Ecology and genomic background shape the probability of parallel adaptation to climate

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Title: Ecology and genomic background shape the probability of parallel adaptation to climate

Short title: Adaptation repeats itself probabilistically

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ABSTRACT

Evolution can repeat itself, resulting in parallel adaptations in independent lineages occupying similar environments. Moreover, parallel evolution sometimes, but not always, uses the same genes. Two main hypotheses have been put forth to explain the probability and extent of parallel evolution. First, parallel evolution is more likely when shared ecologies result in similar patterns of natural selection in different taxa. Second, parallelism is more likely when genomes are similar, because of shared standing variation and similar mutational effects in closely related genomes. Here we combine ecological, genomic, experimental and phenotypic data with randomization tests and Bayesian modeling to quantify the degree of parallelism and study its relationship with ecology and genetics. Our results show that the probability of parallel adaptation to climate among species of Timema stick insects is shaped collectively by shared
ecology and genomic background. Specifically, the probability of genetic parallelism decays with divergence in climatic (i.e., ecological) conditions and genomic similarity. Moreover, we find that climate-associated loci are likely subject to selection in a field experiment, overlap with genetic regions associated with cuticular hydrocarbon traits, and are not strongly shaped by introgression between species. Our findings shed light on when evolution is most expected to repeat itself.

**INTRODUCTION**

To what extent is evolution predictable and repeatable? This was the question posed by Stephen J. Gould’s famous thought experiment on whether repeatedly ‘replaying the tape of life’ would yield similar evolutionary outcomes (Gould 1990). Gould considered similar outcomes unlikely, due to chance events and historical contingency in evolution, and this thought experiment helped launch decades of research on the repeatability of evolution. Indeed, the answer to this question is important because it is central to understanding the processes shaping biological diversification (Stern and Orgogozo 2009; Langerhans 2010; Losos 2011). For example, instances of repeated or parallel evolution in response to similar environmental pressures can provide evidence of evolution by natural selection. In contrast, idiosyncratic outcomes can support a role for chance or contingency in evolution and indicate constraints on the power of selection. The predictability of evolution also has practical implications, for example for forecasting organismal responses to natural and human-induced environmental change (Waldvogel et al. 2020), the planning of plant and animal breeding programs, and the design of medicines and strategies to combat the spread of disease (Lieberman et al. 2011).

It is now known that evolution can repeat itself but does not always do so (Grant and Grant 2002; Bolnick et al. 2018). Moreover, parallelism has been documented at the genetic level, with striking cases of parallel evolution at single genes of major effect, at both the within- and among-species level. For example, the *Ectodysplasin* gene controlling body armor has repeatedly been used by numerous populations of stickleback fish during freshwater adaptation (Colosimo et al. 2005). Likewise, the *Agouti* and *Mc1R* genes control coloration in diverse organisms (Kingsley et al. 2009; Manceau et al. 2010; Linnen et al. 2013). Beyond case studies, meta-analyses have shown that parallel phenotypic evolution often involves the same genomic region (Elmer and
In this context, two general hypotheses have been put forth, which are not mutually exclusive. First, parallel evolution is more likely when shared ecologies result in similar patterns of natural selection in different taxa (the ‘shared ecology’ hypothesis) (Roda et al. 2013; Stuart et al. 2017; Morales et al. 2019). Shared aspects of environmental variation can decline with time since divergence, for example as species come to occupy different geographic areas, thus reducing parallelism (Manousaki et al. 2013; Rennison et al. 2020; Morales et al. 2019). Second, parallelism is expected to be more likely when genomes are similar, because pools of standing variation, the mutations which arise, and mutational effects are more similar in closely-related genomes (the ‘shared genetics’ hypothesis; we use this term to also encompass the role of gene regulation and development) (Schluter et al. 2004; Stern 2013; Roesti et al. 2014). Interactions between genes (i.e., epistasis) might be particularly important here, because the effects of new mutations are then dependent on the mutations which preceded them. Because new mutations are most likely to have different effects in divergent genomes, gene reuse is reduced between divergent genomes.

Both ecological and genetic similarity are expected to decline with time and there is support for both hypotheses in studies of microbial experimental evolution (Matos et al. 2015; Good et al. 2017), protein evolution (Storz 2016), and comparative phylogenetic analyses (Conte et al. 2012; 2016).
Kohler et al. 2015). However, few studies have simultaneously examined ecology and genetics such that the relative contribution of the two hypotheses remains unclear. Parsing these contributions is important because it is required to test the fundamental roles of selection (i.e., shared ecology) and constraint (i.e., shared genetics) in evolution (Haldane 1990; Gompel and Carroll 2003; Schluter et al. 2004; Orgogozo 2015; Blount, Lenski, and Losos 2018). Here we combine ecological data, genomic analyses, a field experiment, and genetic mapping to ascertain the genetic basis and causes of parallel adaptation to climate, thus testing the shared ecology and genetics hypotheses. In other words, rather than focusing on time per se, we conduct analyses that jointly consider the degree of ecological and genetic divergence between taxa, to parse their relative contributions to explaining the degree of parallel evolution observed.

