and Adaptive Traits

We identified a number of SNPs with relatively large effects on phenotypic variation in adaptive dorsal color and pattern in *T. cristinae*. While the point estimates of genetic architecture we report here should be interpreted with caution, our analyses suggest that adaptive dorsal color and pattern in *T. cristinae* is controlled, in part, by loci with large effects. Specifically, estimates of the average effect of a SNP (σ_{AVE}) and the proportion of phenotypic variation that could be explained by these SNPs were larger for dorsal traits than for traits describing lateral color, body size, and body shape (table 2). The estimated effect size of individual SNPs also indicate that at least some of the SNPs underlying variation in dorsal color and pattern have a large effect on phenotypic variation. For example, of the nine SNPs that BVSR analyses identified with estimates of $|\beta| > 0.5$, all but one was associated with dorsal color or pattern traits (fig. 3).

Notably, crossing experiments indicate that the presence versus absence of a dorsal stripe in *T. cristinae* is largely controlled by one or a few loci of large effect (i.e., simple Mendelian inheritance patterns; Sandoval 1993) and this trait can be reliably and repeatedly scored as "present" or "absent" in most populations (e.g., Sandoval 1994*a*, 1994*b*; Nosil 2007, 2009). Indeed, even in the population utilized in this study variation in dorsal traits tended to be more bimodal than for other traits (fig. 2). The large-effect alleles that we identified add to the growing number of examples of adaptive phenotypes that are controlled by loci with large phenotypic effects (e.g., Jiggins and McMillan 1997; Peichel 2001; Bradshaw and Schemske 2003; Colosimo et al. 2004; Hoekstra et al. 2006; Joron et al. 2006; Steiner et al. 2007; Linnen et al. 2009; Chan et al. 2010; Rosenblum et al. 2010). A notable difference between our results and those cited above is that phenotypic variation in dorsal traits in *T. cristinae* is associated with five independent SNPs that had high inclusion probabilities in BVSR models (PIP > 0.5) and large estimated effects ($|\beta|$) on dorsal phenotypic variation, whereas in contrast, the majority of studies cited above report single SNPs or genomic regions with large phenotypic effects. Until the genomic location of the SNPs that we identified here is determined, we cannot say whether these SNPs represent multiple genomic regions. However, low LD in our data set suggests that these SNPs may represent multiple genomic regions. Fu-

Table 2: Parameter estimates and 95% credible intervals (equal-tail probability intervals, given in parentheses) for the genetic architecture of adaptive traits in *Timema cristinae*

Trait	PVE	p_{SNP}	N_{SNP}	σ_{AVE}
D1	.435 (.272–.771)	2.0×10^{-5} (1×10^{-5} to 1.9×10^{-4})	4 (1–38)	1.272 (.601–3.973)
D2	.553 (.372–.774)	3.0×10^{-4} (1×10^{-5} to 1.5×10^{-4})	7 (2–31)	1.239 (.642–3.078)
D3	.444 (.287–.774)	2.0×10^{-4} (1×10^{-5} to 1.4×10^{-4})	4 (1–29)	1.414 (.668–4.095)
L1	.237 (.030–.539)	3.0×10^{-5} (1×10^{-5} to 2.8×10^{-4})	7 (1–59)	.663 (.171–2.479)
L2	.157 (.006–.625)	1.2×10^{-4} (1×10^{-5} to 4.6×10^{-4})	25 (1–95)	.334 (.073–2.071)
L3	.136 (.005–.554)	6.0×10^{-5} (1×10^{-5} to 4.4×10^{-4})	13 (1–92)	.422 (.076–3.063)
BL	.112 (.004–.373)	5.0×10^{-5} (1×10^{-5} to 4.1×10^{-4})	11 (1–87)	.346 (.066–2.394)
BW	.201 (.007–.664)	1.3×10^{-4} (1×10^{-5} to 4.6×10^{-4})	27 (1–95)	.365 (.081–1.848)
HW	.220 (.009–.690)	1.6×10^{-4} (1×10^{-5} to 4.8×10^{-4})	34 (1–97)	.357 (.090–1.886)
SA	.586 (.446–.754)	4.0×10^{-5} (1×10^{-5} to 1.3×10^{-4})	8 (3–26)	1.314 (.736–2.756)

