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LETTER

Experimental evidence for ecological selection on genome variation in the wild

Zachariah Gompert,[1] Aaron A. Comeault,[2] Timothy E. Farkas,[2] Jeffrey L. Feder,[3] Thomas L. Parchman,[4] C. Alex Buerkle[5] and Patrik Nosil[2]*

Abstract

Understanding natural selection's effect on genetic variation is a major goal in biology, but the genome-scale consequences of contemporary selection are not well known. In a release and recapture field experiment we transplanted stick insects to native and novel host plants and directly measured allele frequency changes within a generation at 186 576 genetic loci. We observed substantial, genome-wide allele frequency changes during the experiment, most of which could be attributed to random mortality (genetic drift). However, we also documented that selection affected multiple genetic loci distributed across the genome, particularly in transplants to the novel host. Host-associated selection affecting the genome acted on both a known colour-pattern trait as well as other (unmeasured) phenotypes. We also found evidence that selection associated with elevation affected genome variation, although our experiment was not designed to test this. Our results illustrate how genomic data can identify previously underappreciated ecological sources and phenotypic targets of selection.

Keywords

Adaptation, climate, ecological speciation, genome evolution, genomics, natural selection, next-generation sequencing, population genomics, *Timema cristinae*.

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INTRODUCTION

Natural selection is the mechanism responsible for adaptation and can drive speciation (Schluter 2001; Funk *et al*. 2006). Consequently, understanding the ecological causes and evolutionary consequences of selection is a major goal in biology, especially because historical contingency and stochastic variation in fitness also affect evolution (Gavrilets & Hastings 1996; Gould 2002; Kolbe *et al*. 2012). Of particular interest to ecologists, studies of selection can identify key biological interactions and ecological sources of selection that affect population, community and even ecosystem dynamics on short time scales (Pelletier *et al*. 2009; Post & Palkovacs 2009; Hanski & Mononen 2011; Farkas *et al*. 2013). Such an understanding of evolution by selection in changing environments is especially important in the context of rapid ecological change (Seehausen *et al*. 2008; Pespeni *et al*. 2013).

Accordingly, numerous studies have quantified phenotypic selection within generations in field experiments or natural populations (reviewed in Endler 1986; Kingsolver *et al*. 2001; Siepielski *et al*. 2009, 2013). These studies demonstrate that selection is common and individual episodes of selection can be strong, but that selection varies across space and time. In contrast, less is known about the dynamics of selection at the genome level, particularly in non-laboratory populations (but see Barrick *et al*. 2009; Araya *et al*. 2010; Burke *et al*. 2010; Paterson *et al*. 2010; Burke 2012; Anderson *et al*. 2013; Pespeni *et al*. 2013). For example, whereas patterns of genome variation in natural populations have been used to infer the long-term genomic consequences of selection (Hohenlohe *et al*. 2010; Lawniczak *et al*. 2010; Fournier-Level *et al*. 2011; Hancock *et al*. 2011; Ellegren *et al*. 2012; Heliconius Genome Consortium 2012; Jones *et al*. 2012; Roesti *et al*. 2012), these patterns may tell us little about contemporary selection's immediate effect on genome variation. This gap in our knowledge is important because a genome-level understanding of selection across different time scales is necessary to more fully understand the consequences of the ecological interactions that determine fitness.

Here, we focus on how we can learn about selection's effect on genome variation by quantifying allele frequencies at many loci before and after an episode of phenotypic selection (Figs. 1 and 2). The main premise is that selection on traits is transmitted to causal genetic variants affecting the traits (direct selection) as well as to additional genetic loci correlated with these functional variants (indirect selection). Thus, selection's genome-wide contribution to allele frequency change depends on the genetic basis of variation in fitness and correlations among loci (linkage disequilibrium, Fig. 2a; Nielsen 2005; Barrett & Hoekstra 2011). Because genomes are large and most populations harbour genetic variation, the genome-level response to selection could consist of changes at many loci, including those not tightly physically linked to any of the causal variants (Hermisson & Pennings 2005; Barrett & Schluter 2008). Isolating the contributions of direct versus indirect selection to this genome-level response will be challenging because of the large number of potentially correlated genetic loci relative to the

[1]*Department of Biology, Utah State University, Logan, UT, 84322, USA*
[2]*Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK*
[3]*Department of Biology, Notre Dame University, South Bend, IN, 11111, USA*
[4]*Department of Biology, University of Nevada, Reno, NV, 89557, USA*
[5]*Department of Botany and Program in Ecology, University of Wyoming, Laramie, WY, 82071, USA*
**Correspondence: E-mail: p.nosil@sheffield.ac.uk*

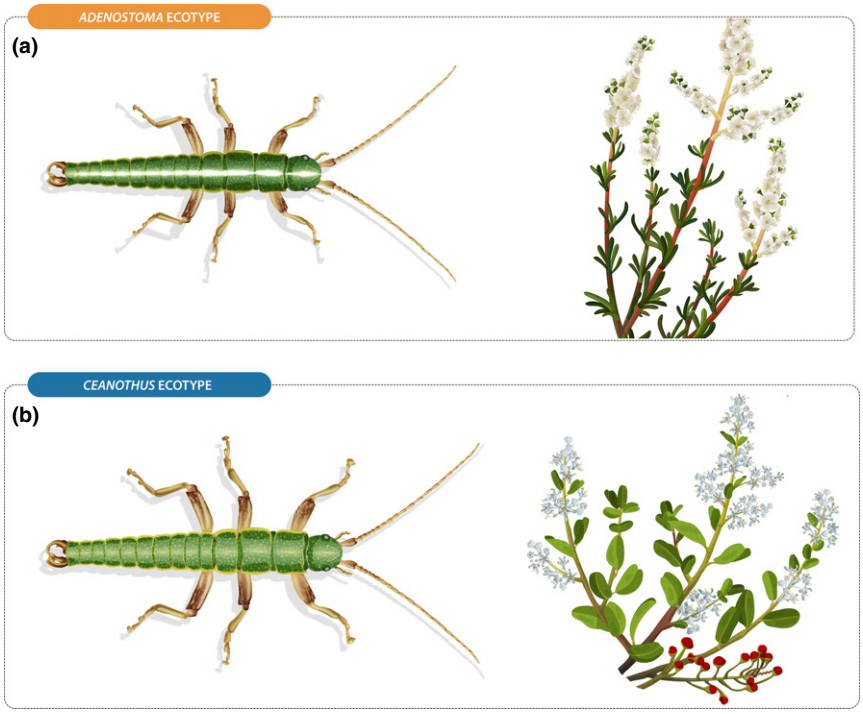

Figure 1 The study system. Illustration of the *T. cristinae* study system, with individuals of each insect ecotype shown on the left and drawings of the host plants that they are adapted to on the right. Illustrations courtesy of R. Marín.

number of individuals that can be sampled from a population (in other words, the number of model parameters will be greater than the number of observations). However, the total selection (direct plus indirect) acting on genome variation, which is analogous to the selection differential in studies of phenotypic selection, can be quantified and is of inherent interest as it determines the genome-level response to selection.