Our study system is the wingless, univoltine, herbivorous stick insects in the genus Timema, many species of which are found in California, USA (Nosil 2007). These insects are perhaps best-studied for their cryptic colours and colour-patterns, which are controlled by the same general genetic region in all species studied to date (i.e., the major locus named Mel-Stripe) (Lindtke et al. 2017; Villoutreix et al. 2020). Timema colouration thus provides a striking example of highly parallel evolution at the level of a single, non-recombining major locus. However, adaptation often involves many genes, including those with alleles of minor effect (Barghi, Hermisson, and Schlötterer 2020; Rockman 2012), where the probability of parallel genetic evolution is less clear (Yeaman 2015). In this context, we study here a novel ecological dimension in Timema, namely climate, motivated by the facts that climate adaptation may be polygenic, and that the genus Timema is distributed across a wide range of habitats in California. For example, the used habitats range from sea-level to thousands of meters of elevation in mountainous regions, and from arid semi-desserts near the Mexican border to wetter, evergreen forests in northern California (Law and Crespi 2002). Moreover, there is climatic variation both within and among species, with several species being distributed along elevational gradients (ranging from 10 ft to ~2800 ft) (Nosil et al. 2020). This variation allows us to find climate-associated genetic regions within species for multiple species that span a range of divergence times from millions to tens of millions of years (here, generations). Moreover, we thus quantify the proportion of the genome that exhibits parallel association to climate across species, to test the shared ecology and shared genetics hypotheses.
In turn, we use a field experiment and genetic mapping to bolster the evidence that climate-associated loci are likely subject to selection and to identify some of the traits involved. Finally, we conduct genomic analyses that test the role of evolutionary history, specifically gene flow and introgression, in observed parallelism. The collective results yield a comprehensive evaluation of parallel evolution at the genome-wide level, in the context of an environmental pressure of high current interest (i.e., climate), and in a system where comparison can be made to parallelism seen at a single, major locus (i.e., Mel-Stripe). In contrast to accumulating examples of adaptive introgression in birds, plants, fish, insects, and other animals (Heliconius Genome Consortium 2012; Roesti et al. 2014; Henning and Meyer 2014; Bay, Taylor, and Schluter 2019; Marburger et al. 2019; Giska et al. 2019; Menon et al. 2021), we find that introgression is unlikely to play a key role in Timema. Thus, parallelism in Timema is likely most strongly influenced by balancing selection on standing variation and the emergence of new mutations, suggesting a diversity of mechanisms for repeated evolution.

RESULTS

Climatic variation within- and among-species. We studied eight Timema species across 53 geographic localities (n = 1420 individuals). Due to high correlations among the 22 studied climate variables, we performed an ordination analysis using principal component analysis (PCA) of the 22 climate variables for all populations included in the study (see Figure 2A for species range map). This revealed that most of the variation in climate variables was explained by the first three principal components (PC) (Total = 92.2%, PC1 = 51.7%, PC2 = 24.4% and PC3 = 16.1%), which we hereafter focus on and refer to as PC1, PC2, and PC3 (Table S2 for PC loadings, Figure S1).

The PC loadings allowed interpretation of the main factors contributing to each PC (variables with PC loadings > 0.25 were considered top variables). For PC1, the top four variables were (i) elevation (Ele), (ii) precipitation of warmest quarter (BIO18), (iii) precipitation of driest quarter (BIO17), and (iv) precipitation of driest month (BIO14) (Figure S1A, Figure S1C, Table S2). Therefore, PC1 is a general axis of elevation and precipitation variation, with high values representing wet localities at high elevation (and conversely, low values representing low-elevation dry sites). For PC2, the top four explanatory variables were (i) mean temperature of
warmest quarter (BIO10), (ii) maximum temperature of warmest quarter (BIO5), (iii) mean temperature of driest quarter (BIO9), and (iv) annual mean temperature (BIO1) (Figure S1A, Figure S1B, Table S2). Therefore, PC2 is a general axis of temperature variation, with high loadings representing localities experiencing high temperatures. Lastly, for PC3, the top four explanatory variables were: (i) precipitation of wettest quarter (BIO16), (ii) precipitation of wettest month (BIO13), (iii) precipitation of coldest quarter (BIO19), and (iv) mean temperature of driest month (BIO9) (Figure S1B, Figure S1C, Table S2). Therefore, PC3 is an axis of contrasting variation in precipitation and temperature, with high values representing localities closer to the coast experiencing greater temperature and precipitation fluctuations.

Overall, precipitation variables consistently loaded strongly on the first 3 PCs, followed by temperature and elevation. One way ANOVA revealed significant between-species variation for all three PCs (PC1: F value 104.5, P-value = < 0.0001; PC2: F value 104.5, P-value = < 0.001; PC2: F value 6.803, P-value = < 0.0001; PC3: F value 28.07, P-value = < 0.0001). We also detected clear within-species variation (range of median PC scores values across the eight species were -3 and 5.8 for PC1, -2.5 to 6.5 for PC2, and -1.6 to 3.5 for PC3; Figure 2C-D). We draw attention particularly now to PC3, as several of the most compelling results that follow concern this PC. We describe a summary of our analyses in Figure 1.

**Identifying climate-associated genomic regions within species**

We first identified the genomic regions most strongly associated with climatic variation within each of the eight species. To do so, we analyzed single nucleotide polymorphisms (SNPs) obtained through previous genotyping-by-sequencing of natural populations (Riesch et al. 2017) to quantify the number of genetic regions associated with climatic variation within each *Timema* species. Because individual SNPs and their numbers differ among species, we focus on 100 kilo base (Kb) SNP windows to allow eventual comparisons among species (n = 9487 windows in each species, across the eight study species).

For each species, we quantified SNP-climate associations for each of the three climate PCs using BayPass (version 1.2). This software controls for background population structure and generates Bayes Factors (BF), which indicate the evidence for an association of each SNP with an environmental variable (in our case, a PC axis). For each PC, we ran four Monte Carlo Markov
Chain (MCMC) simulations, each with a 20,000-iteration burn-in and 500 sampling iterations with a thinning interval of 100. We used the default option of importance sampling to calculate the regression coefficient ($\beta$), which describes the association of each SNP with climate PC scores. These coefficients were then used to calculate Bayes Factors, which were used to compare the marginal likelihoods of models with non-zero versus zero values of $\beta$. For each species, we then calculated the median of logarithmic BF values for all the SNPs in the 100 kb window and identified top SNP windows as those with medians in the top 10% empirical quantile ("climate-associated SNP windows" hereafter). By using this approach, we incorporated multiple loci spread across the genome in our analyses and avoided focusing on a single region of the genome. Additionally, we expected this range of quantile to be enriched for loci truly involved in climate adaptation. Finally, this approach allowed us to make comparisons among species in downstream analysis. In all species, the top climate-associated SNP windows were widely distributed across the genome and found on all 13 linkage groups (LGs) (Figure 3, Figure S2, Figure S3).