Note: Here we report the proportion of variance explained (PVE), the conditional prior probability of a single-nucleotide polymorphism (SNP) being in the model (p_{SNP}), the number of SNPs in the model (N_{SNP}), and the average effect of a SNP on the phenotype (σ_{AVE}). Trait name abbreviations are as given in table 1.

ture work could also test whether the conditions under which the discovered major effect loci evolved (i.e., adaptation to a distant optimum, evolution from new mutations versus standing variation) match theoretical predictions (Orr 1998; Uecker and Hermisson 2011).

While the genetic and biochemical pathways controlling color and pattern variation in *Timema* stick insects are unknown, work conducted in other insects (predominately Drosophilid flies and Nymphalid butterflies) have identified numerous genes and biochemical pathways involved in the generation of insect coloration and patterning (reviewed in Wittkopp and Beldade 2009). Two types of genes, those that regulate the deposition of pigments in space and time and those involved in the synthesis of pigments themselves control phenotypic variation in these systems and regulatory changes are frequently implicated as the genetic driver of variation in color and pattern phenotypes (e.g., Gompel et al. 2005; Reed et al. 2011; van't Hof et al. 2011; Martin et al. 2012). Mapping of the large-effect SNPs we identified here to the draft genome of *T. cristinae* (P. Nosil et al., unpublished data) will provide a first step toward identifying candidate genomic regions and genes controlling variation in color and pattern in *T. cristinae*.

Minor-Effect Loci and Adaptive Traits

Despite the likely role of major effect loci on dorsal color and pattern, our analyses identified many loci that showed relatively weak associations with variation in both dorsal and other adaptive traits. When traits are controlled by multiple loci with additive effects, the strength of the association between genetic variation at any single locus and phenotypic variation will be less than for traits controlled by a few loci of large effect (e.g., Ellison et al. 2011). This suggests that nondorsal traits in *T. cristinae* are controlled by a polygenic genetic architecture composed of individual loci with weak phenotypic effects, but the strength of association of our sequenced SNPs with causative adaptive variants (e.g., the map distance they are separated by) could also contribute to this result.

Many traits that show continuous variation are controlled by a complex and polygenic genetic architecture (e.g., Juenger et al. 2005; Valdar et al. 2006; Albert et al. 2007; Bouck et al. 2007; Buckler et al. 2009; Huang et al. 2009; Brachi et al. 2010; Chan et al. 2011; Ellison et al. 2011; Tian et al. 2011; Gompert et al. 2012; Hung et al. 2012; reviewed by Flint and Mackay 2009). Employing the methods used by GWAS is a powerful way to estimate the genetic architecture of complex traits that are controlled by many loci with minor phenotypic effects (Rockman 2012), as exemplified by recent GWAS in model genetic systems (Valdar et al. 2006; Buckler et al. 2009; Flint and Mackay 2009; Huang et al. 2009; Brachi et al. 2010; Chan et al. 2011; Tian et al. 2011; Hung et al. 2012). GWAS in *Arabidopsis thaliana* provide some of the best examples of the genetic architecture of complex traits in nature. GWAS in *A. thaliana* for traits such as flowering time, glucosinolate secondary metabolite production, and fitness in nature have shown that numerous, sometimes hundreds or more, loci of minor effect underlie trait variation (Brachi et al. 2010; Chan et al. 2011; Fournier-Level et al. 2011; Hancock et al. 2011).