Here, we quantify the contributions of selection and random mortality (genetic drift) to genome-wide allele frequency changes ('genomic change' hereafter) that occurred within a generation after transplanting stick insects to novel and native host plants in a field experiment. The test species is a wingless, herbivorous stick insect (*Timema cristinae*) endemic to southern California that has evolved partially reproductively isolated 'ecotypes' adapted to different host plant species: *Adenostoma fasciculatum* and *Ceanothus spinosus* (Nosil 2007; Nosil *et al.* 2012). These ecotypes differ in a suite of morphological characters, the most obvious being the presence versus absence of a highly heritable, white dorsal stripe, distinguishing the 'green striped' and 'green unstriped' morphs (striped and green hereafter). Previous experiments have shown that striped individuals are more cryptic and suffer less predation from birds on *Adenostoma* than on *Ceanothus,* whereas green individuals are more cryptic and suffer less predation on *Ceanothus* than on *Adenostoma* (Sandoval 1994; Nosil 2004; Nosil & Crespi 2006). Moreover, selection from bird predation can cause rapid and substantial within-generation phenotypic change in *T. cristinae* that affects community composition (e.g. arthropod species richness; Farkas *et al.* 2013).

Our experiment was designed to test the hypothesis that host-related selection causes phenotypic and genomic change in new environments and to quantify selection's effect on genome variation. Genotyping-by-sequencing was used to quantify genomic change in the experiment and whole-genome sequencing was used to generate a reference genome assembly on which we mapped the distribution of these changes. Importantly, our experiment isolates the effects of selection and drift on genomic change because other processes (e.g. recombination and mutation) do not occur within generations. We document the expected host-associated phenotypic response to selection. We find substantial and genome-wide allele frequency change from random mortality during the field experiment. However, we also show that host plant–dependent selection contributes to genomic change at many loci that were widely distributed across the *T. cristinae* genome. This host-associated selection affecting the genome likely acted on both a known colour-pattern phenotype as well as other (unmeasured) phenotypes. Finally, we also find natural selection associated with elevation, which the experiment was not designed to test. The findings demonstrate how genomics-enabled 'reverse ecology' can identify underappreciated sources and phenotypic targets of selection (Li *et al.* 2008).

MATERIALS AND METHODS

Field Experiment

We induced host shifts in nature. To do this, we collected individual *T. cristinae* ($n = 500$) from *Adenostoma* [population code: FHA (Far Hill Adenostoma), 34.51753 N, 119.80125 W] in an area dominated by *Adenostoma*, but in which some *Ceanothus* also occurs. The population FHA is genetically and