**Parallel evolution of climate-associated genomic regions across species**

We next quantified the extent to which climate-associated SNP windows were parallel (i.e., the same) across the eight species of *Timema* that we studied. We did so by testing if such windows exhibit excess overlap across species relative to that expected by chance. We used two related approaches. First, we asked if climate-associated SNP windows exhibited excess overlap between each pair of species ("pairwise-comparison", Figure 1B). Second, we asked if climate-associated SNP windows exhibited excess overlap among multiple species i.e., if the same SNP windows show association with climate PCs between 3, 4, 5, 6, 7 or 8 species ("multi-species comparison", Figure 1B). To do so, we conducted randomisation tests to quantify excess overlap of windows relative to expectations assuming that SNP-climate associations were independent across species, for pairwise as well as multi-species comparisons (Figure 1A). As an example, an x-fold enrichment of 2.0 in the parallelism analyses would indicate that the evidence for overlap of climate-associated SNP windows for a given comparison was two times higher than expected by chance (based on the mean of the null). For this, we focused on windows with the greatest (top 10%) climate association in nature (for all three climate PCs.)
These analyses revealed compelling evidence for parallel climate-associated SNP windows across species. For pairwise comparisons, we detected significant excess overlap in climate-associated SNP windows for all three climate PCs for at least some species pairs (Figure 4A, Figure S4A, and Figure S5A). For example, we observed excess overlap between eight species pairs for PC1 (Figure S4B), one species pair for PC2 (Figure S5B), and five species pairs for PC3 (Figure 4B). The quantitative degree of excess overlap was somewhat variable. For example, for PC1, the overlap of climate-associated SNP windows among the seven species pairs that showed a significant excess of overlap ranged from an x-fold enrichment of ~1.3x for *T. knulli* and *T. poppensis* (observed = 36, expected = 27, x-fold enrichment = 1.31, *P*-value = 0.04) to a maximum x-fold enrichment of ~2x for *T. podura* and *T. chumash* (observed = 23, expected = 11, x-fold enrichment = 2.20, *P*-value = 0.01) (Figure S4A-B). For PC2, there was only one species pair, *T. bartmani* and *T. californicum*, which showed significant excess overlap of SNP windows with largest median Bayes factors (observed = 29, expected = 19, x-fold enrichment = 1.48, *P*-value < .01; Figure S5B). Lastly, for PC3, the overlap of climate-associated SNP windows among the five species pairs that showed a significant excess of overlap ranged from an x-fold enrichment of ~1.2x for *T. cristinae* and *T. landelsensis* (observed = 49, expected = 38, x-fold enrichment = 1.29, *P*-value = 0.03) to ~1.5x for *T. bartmani* and *T. cristinae* (observed = 27, expected = 17, x-fold enrichment = 1.53, *P*-value = 0.01) (Figure 4A-B).

Parallelism was also supported in analyses examining multi-species (i.e., beyond pairwise) comparisons. For PC1, there was significant excess overlap of SNP windows with largest median Bayes factors between three or more species ~2x more than expected by chance (observed = 60, expected = 26.77, x-fold enrichment = 2.25, *P*-value < 0.01; Figure S4C), for four or more species about ~3x more than expected by chance (observed = 4, expected = 1.03, x-fold enrichment = 3.87, *P*-value 0.02; Figure S4C). For PC2, there was significant excess overlap of SNP windows with largest median Bayes factors between three or more species about ~1.5x more than expected by chance (observed = 42, expected = 26.41, x-fold enrichment = 1.59, *P*-value <.01; Figure S5C), for four or more species about ~4x more than expected by chance (observed = 5, expected = 1.19, x-fold enrichment = 4.17, *P*-value = 0.007; Figure S5C). Lastly, for PC3 there was significant excess overlap of climate-associated SNP windows between three or more species ~1.6x more than expected by chance (observed = 43, expected = 26, x-fold enrichment = 1.63, *P*-value < 0.01; Figure 4C). This effect was even stronger for shared
windows among four or more species (observed = 5, expected = 1.10, x-fold enrichment = 4.53,
$P$-value = 0.006; Figure 5C). Similar results were observed for PC1 and PC2 (Figure S4C and
Figure S5C). After finding this evidence for parallelism, we turned to testing our core two
hypotheses, by quantifying the extent to which parallelism decays with ecological divergence,
genomic divergence, or both.

**Parallelism declines predictably with ecological and genomic divergence between species**

We next tested the shared ecology and shared genetics hypotheses for variation in the degree of
parallelism. Shared ecology would cause a higher degree of parallelism due to similar selective
pressures from similar environments. One the other hand, shared genetics would a higher degree
of parallelism due to higher probability of gene reuse. Here, we quantified parallelism as the
degree of excess overlap of climate-associated SNP windows relative to null expectations, for
pairwise comparisons (Figure 2B). We estimated ecological similarity between pairs of species
using climatic data and genetic similarity based on a previously published phylogeny. We then
fit Bayesian linear mixed models to explicitly compare models where the degree of parallelism is
determined by ecological similarity, genetic similarity, and both. Notably, this mixed model
approach accounts for the non-independence of pairwise distances (Gompert et al. 2014 for
details). Specifically, for each climatic PC variable, we modeled parallelism as the x-fold excess
in shared top climate-associated SNP windows (as described in the preceding section) as a
function of climatic distance which was calculated as the average difference in climate PC scores
between a given pair of species (hereafter referred to as ecology indicating “divergence in
ecology”), phylogenetic distance (hereafter referred to as genes indicating “divergence in
genetics”), or both. We tested the effect of both ecology and genetics on parallelism (Figure 5B,
Figure S6B, Figure S7B). The fit of the different models with or without ecology or genetics was
compared using deviance information criterion (DIC), which is a metric of predictive
performance.