Selection, Genetic Architecture, and Population Differentiation

We tested whether estimates of the genetic architecture of adaptive traits in *T. cristinae* could predict levels of adaptive trait divergence in nature. Specifically, for a given number of loci affecting a trait, those traits controlled by alleles with large effects can be more divergent in the face of gene flow than those affected by alleles of small effect. For ex-

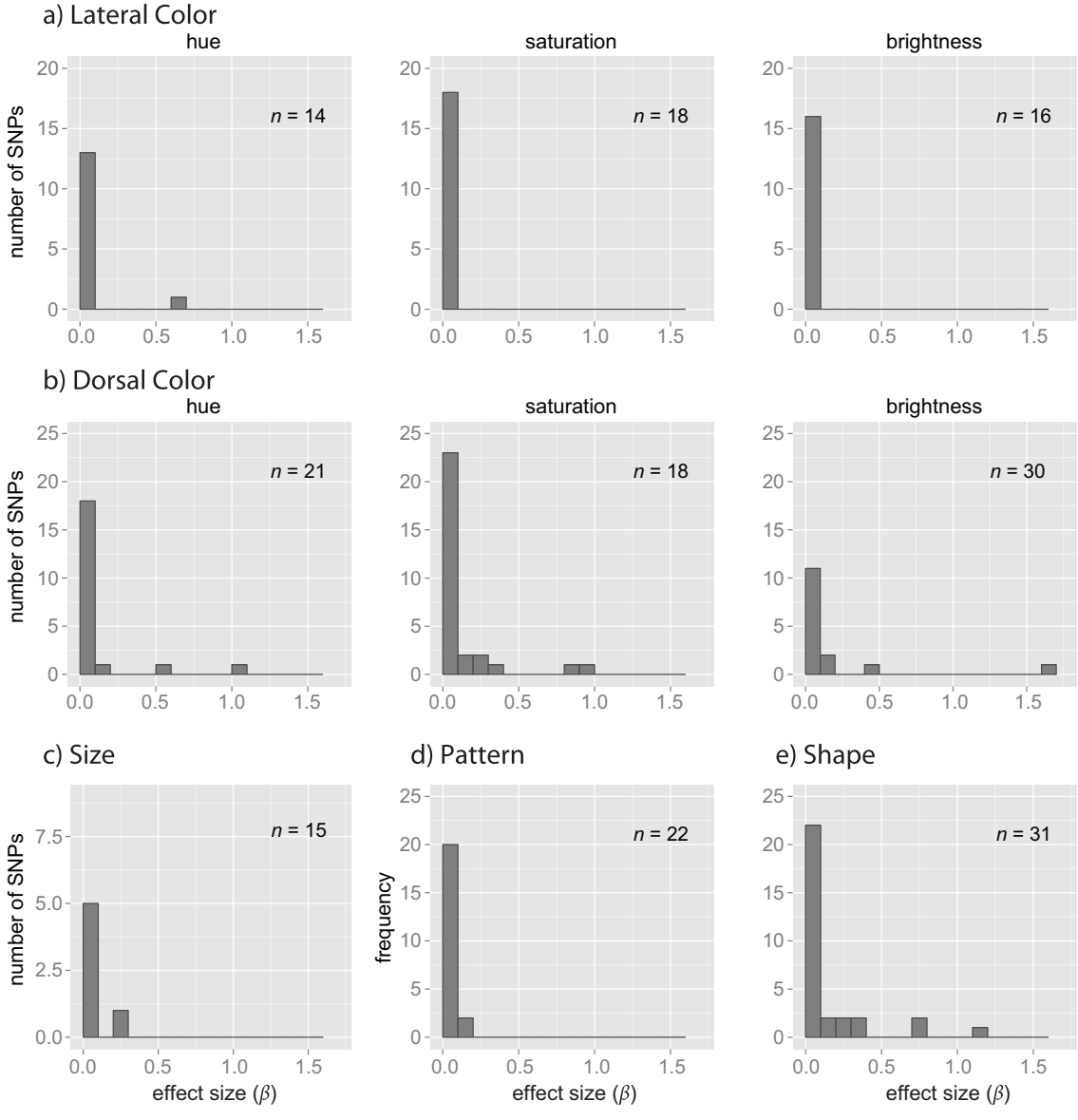

Figure 3: Phenotypic effect sizes of single-nucleotide polymorphisms (SNPs) identified with posterior inclusion probabilities greater than 0.01. Absolute value of effect sizes of SNPs underlying traits describing lateral color (*a*), dorsal color (*b*), size (i.e., body length; *c*), shape (body width; similar results were obtained for head width [see text]; *d*), and pattern (i.e., stripe area; *e*). The number of SNPs with posterior inclusion probability > 0.01 for each trait are given in the top right of each panel.