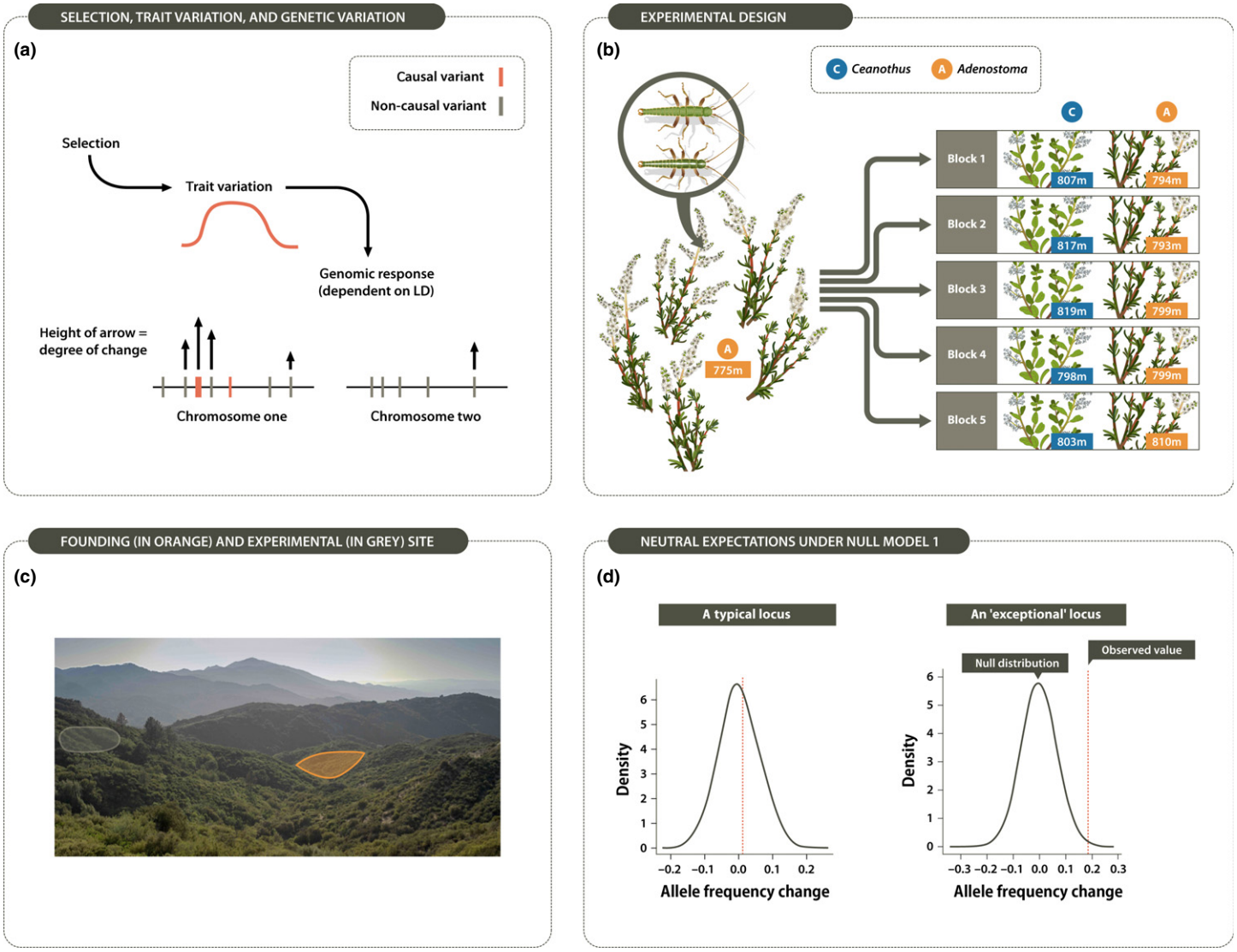

Figure 2 Study species and site, experimental design and predictions. (a) Schematic representation of how selection acting on phenotypic traits affecting fitness can affect allele frequency change in the genome, either directly or via correlations among loci. (b) The source population on *Adenostoma fasciculatum* at 775 m used to found the experiment is depicted in the left of the diagram. In the experiment, individuals were transplanted from the source population to five paired blocks that contained a single plant individual of the native host *Adenostoma* and one of the novel host species *Ceanothus* (illustration credit: Rosa Marín). Different bushes were also at different elevations as indicated. (c) A photo depicting the location of the founding population (orange oval) and the experimental field site (grey oval). (d) Null distributions of allele frequency change between release and recapture at individual loci in the absence of selection. A typical locus is depicted as well as one with exceptional change inconsistent with neutral expectations.

phenotypically variable due to gene flow between populations on different hosts (Nosil *et al.* 2012).

Individuals were collected on April 14, 2011, phenotyped as either striped or green, and placed in 500-mL plastic containers at a density of 50 individuals per container. The following day we randomly assigned individuals to one of 10 experimental bushes (five of each host species). Each individual had a portion of one leg removed as a tissue sample using sterile scissors (no effect of tissue sampling on survival was seen in either laboratory and field experiments, see Supporting Information). Tissue samples were placed in 100% ethanol and stored at −20°C. Individuals were monitored for 12 h prior to their release on the experimental bushes. No adverse effects of tissue sampling were observed in the physical appearance or behaviour of individuals. Notably, the tissue sample allowed us to identify experimental animals in the future, and thus distinguish them from wild *T. cristinae* that were not part of our experiment.

We moved each group of 50 individuals onto either an individual of their native host plant (*Adenostoma*) or the alternative host (*Ceanothus*) on April 16, 2011. All plant individuals used in the experiment were separated from individuals of both host species by regions of grassy, bare ground. Distances between plants within blocks were ranged from 6 to 10 m and distances between blocks from 12 to 30 m (Fig. 2, Fig. S1).

We were interested in rapid changes in these populations because phenotypic studies in this system have documented

adaptive divergence between experimental populations within a week upon transplantation to new environments and because adult and penultimate instar *Timema* tend to live for only 1–3 weeks in the field, with bird predation being a major source of selective mortality (Nosil 2004; Nosil & Crespi 2006; Nosil *et al.* 2012). Thus, after 8 days, we recaptured surviving insects in our experiment using sweep nets and visual surveys during April 24th and 25th (2011) and took a second tissue sample (n = 140). Past mark recapture work has shown this protocol is highly effective at recapturing the overwhelming majority of surviving individuals and that dispersal across 'bare ground' (grassy regions not containing suitable hosts) is near absent (Sandoval 2000; Nosil 2004; Nosil & Crespi 2006; Nosil *et al.* 2012). Nonetheless, we examined the potential for dispersal in our experiment and found only a single instance (Supporting Information). Thus, mortality resulted in the individuals in each population at the end of the experiment being a subset of the initially released individuals (range of surviving individuals = 7–23).

Mortality and phenotypic divergence

We tested whether percent recaptured (survival) differed between hosts using a t-test and whether phenotypic divergence in the stripe phenotype occurred between hosts within blocks using a paired t-test. If host-related selection acted on the stripe phenotype in the experiment, we predict that the difference in the frequency of striped individuals within each block should increase between release and recapture, with striped individuals increasing in frequency on *Adenostoma* but decreasing on *Ceanothus*. We also report changes in the stripe phenotype on each experimental bush and treatment.

Genotype-by-sequencing

We isolated genomic DNA from the 500 tissue samples we took from each released individual and again from legs of the 140 individuals that were recaptured using Qiagen's DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). We constructed reduced-complexity genomic libraries following published protocols (see Nosil *et al.* 2012 and Supporting Information for a complete description). Genotype-by-sequencing (GBS) data gathered from the released and recaptured individuals allowed quantification of allele frequency changes caused by mortality during the experiment.

Sequence assembly, variant calling and genotype estimation

After quality filtering, identifying and removing individual identifier (barcode) sequences, and removing DNA sequences with corrupt barcode sequences or *Mse*I adaptor sequence, we retained 949 227 283 85 bp reads. We assembled these onto an artificial reference that was created from assembly of GBS data presented in Nosil *et al.* (2012), by executing a reference-based assembly using Seqman Ngen (DNAstar, Inc.). We used a minimum match percentage of 93%, a gap penalty of 25 and a mismatch penalty of 20. This assembled 575 583 024 reads onto the artificial reference, resulting in an average coverage depth of 3199× per genetic region, or 5× per stick insect per genetic region. We used samtools (Li *et al.* 2009) in conjunction with custom Perl scripts to identify variable sites and obtained a final set of 186 576 single-nucleotide polymorphisms (SNPs). As in past work (Nosil *et al.* 2012) we used a Bayesian model and Markov chain Monte Carlo (MCMC) to estimate genotypes and allele frequencies in each experimental population (Supporting Information for details).

Whole-genome sequencing and *de novo* assembly

To assemble a first draft of the *T. cristinae* genome we constructed one each of four paired-end libraries with insert sizes of 170 500 800, and 5000 bp and two paired-end libraries with an insert size of 2000 bp for sequencing on seven lanes of the Illumina HiSeq 2000 platform with V3 reagents (Table S1). The assembly we used for further analysis was obtained by invoking the HAPLOIDIFY=T option in ALLPATHS-LG (version 43375; Butler *et al.* 2008). This assembly included 190,773 contigs in 14 221 scaffolds and covered ~ 80% of the genome (Supporting Information for details). We assembled the GBS contig consensus sequences to this draft genome as described in the Supporting Information.

Linkage Disequilibrium

We estimated Burrow's composite measure of Hardy–Weinberg and linkage disequilibrium (Δ) for each pair of variable sites. This measure does not assume Hardy–Weinberg equilibrium or require phased data, but instead provides a joint metric of intralocus and interlocus disequilibria based solely on genotype frequencies. Thus, Δ is equivalent to the linkage disequilibrium parameter D under Hardy–Weinberg equilibrium (Weir 1979). We estimated Δ at the onset of the experiment for all 17 405 208 600 locus pairs within each experimental population. We used a Monte Carlo algorithm to incorporate uncertainty in genotype into our Δ estimates (Nosil *et al.* 2012 and Supporting Information for details). We tested whether levels of Linkage Disequilibrium (LD) within populations differed between SNPs on the same versus different GBS contigs using a paired t-test.