Our analyses revealed evidence for effects of both ecology and genes on the probability of
parallelism, with results that varied among the climate PCs (Figure 5C-D for PC3, Figure S6C-D
for PC1, Figure S7C-D for PC2). For PC3, the full model (ecology and genes) was the best
model, with similar, negative effects on parallelism of divergence in ecology (standardized beta
= -0.47, 95% CI = -0.80 to -0.14) and divergence in genes (standardized beta = -0.55, 95% CI = -
0.87 to -0.21; Figure 5E; Table S3). For PC1, the genes-only model was the best model (standardized beta = -0.55, 95% CI = -0.8 to -0.25; Figure S6E, Table S3). The second-best model was the full model, but this included a positive rather than negative effect of ecological distance on parallelism. Lastly, for PC2 the best model was a null model of no effect of genes or ecology on parallelism (Figure S7E, Table S3). The results thus provide variable support for both the shared ecology and shared genetics hypotheses, dependent on climate PC and strongest for PC3. Having tested these hypotheses, we next tested for additional evidence, beyond parallelism, that the climate-associated SNP windows have been affected by natural selection.

Climate-associated regions exhibit elevation-dependent allele-frequency change in a field experiment and co-vary with CHCs in *T. cristinae*

To potentially bolster the evidence that climate-associated SNP windows are enriched for genetic variants experiencing natural selection, we next tested if such windows exhibited exceptional patterns of allele-frequency change in a transplant-and-sequence field experiment and if they overlap with regions associated with phenotypic variation in genetic mapping analyses. Here, an x-fold enrichment of 2.0 in the CHC analysis would indicate that the evidence for SNP-CHC associations in climate-associated SNP windows was two times higher than expected by chance (based on the mean of the null, see below for details).

The transplant experiment measured 8-day survival and associated genome-wide allele frequency change during this period in 500 *T. cristinae* transplanted to 10 experimental bushes comprising two host plants and all occurring at a gradient of higher elevations than the source population for the experiment. A previous analysis of this experiment documented evidence of selection associated with elevation. Here, as a metric of possible elevation (environment)-dependent selection, we calculated the Pearson correlation between transplant elevation and allele frequency change caused by mortality during the experiment for each SNP. In this current analysis, we found that 100 kb windows exhibiting patterns of allele frequency change most strongly associated with elevation in the transplant experiment coincided modestly with climate-associated SNP windows, but more than expected by chance. Specifically, when focusing on the windows with the greatest (top 10%) correlation between change and elevation in the experiment and with the greatest (top 10%) climate association in nature, windows associated with all three climate PCs corresponded with those where change was most strongly associated with elevation.
~1.2-1.3 times more than expected under the null hypothesis of independence (constrained randomization test controlling for SNP density within windows based on 1000 randomizations; PC1: observed = 108 shared windows, \( P = 0.005 \); PC2: observed = 101 shared windows, \( P = 0.015 \); PC3: observed = 105, \( P = 0.021 \) windows) (Figure 6). Similar patterns were observed where more extreme top percentiles were considered, and when using an unconstrained randomization test (Table S4). These patterns are consistent with the hypothesis that multiple genetic variants in these windows are subject to selection in nature.

For the CHC analyses, we considered three compound classes - pentacosanes, heptacosanes, and nonacosanes - in males and in females (i.e., six CHC traits total). We found evidence of heritable variation for each compound in both male and female \( T. cristinae \), with 50.8% (male nonacosanes) to 89.7% (female pentacosanes) of the variability in these traits explained by ~176 thousand sequenced SNPs in a mapping population (these values denote Bayesian point estimates; these results are based on 602 \( T. cristinae \) from a single population, FHA) (see Table S5 for details). We summarized the evidence that each 100 kb window included CHC-associated SNPs by computing the mean posterior probability of association (i.e., the mean probability of a non-zero genotype-phenotype association, also known as the posterior inclusion probability or PIP) across SNPs in 100 kb windows (i.e., the same 100 kb windows used for summarizing SNP-climate associations). Then, based on a randomization test, we found that for some CHC traits, the average posterior inclusion probability for SNPs in the top climate-associated SNP windows in \( T. cristinae \) was marginally but significantly greater than expected by chance. Specifically, the average probability of SNPs being associated with female pentacosanes was ~1.05 times higher than expected by chance for both the top 10% of PC2 and PC3 climate-associated SNP windows (\( P \)-value = 0.009 for PC2 and \( P \)-value = 0.010 for PC3 based on 1000 permutations; Figure 5B, Table S7 and S8). We also detected a marginally non-significant increase in the average posterior inclusion probability for SNP associations with female nonacosanes in the top 10% of PC3 climate-associated SNP windows (x-fold increase in mean inclusion probability = 1.03, \( P \)-value = 0.051, 1000 permutations, Table S8). These results from CHCs support the hypothesis that at least a subset of the top climate-associated SNP windows are associated with traits involved in climatic adaptation in \( Timema \). Thus, together with the results presented in the previous paragraph, these results suggest a polygenic basis for climatic adaptation in \( T. cristinae \) with at least a modest correspondence between our top climate-associated windows and the
actual loci involved in climate adaptation. With this evidence, we next turn to additional analyses concerning the evolutionary history and mechanisms of parallelism, namely the potential role of introgression between species in promoting parallelism.