ample, theoretical studies indicate that divergence in the face of gene flow can be enhanced when selection acts on traits controlled by a few loci of large effect (Gavrilets 2004; Nosil et al. 2009; Yeaman and Whitlock 2011; Flaxman et al. 2012). Our analysis did not find a relationship between any of the parameters describing the genetic architecture of traits we explored here and levels of divergence in natural populations for those same traits. This lack of relationship could be due to a number of factors including variation in heritability among traits or uncertainty surrounding parameters included in these models. Additionally, if traits vary widely in their genetic architecture and many alleles of small effect often underlie trait variation (e.g., the infinitesimal model; Fisher 1918), relationships between gene flow, selection, genetic architecture, and divergence may be more nuanced than the predictions generated by (necessarily) simplified simulation studies. Reciprocal causation between selection and patterns of variation at the genotypic and phenotypic level further complicates inferences concerning the relationships be-

tween these factors. For example, selection requires heritable phenotypic variation on which to act and the presence and nature of that variation will be dependent on many factors, including mutation rate, population size, and the selective environment in which a mutation arises. It is also important to note that the strength of selection acting on the traits included in this study has already been shown to account for the majority of variation in levels of trait divergence ($R^2 = 0.82$; Nosil and Crespi 2006). Therefore, there was little residual variation in trait divergence for genetic architecture to account for once the effects of selection had been considered. Despite a lack of support for the genetic architecture of traits predicting levels of trait divergence observed in nature, this analysis highlights how combining information on both the genetic architecture and natural history of traits can help to test theoretical predictions of the genetics of adaptation. In *T. cristinae*, a very tentative conclusion is that the strength of selection on a trait is of greater importance for its divergence in the face of gene flow than is its underlying genetic architecture. Further work is required to substantiate this claim, but if true, it highlights how ecological field studies of selection are an important component of understanding the evolution of phenotypic traits.

Table 3: Estimates of the posterior inclusion probability (PIP) and the magnitude of effect on phenotype (β) for the three single-nucleotide polymorphisms identified with the highest PIP for each trait

Trait	PIP1	β_1	PIP2	β_2	PIP3	β_3
D1	.625	1.057	.382	.526	.094	.104
D2	.773	.989	.640	.810	.450	.394
D3	.925	1.648	.310	.479	.237	.132
L1	.792	.624	.122	.085	.062	.043
L2	.054	.029	.027	.019	.021	.010
L3	.054	.078	.053	.049	.039	.032
BL	.400	.227	.028	.011	.021	.010
BW	.268	.162	.190	.153	.166	.080
HW	.062	.023	.053	.056	.044	.019
SA	.824	1.181	.538	.742	.533	.740

Note: Trait name abbreviations are as given in table 1.

Future Directions

The genetic architecture of adaptive traits can influence how genomic divergence unfolds. However, studies describing the genetic architecture of adaptive traits are only the first step toward a better understanding of the process of adaptation. For example, GWAS are strictly statistical, and evidence of an association between a SNP and a phenotype of interest does not indicate causation. Thus, making causative links between both phenotype and genotype is important to confirm the role of a given genetic region in underlying adaptive trait variation (Barrett and Hoekstra 2011). This can be accomplished through functional analyses (Manceau et al. 2011) or by introgressing genomic regions of interest into alternate genomic backgrounds (e.g., Lowry and Willis 2010; Ellison et al. 2011). It is also of interest to ask whether genetic regions associated with traits that differ between populations exhibit high divergence (e.g., F_{ST}) between populations (Jones et al. 2012). Finally, field experiments could also be used to measure the response of trait-associated SNPs to selection in nature. Despite long-standing interest, direct experimental estimates of selection acting on adaptive genetic variation are still relatively rare (Bradshaw and Schemske 2003; Weinig et al. 2003; Barrett et al. 2008; Lowry and Willis 2010; Fournier-Level et al. 2011; Hancock et al. 2011; Mojica et al. 2012), especially at the level of individual genes or SNPs. Additional research in *T. cristinae* taking these approaches is underway and could yield a better understanding of the