Allele Frequency Change

We estimated the observed change in allele frequency at each locus as $\Delta p_i = (1/(2\ \Sigma_j\ \lambda_j))\ (\Sigma_j\ g_{ij}\ \lambda_j) - (1/2N)\ (\Sigma_j\ g_{ij})$, where $g_{ij} = \{0, 1, 2\}$ is the number of gene copies containing the reference allele for locus i and individual j, and λ is a vector of binary indicator variables designating whether each individual survived ($\lambda_j = 1$) or died ($\lambda_j = 0$). We estimated the Bayesian posterior probability distribution of observed allele frequency change by repeatedly (1000 times) sampling genotypes (g) according to their posterior distributions and calculating Δp_i based on the sampled genotypes. Thus, the posterior probability distributions of allele frequency change incorporated uncertainty in genotypes. This is the only source of uncertainty because we sequenced all individuals in each experimental population. We determined the number of SNPs that fixed for a single allele during the experiment based on our estimates of allele frequency change and considering only SNPs

with initial minor allele frequencies > 5%. We considered fixation in individual populations and within treatments.

Null models of random mortality

We wanted to distinguish between random mortality and natural selection as the causes of allele frequency change during the experiment. We thus developed two null models for allele frequency changes expected by random, genotype-independent mortality (i.e. genetic drift; Fig. S2). We used these null models to test the hypotheses that survival in individual populations or host plant treatments was: (1) independent of genotype at each individual locus, and (2) independent of genotype at all loci.

The first of these null models tests whether the direction and magnitude of allele frequency change at each locus is consistent with evolution by genetic drift (i.e., neither direct nor indirect selection affected the locus). We refer to loci for which this null model was rejected as those with 'exceptional change' (details below). The second null model tests whether the number of loci with exceptional change in an experimental population or host plant treatment (based on null model 1) is greater than expected if selection did not affect any of the loci. This second model accounts for the large number of genetic loci we examine (i.e. addresses the issue of multiple comparisons) and provides a genome-level test that selection had a detectable effect on genomic composition. Both null models incorporate the genetic composition of the experimental populations (e.g. starting allele frequencies), the number of released and recaptured *T. cristinae*, and statistical uncertainty in genotypes and allele frequencies. We describe these null models below and the Supporting Information provides details.

Absence of selection on individual loci (null model 1)
We used a Monte Carlo-Bayesian method to obtain the distribution of expected allele frequency change at each locus under the null hypothesis that survival (λ) and genotype ($\mathbf{g}$) were independent by permuting the elements of λ within each experimental population, and calculating the allele frequency change (as described previously) based on the permuted survival vector. We generated the null distribution of expected allele frequency change (Δp^{drift}) by repeatedly (1000 times) sampling genotypes ($\mathbf{g}$) according to their posterior distributions and permuting λ. This procedure thus incorporated uncertainty in genotypes and the stochastic nature of drift. We then defined and calculated a selection index ($s^{index}{}_i$) that summarizes the Bayesian-Monte Carlo probability that a locus was affected by selection. We equated 'exceptional change' with a selection index of 97.5 or greater; this is equivalent to a two-tailed probability of 95% or greater that the allele frequency change at the locus was not caused by drift alone.

Parallel evolution is often used to infer selection, as it is unlikely to arise by drift (Schluter & Nagel 1995). We thus estimated the probability that each locus exhibited exceptional parallel allele frequency change across populations within a host treatment. Specifically, we estimated summed values of Δp_i and $\Delta p^{drift}{}_i$ across the set of experimental populations transplanted to each host (we still limited permutations of λ to individuals within the same experimental population to maintain population-specific survival rates). We then identified loci with exceptional change and calculated selection indexes as described previously. This is a more powerful test for selection as it combines evidence across replicate populations.

Absence of selection on any loci (null model 2)
We then generated a null model for the number of loci expected to exhibit exceptional change under genome-wide genetic drift. We did this analysis by generating 100 permutations of the survival vector λ (at the level of individuals), and estimating the number of loci exhibiting exceptional allele frequency change based on each of these permuted data sets using the methods described in the previous paragraphs. This generated a null distribution for the number of loci with exceptional allele frequency change within each experimental population and treatment if survival was wholly independent of genotype at all loci (to which we compared the observed numbers of exceptional loci). We rejected null model 2 at the treatment level for parallel change on *Ceanothus*, but not in other instances (see Results). Thus, we conservatively focus our analyses on the *Ceanothus* treatment, but also report results for parallel change on *Adenostoma* for completeness.

Selection coefficients

We estimated a selection coefficient that measures the strength of selection acting on each locus for which null model 1 was rejected in the analyses of parallel change (thus, coefficients were estimated separately for each host plant treatment). These selection coefficients (s) incorporate both direct and indirect selection (i.e. $s = s_{direct} + s_{\text{indirect}}$) and denote the difference in the absolute expected marginal fitness between alternative homozygotes. In other words, this coefficient describes the absolute difference in survival probability between homozygotes averaged over all populations within a host plant treatment and over all genomic backgrounds (in terms of phenotypic selection, this is analogous to the selection differential rather than the selection gradient). We estimated these coefficients using a Bayesian generalized linear model as described in the Supporting Information.

Physical dispersion of regions with exceptional change

We estimated Moran's I (Moran 1950) at a series of physical distances (from 0 to 10^9 bp) as a measure of genomic autocorrelation of selection indices in the *Ceanothus* treatment (i.e. spatial autocorrelation along chromosomes; Moran's I ranges up to +1, with high positive values indicative of clustering and zero of random dispersion). We calculated Moran's I as:

$$I_{(k1,k2)} = (L)/\left(\sum_i \sum_{i'} r_{ii'}\right)\left(\sum_i \sum_{i'} r_{ii'}\Theta_i\Theta_{i'}\right)/\left(\sum_i \Theta_i^2\right)$$

where $r_{ii'}$ is a binary indicator variable that is 1 if the physical distance between SNPs i and i′ is $k1 < r_{ii'} \leq k2$, and is 0 otherwise and Θ is the zero-centred parameter of interest (i.e. the selection index). This was done using a program written in C++ and using the GNU Scientific Library. We tested whether the frequency of SNPs with exceptional allele frequency change varied among the genomic scaffolds as described in the Supporting Information.

Connecting genotypic and phenotypic changes

We asked whether the SNPs most strongly associated with the stripe phenotype showed greater evidence of exceptional allele frequency change than random sets of SNPs and whether the loci exhibiting exceptional parallel change on *Ceanothus* were associated with the stripe phenotype using permutation methods described in the Supporting Information.