**Introgression between species does not contribute strongly to parallel evolution**

We conducted two analyses, focused on different time scales, to ask if introgression and gene flow between species promotes gene sharing and thus climate-associated parallel evolution. First, we identified historical patterns of introgression using a population tree-based approach. Second, we identified contemporary patterns of gene flow using an admixture model. Both these analyses helped us to assess the degree of genetic independence in adaptation to climate within each species.

To identify historical patterns of introgression, we used TREEMIX to generate a population tree for all populations and species while allowing for historical admixture or gene flow among intra-specific or inter-specific populations. For this analysis, we realigned GBS sequence data for all 1420 individuals included in this study to the *T. cristinae* genome. We then called and filtered single nucleotide polymorphisms (SNPs) to identify final set of 8787 SNPs for this analysis. Our results from TREEMIX yielded a population graph or bifurcating tree depicting relationships between focal localities in this study. The best bifurcating tree explained 99.6% of the variation in the population allele-frequency covariances. In this tree, *Timema* populations formed eight major clades that grouped populations by species (Figure 5A). Adding migration edges to the tree increased the variance explained by a negligible extent (Table S9), a logical result given that the tree with no migration edges explained the overwhelming majority of the variation in the data. These results are consistent with little to no introgression between species, a result reported previously for analogous analyses that focused on only the *Mel-Stripe* locus (Villoutreix et al. 2020).

We further used the admixture model from ENTROPY (version 1.2) to infer contemporary gene flow. This analysis was based on the full data set of 1420 individuals and species-specific SNPs (see methods for details). Here we focused on admixture proportions for k=2, as we were interested in the two nominal species and hybrids between them. Additionally, all our analyses focused on pairs of species. We summarized patterns of population structure and admixture
across the sampled populations and individuals based on these admixture proportions and a principal component analysis (PCA) of the genotypic data (Figure S8-S10). As previously reported (Riesch et al. 2017; Villoutreix et al. 2020), we saw minimal evidence of contemporary admixture between species. Together these results suggested that introgression and gene flow do not strongly or regularly influence the dynamics of parallel adaptation to climate in these species.

**CONCLUSION**

We used GBS data from 1420 individuals across eight species, combined with other forms of data, to show that adaptation to climate is occurs in parallel across species but as a function of the ecological and genomic divergence between species. Our results inform three fundamental issues in biology, namely the repeatability of evolution, the effect of ecology and genetics on parallelism, and the processes promoting parallelism. We treat these in turn.

Firstly, we show that evolution in response to climate occurs in parallel among eight species and that parallelism likely involves multiple loci. These findings fill a gap in our knowledge of parallel evolution because many studies, including past work in *Timema*, have mostly focused on parallelism driven by single-genes or specific regions of the genome (Colosimo et al. 2005; Kingsley et al. 2009; Villoutreix et al. 2020). These results agree with other cases of parallel or convergent climate adaptation which are also driven by polygenic interactions, as for example observed in plants (Yeaman et al. 2016; Walden, Lucek, and Willi 2020; Rose et al. 2018; Blanco-Pastor et al. 2021). Our study demonstrates that repeatability of evolution can be driven by numerous genetic paths, but the magnitude of repeatability can be highly variable, specifically when considering inter-species comparisons.

Second, our results also show that parallelism decays with ecological and genetic divergence, suggesting that both shared ecology and shared genetics can affect parallel evolution. Similar ecological settings can exert similar selection pressures which drive parallel evolution in populations inhabiting similar geographical niches even among species (Stuart et al. 2017). In addition, genetic similarity could lead to access to the same standing genetic variation in closely related taxa, which allows gene reuse in response to similar environmental pressures (Bohutínská et al. 2021). Our study demonstrates that both these aspects can affect parallelism, with a perhaps stronger or more consistent effect of genetics. This makes sense as ecological settings are
perhaps more complex compared to genetics and the ecological variation considered can be context-dependent.

Third, our collective results inform how two core evolutionary processes, namely introgression/gene flow and selection, might affect parallelism. For example, we show that parallel evolution and adaptation to climate occurs despite limited or minimal gene flow among and within species, wherein we show little to no introgression among our focal study species. While introgression can facilitate parallel adaptation to similar environmental pressures by providing novel genetic material (Heliconius Genome Consortium 2012; Roesti et al. 2014; Henning and Meyer 2014; Bay, Taylor, and Schluter 2019; Marburger et al. 2019; Giska et al. 2019; Menon et al. 2021), a lack of introgression or gene flow demonstrates independent instances of adaptation and the role of selection in driving parallel evolution (Zhang et al. 2021). In summary, our study shows how local adaptation even among species with minimal gene flow can occur and consequently be crucial for predicting evolution in response to rapidly changing environments and climate. Furthermore, our results bolster evidence for selection beyond a correlational genome scan because we found that the genomic regions which underlie parallelism also showed marked allele-frequency change in an experiment and were associated with ecologically relevant CHC traits. Thus, together these results suggest that allele reuse through standing genetic variation, new mutations, and selection can be powerful drivers of local adaptation.

METHODS

Below we describe details of all our methods and analyses, and we provide a graphic summary in Figure 1 of the main text.

Samples and DNA sequences from natural populations

For this study, we analyzed genotyping-by-sequencing (GBS) data from 1420 Timema stick insects from 53 localities from eight species: 6 T. bartmani populations (N = 195 individuals), 3 T. californicum populations (N = 77 individuals), 12 T. chumash populations (N = 358 individuals), 6 T. cristinae populations (N = 205 individuals), 5 T. knulli populations (N = 89 individuals), 4 T. landensensis populations (N = 125 individuals), 12 T. podura populations (N = 12 individuals) and 5 T. poppensis populations (N = 116 individuals) (Table S1). GBS data for
this study has been previously published in a study of speciation continuum in *Timema* (Riesch et al. 2017) (also see data availability for more details). The genomic data in the transplant experiment and used for genetic mapping of cuticular hydrocarbons is independent from these data and is described in detail below.