Table 4: Shared $PIP_{0.01}$ (posterior inclusion probability) single-nucleotide polymorphisms (SNPs) for dorsal color and pattern traits

	SNP ID									
Traits	15307	23472	117994	122740	122741	130019	155501	169259	171787	193911
D1 and D2		+			+				+	
D1 and D3		+			+		+	+	+	
D2 and D3		+			+	+			+	
SA and D1		+			+					
SA and D2		+		+	+					
SA and D3		+	+		+					
SA and L3										+
BW and HW	+									

Note: SNP identification numbers are given for each $PIP_{0.01}$ SNP that was shared between two or more traits. The only traits that shared any $PIP_{0.01}$ SNPs were those describing variation in dorsal color and pattern. The plus sign denotes shared SNP. Trait name abbreviations are as given in table 1.

interplay between phenotypic variation, the underlying genetic variation, and fitness in nature (Gompert et al. 2014).

Acknowledgments

We thank J. Feder, M. Muschick, and members of the "Speciation Reading Group" at the University of Sheffield for constructive discussion on previous drafts of this manuscript. This research was funded by the European Research Council (Starter Grant NatHisGen to P.N.). A.A.C., V.S.-C., T.E.F., and P.N. were supported by the European Research Council, and A.A.C. was supported by a postgraduate scholarship from the Natural Sciences and Engineering Research Council of Canada.

APPENDIX A

Supplementary Information

Kingsolver et al. (2001) Literature Search

We searched the literature for studies that supplemented estimates of selection acting on traits in the study systems reviewed by Kingsolver et al. (2001) with estimates of the molecular genetic basis of those traits under selection (we did not include studies that estimated heritability of additive genetic variation for traits). Specifically, we reviewed the abstract of each study examined by Kingsolver et al. (2001) for phenotypic selection. We recorded the species names and traits for which the strength of selection was estimated. We then queried the Web of Science database for studies that included the name of the study organism for which estimates of selection were available and the terms "genetic" or "mapping." We found studies that estimated the genetic architecture of the traits under selection for only 6 of the 51 study systems examined by Kingsolver et al. (2001). For 5 out of these 6 study systems, estimates of the genetic architecture of traits under selection stem from quantitative trait loci mapping. The one other study that had genetic information examined the developmental pathway underlying variation in beak morphology in Darwin's finches (Abzhanov et al. 2006).

Repeatability Estimates for Phenotypic Measurements

We estimated the repeatability of our trait measurements as described by Whitlock and Schluter (2011). Specifically, repeatability is estimated by dividing the difference between the mean squared treatment and the mean squared error terms by the number of repeated measures (here we measured each individual five times) and then taking the proportion of this term over itself plus the mean squared error term. This estimate of repeatability gives the proportion of variance that is distributed among treatment levels (i.e., individuals). We found that all traits examined were statistically variable among individuals and typically had moderate to high estimates of repeatability (i.e., the majority of variation was partitioned among rather than within individuals; table B1).

Correcting for the Effects of Variation in Lighting, Sex, and Body Size on Phenotypic Measurements

We used ANCOVA to extract residuals controlling for slight variation in the ambient lighting conditions among photographs taken on different days and differences in color between the sexes. The dependent variable in all cases was the color measurement of interested. We carried out ANCOVAs that modeled the effect of variation in lighting (i.e., the color measurement of interest obtained from the standard green color chip found in each photograph was used as a covariate) and sex on each color measurement separately. Slopes of all ANCOVAs were not significantly different between the sexes (all $P > .05$) except for the measure of lateral hue ($F_{1, 211}$, $P = .008$). For our measurement of dorsal pattern we controlled for differences in stripe area between the sexes ($F_{1, 256} = 20.89$, $P < .0001$) by extracting residuals from linear models. We removed the effect of sex on overall size ($F_{1, 262} = 1{,}211$, $P < .0001$) by extracting the residuals from linear models with sex as the independent variable. Finally, we accounted for the effects of size and sex on head width (effect of size: $F_{1, 260} = 3{,}344.32$, $P < .0001$, sex: $F_{1, 260} = 344.61$, $P < .0001$) and body width (effect of size: $F_{1, 260} = 528.06$, $P < .0001$, sex: $F_{1, 260} = 19.56$, $P < .0001$) by extracting residuals from ANCOVAs that modeled shape measurements as a function of body size and sex.