RESULTS

Mortality and phenotypic divergence

Overall mortality did not differ between hosts, with 69 and 71 individuals recaptured on *Adenostoma* and *Ceanothus* respectively ($t_8 = 0.12$, $P = 0.91$). However, phenotypic frequencies shifted between release and recapture such that we detected evidence for non-random mortality with respect to phenotype (i.e. selection). Specifically, the mean difference in the frequency of striped individuals between host species within a block (frequency on *Adenostoma* minus frequency on *Ceanothus*) was slight upon insect release (Mean = 0.012, SD = 0.058) and increased upon recapture (Mean = 0.155, SD = 0.149). Increases in divergence between release and recapture in the predicted direction were observed for four of the five paired blocks and statistically significant overall ($t = -2.95$, d.f. = 4, $P = 0.021$, paired t-test, Fig. 3). Changes in morph frequencies occurred on both hosts (Table S2). Thus, phenotypic selection acted in the experiment.

Genetic variability at the onset of the experiment

We identified 186 576 bi-allelic SNPs in the released stick insects, and we mapped 155 920 (84%) of these to the first assembly of the $\sim 1.3 \times 10^9$ bp *T. cristinae* genome. Genetic variability at the onset of the experiment affects the potential for allele frequency change at individual loci and the independence of changes at different loci. Accordingly, we quantified genetic variability in the released stick insects by estimating genotypes and allele frequencies at all SNPs, and statistical associations within and between all pairs of SNPs.

The minor allele frequency (MAF) distribution in each population was roughly L-shaped with many relatively low-frequency alleles (mean MAF = 14%, Fig. 4a, Table S3). Estimates of Δ indicated that deviations from Hardy–Weinberg and linkage equilibrium were low at the onset of the experiment (Fig. 4b). Consistent with expectations, estimates of Δ were on average higher for nearby SNPs (defined as those on the same GBS contig) than for other SNPs (mean Δ: nearby SNPs = 0.007, other SNPs = 0.004, $t = 52.41$, d.f. = 9, $P < 0.001$, paired t-test). However, the strongest statistical associations observed were rarely between nearby SNPs. Specifically, the maximum estimate of Δ for each SNP was approximately 10 times higher than the mean Δ, and only involved a nearby SNP for 2.0–2.5% of the SNPs (Mean = 2.3%, $n = 10$). These results indicate that substantial genetic variation existed at the onset of the experiment and that most loci we sequenced could evolve relatively independently from one another. However, statistical associations among some physically linked or unlinked genomic regions could cause weakly to moderately correlated allele frequency changes at different loci.

Allele frequency changes in the field experiment

We observed pronounced genome-wide allele frequency changes in the experimental populations between release and recapture (Fig. 5). The mean change in each experimental population varied from 2.2 to 5.1% (Mean = 3.5%). Numerous loci exhibited much larger allele frequency changes. For example, the 97.5th quantile of allele frequency change varied

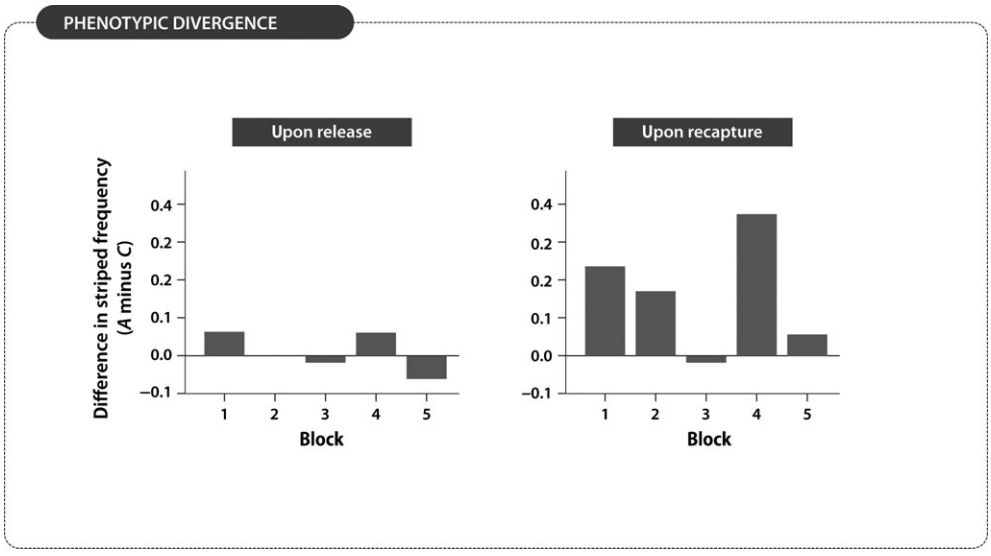

Figure 3 Phenotypic divergence of *T. cristinae* between host plant species in the field experiment. Each bar represents the difference between hosts within a paired block in the proportion of individuals that are striped (proportion striped on *Adenostoma* minus the proportion striped on *Ceanothus*). Increases in divergence between release and recapture in the predicted direction were observed for four of the five paired blocks and were statistically significant overall ($t = -2.95$, d.f. = 4, $P = 0.021$, paired t-test).