**Sequence alignment and variant calling**

To incorporate variants typed for individuals of each species, we built a consensus reference sequence for each species (similar to (Comeault et al. 2016; Villoutreix et al. 2020)). To do this, we first aligned all reads from all our samples to the *T. cristinae* reference genome (draft version 0.3) using the MEM algorithm of BWA (Version: 0.7.17-r1188) (Riesch et al. 2017). We ran BWA MEM with a minimum seed length of 15 (-k), internal seeds of longer than 20 bp, and only output alignments with a quality score of $\geq 30$ (-T). We then used SAMTOOLS (version 1.5) to view, sort and index the alignments (Li et al. 2009). We called variants using SAMTOOLS and BCFTOOLS (version 1.6) (Li et al. 2009; Danecek et al. 2021). For variant calling, we used the mapping quality adjustment of 50 (-C), skipped alignments with mapping quality 0, skipped bases with base quality 13, and ignored insertion-deletion polymorphisms. We then set the prior on single nucleotide polymorphisms (SNPs) to 0.001 (-P) and called SNPs when the posterior probability that the nucleotide was invariant was 0.01 (-p). We then performed two rounds of filtering to retain final sets of SNPs. In the first round, we filtered the initial set of SNPs to retain only those with sequence data for at least 80% of the individuals, a mean sequence depth of two per individual, at least four reads of the alternative allele, a minimum quality score of 30, a minimum (overall) minor allele frequency of at least 5%, and no more than 0.01% of the reads in the reverse orientation. In the second round of filtering, we removed SNPs with excessive coverage (2 standard deviations above the mean) or that were tightly clustered (within 5 base pairs (bp) of each other) and removed variants with poor alignments. This left us with the following number of SNPs for each species: 10,036 SNPs for *T. bartmani*, 14,955 SNPs for *T. californicum*, 20,478 SNPs for *T. chumash*, 3,43,746 SNPs for *T. cristinae*, 25,835 SNPs for *T. knulli*, 21,314 SNPs for *T. landelsensis*, 21,986 SNPs for *T. podura*, and 18,237 SNPs for *T. poppensis*.

We used these filtered variants for each species to construct consensus reference sequences for each species using the CONSENSUS algorithm of BCFTOOLS (version 1.6) (Danecek et al. 

15
We then used the consensus reference of each species to redo alignments for GBS sequences of individuals for each species separately. Following this, we repeated variant calling and two rounds of variant filtering as described above. This left us with the following number of SNPs for each species: 3074 SNPs for *T. bartmani*, 7858 SNPs for *T. californicum*, 4172 SNPs for *T. chumash*, 1,96,252 SNPs for *T. cristinae*, 11,139 SNPs for *T. knulli*, 8548 SNPs for *T. landelsensis*, 6000 SNPs for *T. podura*, and 7157 SNPs for *T. poppensis*. We used this second set of SNPs noted directly above for all downstream analyses.

**Climate variables and identifying SNP by climate associations in nature**

We used 22 climate layers values associated with our 53 study localities (Table S2), which were extracted from the WorldClim database version 1.4 (https://www.worldclim.org/data/v1.4/worldclim14.html; climate data for 1960-1990). Since the first three PC scores explained the overwhelming majority (92.4%) of variation in the climate variables (Figure 2B, Table S2, Figure S1), we used these three PCs to study genomic associations with climate in all further analyses.

We used BayPass version 2.1 (Gautier 2015) to identify genomic regions associated with the three sets of PC scores for the climate variables. The BayPass software controls for background population structure and is based on the BAYENV method introduced by Gunther and Coop (Günther and Coop 2013). We ran this program separately for each species and for each PC (eight species by three PCs). We treated each PCs scores as the environmental covariate and ran the standard covariate model. For each data set, we ran four Monte Carlo Markov Chain (MCMC) simulations, each with a 20,000-iteration burn-in and 500 sampling iterations with a thinning interval of 100. We used the default option of importance sampling to calculate the regression coefficient ($\beta_i$), which describes the association of each SNP with climate PC scores. These coefficients were then used to calculate Bayes Factors which were used to compare the marginal likelihoods of models with non-zero versus zero values of $\beta_i$. Finally, since we had different number of focal SNPs for each species, we calculated median of logarithmic Bayes Factors for 100 kilobase (kb) non-overlapping SNP windows (i.e., the same windows were used in every species, facilitating comparisons among them). Our downstream analyses described below focus on these windows. We delimited climate-associated SNP windows as those with...
greatest association with the three climate PCs, specifically as the windows in the top 10% quantile. We refer to such windows as “climate-associated SNP windows” hereafter.

Quantifying parallel genomic associations with climate across species

We quantified parallel genomic associations with climate across species (using the results described above from BayPass) and used randomization tests to measure the extent to which the observed parallelism exceeded that expected by chance. We report this excess as ‘x-fold’ enrichments, relative to null expectations, also reporting associated P-values for statistical significance.

We first tested for excess overlap of climate-associated SNP windows between pairs of species (“pairwise comparisons”), for each of the three climate PCs. To do this, we used randomization tests (10,000 randomizations per test) to generate null expectations for the proportion of top climate-associated SNP windows shared between a given pair of species and tested whether this was significantly more than expected by chance (x-fold enrichments and P-values). As an example, an x-fold enrichment of 2.0 would indicate that twice as many climate-associated SNP windows showed overlap between a species pair than was expected by chance (based on the mean of the null). We then quantified overlap in climate-associated SNP windows between multiple species (“multi-species comparisons”) i.e., we tested if similar SNP windows show association with climate PCs between 2, 3, 4, 5, 6 or 8 species. We did this by using similar randomization tests as for pairwise-comparisons, to generate x-fold enrichments and P-values.