Trait Variation Relative to the Total Amount of Variation between Ecotypes

To estimate the total amount of phenotypic variation present among populations in nature in the traits we conducted GWAS for in this study, we measured each trait for individuals sampled from two populations, one sampled from *Adenostoma fasciculatum* and the other from *Ceanothus spinosus*. The two populations we used in this analysis were chosen based on high levels of adaptation to their respective host plant environment, as determined by qualitatively observing the phenotypes of individuals upon capture and phenotypic measurements collected from these individuals in previous studies (Nosil and Crespi 2004, 2006). We sample and measured each of the 10 traits described above for a total of 22 individuals from *A. fasciculatum* and 33 from *C. spinosus*. All phenotypic

measurements were recorded as described for our focal population.

The variation we sampled in our focal population spanned the range of mean trait values observed in the two populations we sample for this comparison. Six of the ten traits (lateral brightness, dorsal hue, dorsal saturation, dorsal brightness, stripe area, and body width) that we measured in our focal population had mean trait values that were closer to mean trait values of the population we sampled from *A. fasciculatum*, while 4 (lateral hue, lateral saturation, body length, and body head) had mean trait values that were closer to those found in the population we sampled from *C. spinosus*. These data provide evidence that the phenotypic variation found in the population we focused on to conduct genome-wide association mapping studies presented here is comparable to the total amount of phenotypic variation that exists between the two ecotypes of *Timema cristinae*.

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APPENDIX B

Supplementary Tables

Table B1: Repeatability information

Variable	df	MS groups	MS error	*F*	*P*	Repeatability
Dorsal hue	9, 40	13.181	1.135	11.62	<.001	.680
Dorsal saturation	9, 40	32.910	2.810	11.72	<.001	.682
Dorsal brightness	9, 40	52.140	.960	54.55	<.001	.914
Lateral hue	9, 40	46.300	1.480	31.21	<.001	.858
Lateral saturation	9, 40	6.087	1.897	3.208	.005	.306
Lateral brightness	9, 40	8.217	.776	10.59	<.001	.657
Area of stripe	9, 40	.000	.000	9.687	<.001	.635
Body length	9, 40	1.903	.001	3,558	<.001	.999
Body width	9, 40	.067	.000	1,125	<.001	.996
Head width	9, 40	.001	.000	9.839	<.001	.639

Table B2: $PIP_{0.01}$ SNPs that were shared among at least three traits and the magnitude of their effect on phenotype (β) for each trait

Trait	SNP	PIP	β	Trait	SNP	PIP	β
BW	15,307	.028	.011	SA	122,741	.533	−.740
HW	15,307	.019	.007	D2	130,019	.097	.128
D1	23,472	.382	.526	D3	130,019	.053	−.071
D2	23,472	.773	.989	D1	155,501	.625	1.057
D3	23,472	.310	−.479	D3	155,501	.925	−1.648
SA	23,472	.824	−1.181	D1	169,259	.016	.021
D3	117,994	.018	−.024	D3	169,259	.091	−.149
SA	117,994	.538	−.742	D1	171,787	.016	.013
D2	122,740	.217	.285	D2	171,787	.640	.810
SA	122,740	.014	−.016	D3	171,787	.018	−.014
D1	122,741	.012	.014	SA	193,911	.039	−.026
D2	122,741	.027	.037	L3	193,911	.026	.018
D3	122,741	.050	−.071				

Note: PIP = posterior inclusion probability; SNP = single-nucleotide polymorphisms.

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Symposium Editor: Hopi E. Hoekstra