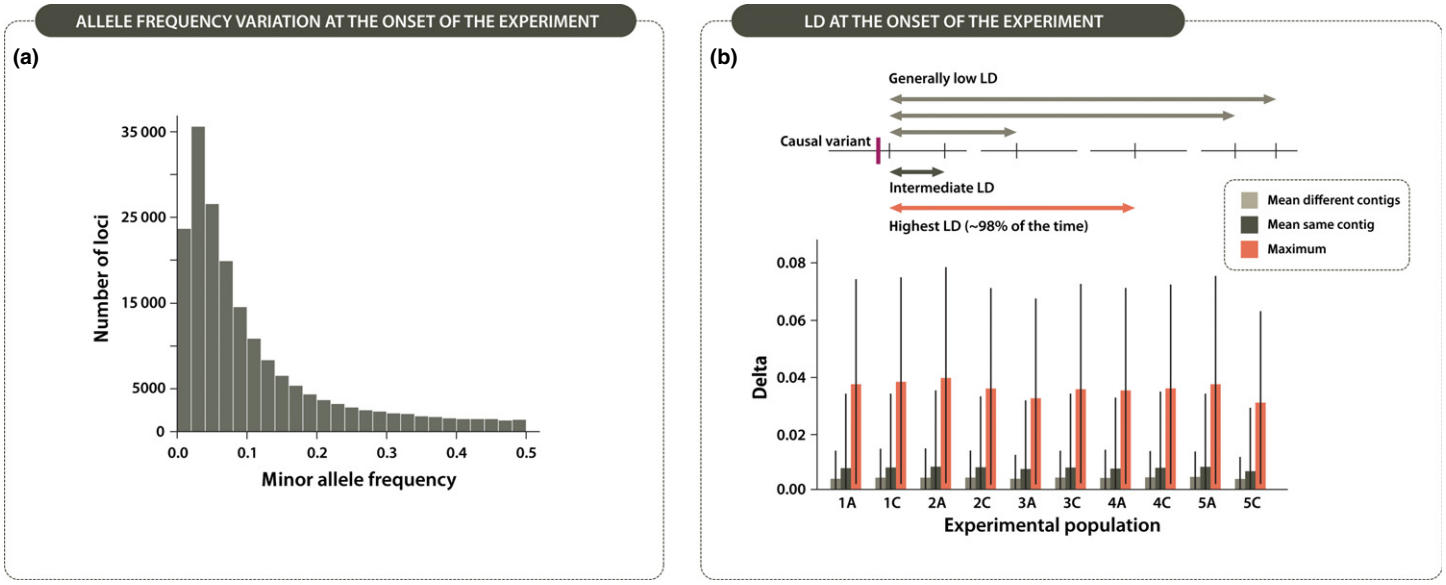

Figure 4 Genetic variability at the onset of the experiment. (a) Distribution of minor allele frequencies at the onset of the experiment within one experimental population (1A). Similar distributions were seen in all populations (Table S3). (b) Levels of linkage disequilibrium (LD, estimated using Δ, text for details) within each experimental population at the onset of the experiment (> 17 billion pairwise comparisons per population, bars depict means and error bars 5% and 95% quantiles). Results are shown for mean LD for single-nucleotide polymorphism (SNP) pairs on the same vs. different genotyping-by-sequencing contigs and the maximum LD between SNPs. The diagram above the bars depicts the general results is schematic form (horizontal grey lines denote genotype-by-sequencing (GBS) contigs and vertical grey lines denote SNPs): specifically it illustrates that although LD was generally higher for SNP pairs on the same vs. different GBS contigs, maximum LD tended to occur between SNPs on different contigs.

among populations from 8.1 to 18.6% (Mean = 12.6%) and the maximum allele frequency change observed was very high (Mean = 44.1%, range = 27.8–61.9%). We detected numerous instances of fixation of one allele within experimental populations, even when considering only loci with MAF > 5% (mean number of loci exhibiting fixation = 7956, SD = 6950, n = 10; Table S4). Many cases of fixation stemmed from loci with modest MAFs (e.g. 5–15%) but fixation involving more common minor alleles was also observed (Fig. 5).

Tests for selection

Consistent with the null hypothesis of random mortality, allele frequency changes at individual loci were not greater than expected by genetic drift for most loci (Fig. 5c). Nonetheless, a modest number of loci exhibited exceptional allele frequency change relative to null model 1 expectations (1–49 within individual populations) (Figs. 5 and 6). We compared the observed number of such loci with exceptional change to the number expected by chance due to genome-wide genetic drift (null model 2). Within individual populations and across the five populations transplanted to the native host *Adenostoma*, the number of loci with exceptional change was not different from that expected under the null model of genome-wide drift (all $P > 0.10$, Fig. 5).

In contrast, we detected evidence that selection contributed to genomic change in the five populations transplanted to the novel host *Ceanothus*. Specifically, the results considering parallel allele frequency change across these five populations revealed that the observed number of loci with exceptional change (= 129) was significantly greater than predicted by a null model of genome-wide genetic drift ($P = 0.01$, Fig. 5c). Thus, significantly more loci exhibited evidence for selection on *Ceanothus* than expected by chance (i.e. this is a genome-wide rather than locus-specific measure of significance).

Selection coefficients

Selection coefficients for 116 of the 129 (90%) loci that showed exceptional parallel change in the *Ceanothus* treatment were significantly different from zero (i.e. the 95% CI of s excluded zero for these loci; Fig. 5d). Thus, we have evidence of selection affecting 116 genetic variants in the *Ceanothus* treatment. Based on the point estimates of selection the average absolute strength of selection experienced by these loci was = 0.32 (SD = 0.08, 2.5% quantile = 0.15, 97.5% quantile = 0.42). Results for the 171 loci showing exceptional parallel change on *Adenostoma* were similar but with fewer loci with significant selection coefficients (55%) and weaker overall selection (Mean = 0.25, SD = 0.04, 2.5% quantile = 0.14, 97.5% quantile = 0.31, Fig. S3). Thus, the upper levels of selection estimated on *Adenostoma* mirrored the mean selection on *Ceanothus*. We stress there is considerable uncertainty in these locus-specific estimates of selection such that the actual selection experienced could be much weaker or stronger than the point estimates (Fig. 5d). Furthermore, by focusing on loci with evidence of selection from null model 1, we likely missed loci that experienced weaker selection.

Genomic distribution of exceptional allele frequency changes

Genetic variants putatively affected by selection were distributed widely across the *T. cristinae* genome (Fig. 6). For example, the 109 mapped SNPs with exceptional change in the