Testing the shared ecology and shared genetics hypotheses

We tested the contribution of shared ecology versus shared genetics to the observed degree of parallelism. We expect both shared ecology and genetics to have an effect on the probability of parallelism. To do so, we fit Bayesian linear mixed models to explicitly compare models where parallelism is determined by ecological similarity, genetic similarity, or both. This Bayesian regression analysis is based on the mixed model framework proposed by (Clarke, Rothery, and Raybould 2002) and extended by (Gompert, Lucas, et al. 2014). Our method accounts for the correlated error structure inherent in pairwise covariates and response variables (e.g., ecological or genetic distances). In this analysis, our response variable was the x-fold excess in shared top climate-associated SNP windows for a given PC (we did analyses separately for each climate
PC). Our independent variables were ecological and genetic distances, estimated as follows. Climatic (i.e., ecological) distance was calculated as pairwise absolute mean difference of PC scores of each species. We calculated genetic (i.e., phylogenetic) distances based on the previously published phylogeny described in Riesch et al. 2017. Briefly, we used the data from this previous phylogeny constructed using Bayesian phylogenetic inference with BEAST (version 2.1.387) for 11 *Timema* species based on GBS data of curated dataset of 19,556 single-nucleotide variants. For our current study, we used pairwise phylogenetic distances for the eight *Timema* species as our metrics of genetic distances for this analysis. All variables were standardized (given mean 0 and standard deviation of 1) before analysis.

We then considered four alternative models: (i) a null model without covariates, (ii) a model including only phylogenetic distance, (iii) a model with only climatic distance, and (iv) a model with both climate and phylogenetic distance. We fit the models in R using the rjags (version 4.8) interface with Jags (version 4.3.0). We used minimally informative priors for the regression coefficients (i.e., normal with $\mu = 0$ and precision $\tau = 0.001$) and for the population random effects and residual errors, all gamma (1, 0.01). Deviance information criterion was used for model comparison. Parameter estimates and DIC estimates were obtained via MCMC. For each analysis and model, we ran three chains each comprising 10,000 sampling iterations, a 2000-iteration burn-in, and a thinning interval of 5.

**Overlap of climate-associated SNP windows with genetic regions showing elevation-associated change in a field experiment**

We quantified overlap between climate-associated SNP windows and windows that exhibited elevation-dependent allele-frequency change in a previously published release-recapture field experiment. We then tested if this overlap was greater than expected by chance. Full details of the experiment can be found in the original publications (Gompert, Comeault, et al. 2014; Nosil et al. 2018) but those relevant for the current study are as follows. The experiment involved releasing 500 *T. cristinae* (from which a tissue sample was taken) onto 10 experimental bushes (five blocks, each with one plant of *Adenostoma fasciculatum* and one of *Ceanothus spinosus*). Survivors were recaptured eight days later. Whole-genome sequence data, which we analyze here, was obtained from 491 of the 500 stick insects (Nosil et al. 2018).
For the current study, we estimated allele frequencies in the released and recaptured stick insects at the 6,175,495 bi-allelic SNPs identified by Nosil et al. 2018. This was done using an expectation-maximization (EM) algorithm as implemented in the program estpEM (version 0.1) with tolerance of 0.001 and a maximum of 50 EM iterations (Soria-Carrasco et al. 2014). We then used these estimates to compute allele-frequency change between the start and end of the experiment. Then, for each SNP we calculated the Pearson correlation between allele frequency change and the elevation at each of the ten transplant sites. Finally, we determined the average correlation between change and elevation for the 100 kb windows across the genome. Windows with fewer than four SNPs were ignored. These steps were done using R (version 3.4).

We then calculated the number of 100kb windows that were among the top 10% for both elevation-dependent change during the experiment (highest average absolute correlation) and for climate-association (highest average Bayes factor for each climate PC). We used a constrained randomization procedure to generate null expectations for such concordance between change and climate-association windows, using a separate randomization for each PC. Specifically, we randomized mean change metrics across windows, but only among windows with similar SNP densities (10 equally sized bins were used for this). This was done because we observed a positive correlation between SNP density and mean change-elevation correlations per window (Pearson R = 0.069, 95% CI = 0.047-0.091, P < 0.001), and we wanted to control for this. Null distributions and P-values were based on 1000 randomizations and are reported for each climate PC.

**Overlap of climate-associated SNP windows with genetic regions associated with cuticular hydrocarbon variation**

Our next set of analyses concern climate-associated SNP windows and genomic regions associated with cuticular hydrocarbon (CHC) variation. The logic here is that CHCs tend to play a role in desiccation tolerance and climatic adaptation in insects (e.g., (Rajpurohit et al. 2017)), such that genetic regions associated with climate versus CHCs might overlap. We thus specifically quantified the extent to which climate-associated SNP windows overlapped with windows harbouring SNPs associated with CHCs, and whether this overlap was greater than expected by chance. The CHC data were originally described and analyzed by Riesch et al. (2017). Specifically, for each insect we had quantified the proportional abundance of 26 different
mono- and di-methylated CHCs, which comprised eight pentacosanes, eight heptacosanes and ten nonacosanes, and then applied log-contrasts. For the current dataset, we used those values to calculate the proportional abundance of the sum of all pentacosanes, the sum of all heptacosanes and the sum of all nonacosanes (henceforth: pentacosanes, heptacosanes and nonacosanes). Therefore, the six CHC traits considered were pentacosanes, heptacosanes, and nonacosanes in males and females (i.e., three molecule types in each of two sexes).