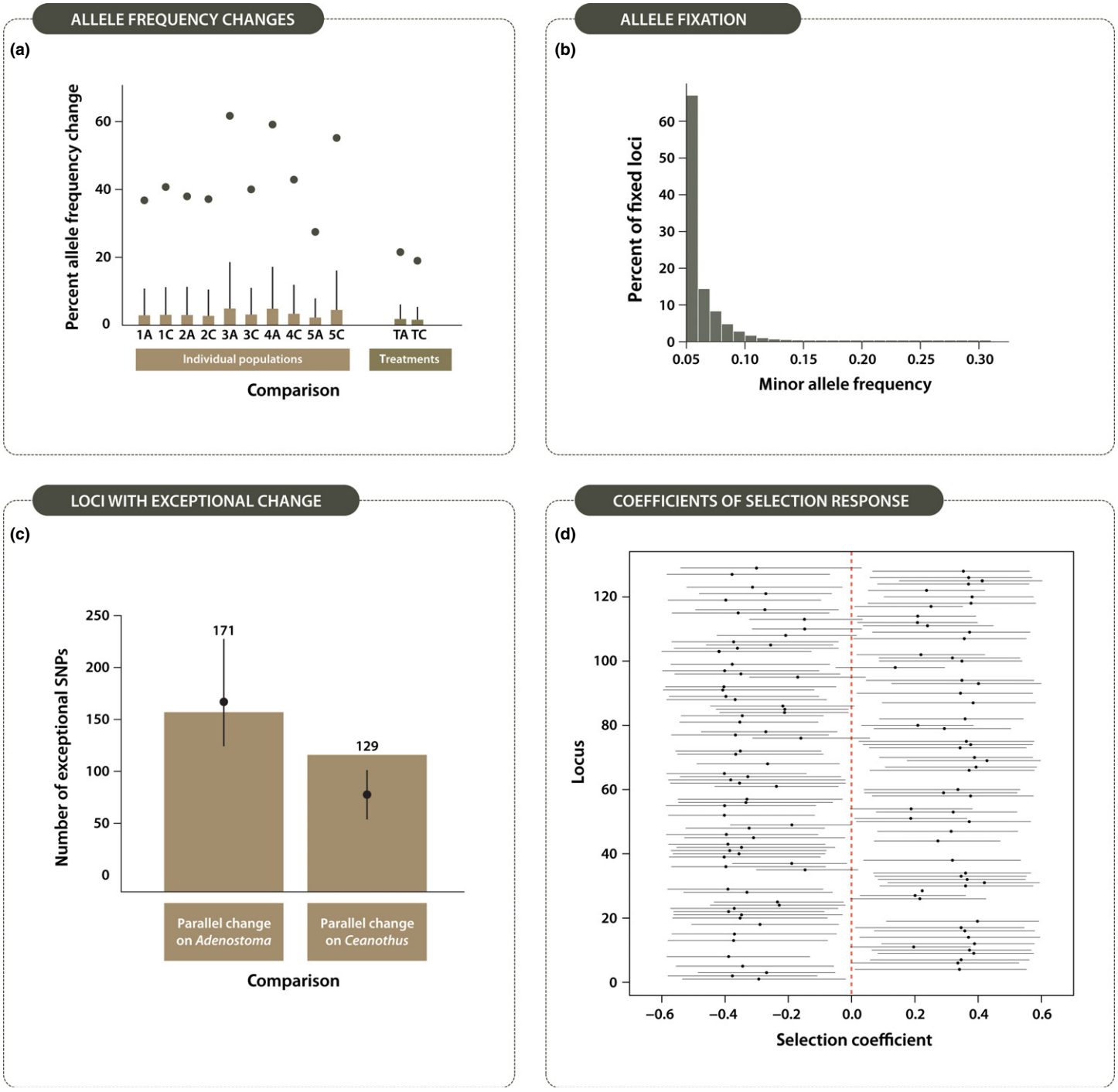

Figure 5 Genomic change in the field experiment. (a) Observed allele frequency changes for each experimental population and treatment (C = *Ceanothus*, A = *Adenostoma*). Bars represent means and error bars the 97.5% upper quantile. Grey circles are maximum change. (b) Fixation of alleles as a function of minor allele frequency. Results are depicted for one experimental population (1A), but were similar among experimental populations (Table S4 for details). (c) Numbers of loci with 'exceptional change'. Bars and numbers above them indicate observed numbers of exceptional change loci. The numbers expected given the number of comparisons made under a null model of genome-wide drift are depicted by the error bars (mean, 2.5%, 97.5% quantiles). (d) Selection coefficients and 95% credible intervals for the 129 loci exhibiting exceptional parallel change in the populations transplanted to *Ceanothus* (for comparable results on *Adenostoma* see Fig. S3).

Ceanothus treatment were distributed among 95 of the 3950 largest (> 50 000 bp) genome scaffolds. Moreover, we detected only weak and short-range genomic autocorrelation in selection indexes. Specifically, our estimate of Moran's I for selection indexes in the *Ceanothus* treatment was 0.033 for SNPs less than 100 bp apart, but near zero for more distant SNPs. Similarly, Bayesian model comparison methods revealed no evidence for genomic clustering of selection index values

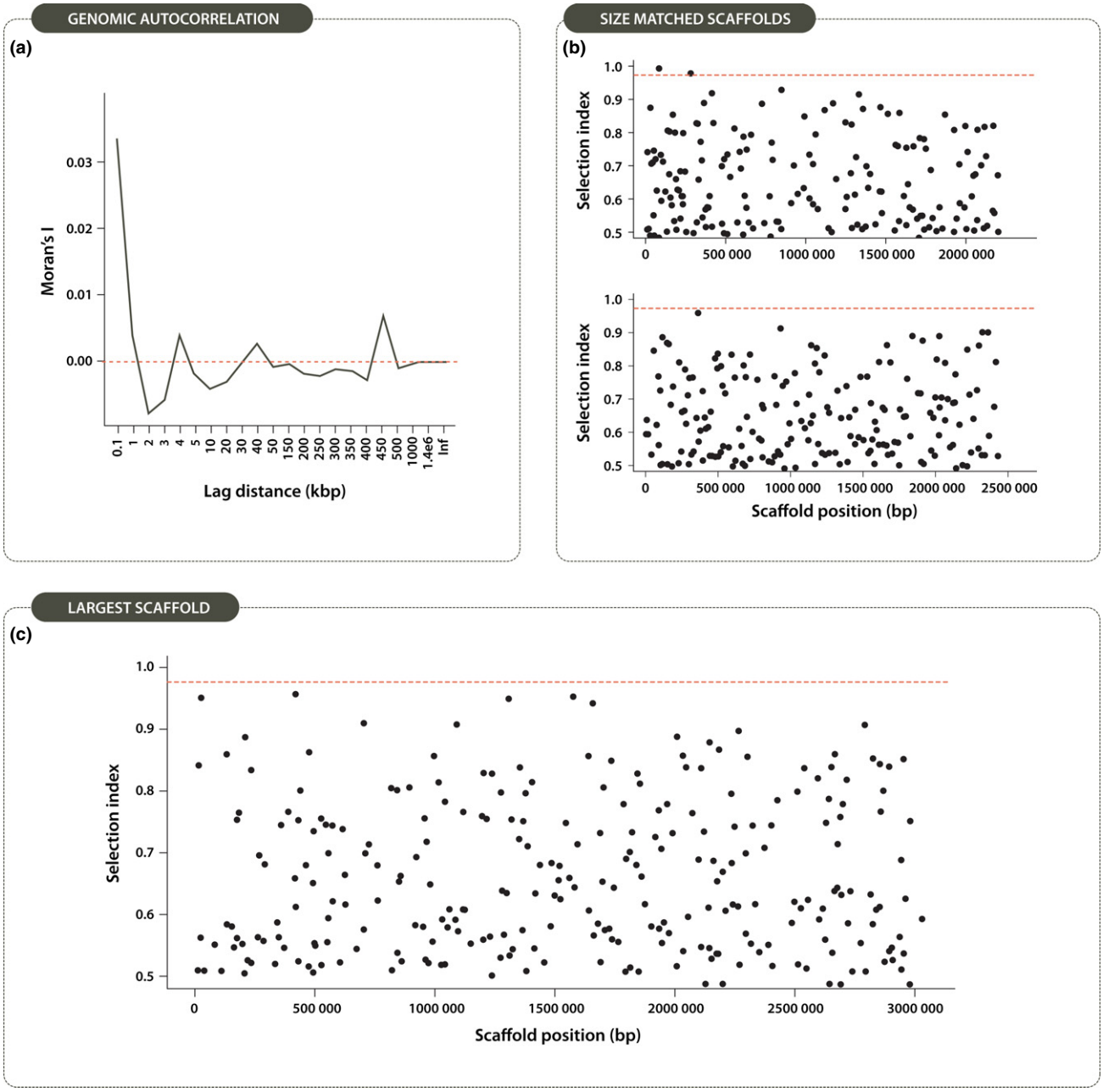

Figure 6 Variation in selection index across the genome. Results are shown for parallel divergence across the five populations on *Ceanothus* (results on the other host were comparable). (a) Correlogram of genomic autocorrelation of selection index values across the genome, at different physical distances. Genomic autocorrelation is near zero (dashed red line) at distances >100 bp. 'Inf' represents loci on different scaffolds. (b) The genomic distribution of selection index values along two size-matched scaffolds in the *T. cristinae* whole-genome assembly. The top panel depicts a scaffold with one exceptional change locus, whereas the bottom panel depicts a scaffold with no exceptional change loci. This is the maximum difference observed for size-matched scaffolds. (c) The genomic distribution of selection index values along the longest scaffold in the *T. cristinae* whole-genome assembly. High and low parameter estimates are widely distributed, with pronounced local increases and decreases in parameter values (SI for statistics considering numerous scaffolds).

among scaffolds (Supporting Information). Accordingly, LD at the onset of the experiment for loci showing exceptional change on *Ceanothus* was low (mean $\Delta = 0.005$, average maximum $\Delta = 0.022$) and comparable to genome-wide LD.

Connecting genotypic and phenotypic changes

The joint selection index for the 10 SNPs with the greatest allele frequency difference between striped and green stick insects was significantly greater than expected under the null hypothesis that selection did not affect these top stripe-associated loci (mean selection index = 0.76, with $s > 0.9$ for three SNPs, $P = 0.0012$). We then asked whether any of the *Ceanothus* exceptional change loci were associated with the stripe phenotype and found no evidence for such an association [six loci were nominally associated with stripedness at $P < 0.05$, but not after FDR (False Discovery Rate) correction]. Thus, whereas none of the 129 loci with the most

evidence of selection was associated with the stripe phenotype, we do have evidence that the 10 loci most associated with stripedness were affected by selection.

Natural selection associated with elevation

The experimental populations varied in elevation, each being uphill from the founding population (Fig. 2). We observed that the number of loci exhibiting exceptional change within individual populations increased with transplant elevation (rho = 0.70, $P = 0.025$, $n = 10$, Spearman Rank Correlation, Fig. S4). This ecological pattern is unlikely to arise by genetic drift and was not caused by variation in the number of insects recaptured at each site, as mean mortality was unrelated to elevation (% recaptured versus elevation, $r = 0.05$, $P = 0.90$, $n = 10$; Fig. S1). Thus, we detected more evidence for selection at higher elevations.

DISCUSSION

We demonstrated that selection contributed to allele frequency change at multiple genetic loci distributed across the genome in experimental *T. cristinae* populations transplanted to a novel host plant. Combined with recent studies demonstrating genome-wide evolution in sea urchins in response to experimental ocean acidification (Pespeni *et al.* 2013) and in plant populations transplanted to new environments (Anderson *et al.* 2013), our results indicate that newly founded populations can harbour sufficient genetic variation for rapid adaptation to a novel environment, as might be required in the face of human caused ecological change (Hendry *et al.* 2006; Seehausen *et al.* 2008; Pelletier *et al.* 2009). However, the existence and maintenance of such variation, and for it to involve multiple statistically independent loci, might depend on specific factors such as gene flow among populations and large effective population sizes, both of which occur in the stick insect system.

Our results indicate that individual variants might experience moderate to strong selection even when multiple variants are affected by selection (Barrett & Hoekstra 2011). This does not mean that each of these variants had a large effect on variation in fitness, as the total selection experienced by each locus likely includes indirect selection. Moreover, considerable uncertainty exists in these estimates because these loci were probably affected by both genetic drift and selection, and it is difficult to parse the relative contributions of these forces. We note that the selection coefficients inferred in this study are not indicative of the genome-wide distribution of selection, but rather are for the small subset of loci with the strongest evidence for selection in our experiment. More generally, a publication bias where studies documenting strong selection are easier to publish will lead to overestimates of the genome-wide strength of selection.

Our results are consistent with the hypothesis that ecologically mediated selection can cause substantial allele frequency change at dozens of loci on a short-time scale (and note that our GBS data covered ~ 10% of the genome such that many non-sequenced regions might have been affected). This short-term response to selection highlights the need for additional research on the interaction of ecological and evolutionary dynamics, even at the genomic level. Although we were primarily interested in selection, we also documented substantial and genome-wide allele frequency change from genetic drift. This result is consistent with theoretical expectations and with founder effects in island lizards (Kolbe *et al.* 2012).

Finally, our results illustrate how genomics can be used to identify previously underappreciated determinants of ecological or evolutionary dynamics (i.e. 'reverse ecology'). Specifically, we found that although there was evidence for selection affecting genomic regions associated with a colour pattern phenotype, the most exceptional responses to selection were not associated with this phenotype. Thus, host-related selection in this system likely involves both colour pattern and other phenotypes. We also discovered a relationship between the number of variants putatively affected by selection and elevation. Thus, host plant differences could be one of many causes of selection acting on *T. cristinae* populations. Coupled with evidence that rapid phenotypic evolution in this system affects population, community and ecosystem properties (Farkas *et al.* 2013), these results demonstrate strong coupling of ecological and evolutionary dynamics at multiple levels of biological organization, and how genomics and experiments might be integrated to study such eco-evolutionary dynamics.

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STATEMENT OF AUTHORSHIP

PN and JF initially conceived the experiment and all authors helped refine the experiment and genomic design. PN, AAC and TEF performed the field experiment. AAC and TLP conducted the laboratory work. ZG, CAB, TLP ad PN conducted analyses. ZG and CAB provided new methods. ZG and PN wrote the initial manuscript and all authors contributed to revisions.

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