Here, we first re-aligned the GBS data from Riesch et al. (2017) to the current (i.e., more recent and less fragmented) *T. cristinae* genome (draft version 0.3). This included GBS data from 395 male and 195 female *T. cristinae* all collected from a single population (FHA), and all of which for CHC data was also collected. These data were aligned to the genome using the BWA ALN algorithm (version 0.7.17-r1188) (Li 2013). We allowed for 5 miss-matches total, and not more than 2 miss-matches in the first 20 bp. Only reads with a mapping quality greater than 10 were retained. We then compressed, sorted and indexed the alignments with SAMTOOLS and BCFTOOLS (version 1.2) (Li et al. 2009; Danecek et al. 2021). Next, we used SAMTOOLS and BCFTOOLS to identify SNPs and calculate genotype likelihoods. For this, we used the recommended mapping quality adjustment (-C 50), only considered alignments with mapping qualities of 20 or more and SNPs with base qualities of 30 or more, and only called variants when the posterior probability that locus was invariant was less than 0.01 given a prior mutation rate parameter of 0.001. We then used custom perl scripts to filter out variants with a mean coverage of less than 2x, fewer than 10 non-reference reads total, mapping quality less than 30, minor allele frequency less than ~0.005, more than 1% of reads in the reverse orientation (with our GBS method, all reads should have the same orientation), missing data (no reads) for more than 20% of individuals, SNPs with more than two alleles, and SNPs with coverage exceeding three standard deviations above the mean. Finally, we obtained Bayesian point estimates (posterior means) of genotypes for each locus and individual based on the genotype likelihoods and used the estimated allele frequencies to parameterize a binomial prior.

We then conducted genetic mapping of CHC variation using a polygenic genome-wide association (GWA) mapping approach, that controls for linkage disequilibrium among SNPs and background population structure as detailed below. We specifically fit Bayesian sparse linear mixed models (BSLMMs) to determine the contribution of additive genetic variation (as
captured by our collective SNP data set) to each of six CHC traits, and to determine the
probability of association (posterior inclusion probability, PIP) of each individual SNP with each
trait (this PIP value is computed from, i.e., equal to, the proportion of MCMC samples that
included each SNP in the polygenic regression model). We fit this model using gemma (version
0.95a) (Zhou, Carbonetto, and Stephens 2013), a polygenic GWA mapping method that fits a
single model with all SNPs while accounting for uncertainty and redundancy in genotype-
phenotype associations, for example by controlling for linkage disequilibrium among SNPs, and
background polygenic effects. The latter is inferred based on a kinship matrix derived from the
collective SNPs, which also serves to control for population structure when estimating effects for
individual SNPs. Models were fit using MCMC, with each mapping exercise involving 10
independent chains each comprising 1 million sampling iterations and a 200,000-iteration burn-in.

Based on these analyses, we then computed the mean PIP (i.e., probability of a genotype-
phenotype association) across all SNPs in 100 kb windows for each of the six CHC traits. Then,
we asked whether the average association with CHCs (averaged over windows) was higher for
the climate-associated SNP windows than expected by chance. Randomizations (1000) were
used to generate a null distribution. Specifically, mean posterior probabilities for SNP-CHC
associations were permuted across 1000 kb windows and the number windows in the top 10%
for climate association and (permuted) CHC posterior inclusion probabilities was determined.
Note that we conducted this test independently for each of the six CHC traits and each of the
three climate PCs. We then examined the combination of these results to assess the total
evidence that SNP windows associated with climate adaptation are enriched for those regions of
the genome possibly affecting CHC variation.

**Testing for introgression and quantifying population structure**

We quantified both historical and contemporary gene flow patterns, respectively as follows. For
identifying historical introgression, we used TREEMIX (version 1.13) (Pickrell and Pritchard
2012) to construct a population-based phylogeny to identify historical admixture or gene flow
among our 53 focal populations. This differed from previous TREEMIX analysis done for
Timema species where we used the data only from the Mel-Stripe locus (Villoutreix et al. 2020).
For the analysis in our study here, we re-aligned the GBS sequences for 1420 individuals (across
53 populations) included in this study to the *T. cristinae* genome (draft version 0.3). We did this by using the MEM algorithm from BWA (version 0.7.17-r1188). We ran BWA MEM with a minimum seed length of 15, internal seeds of longer than 20 bp, and only output alignments with a quality score >= 30. We then used SAMTOOLS (version 1.6) to compress, sort and index the alignments (Li et al. 2009). We then identified SNPs using SAMTOOLS and BCFTOOLS (version 1.6). For variant calling, we used a mapping quality of 50, skipped alignments with mapping quality lower than < 20, skipped bases with base quality <15, and ignored insertion-deletion polymorphisms. We set the prior on SNPs to 0.001 and called SNPs when the posterior probability that the nucleotide was invariant was <=0.01. After we got the initial set of variants, we filtered them to retain only those SNPs with sequence data for at least 80% individuals, a mean sequence depth of two per individual, at least 4 reads of the alternative allele, a minimum quality score of 30, a minimum overall) minor allele frequency of at least 0.005, and no more than 1% of the reads in the reverse orientation (this is an expectation for our GBS method). We further removed SNPs with excessive coverage (3 standard deviations above the mean) or that were tightly clustered (within 3 bp of each other), as these could be poor alignments (e.g., reads from multiple paralogs mapping to the same region of the genome). This left us with 8787 SNPs for this analysis. We used these variants to run TREEMIX to fit trees allowing 0-9 admixture events and calculate the proportion of variance in allele frequency variances explained by the population tree with the varying numbers of admixture events. This way we could determine the extent to which individual admixture events improved model fit.

For estimating contemporary gene flow, we implemented the admixture model from ENTROPY (version 1.2) (Gompert, Lucas, et al. 2014). This analysis yielded similar results as previously reported using the same model (Riesch et al. 2017). From ENTROPY, we obtained Bayesian estimates of genotypes and admixture proportions. This analysis was performed separately for each species- and species-specific set of SNPs. We did this to identify contemporary gene flow within species to understand if gene flow could affect parallelism in response to climate. The admixture model in ENTROPY is similar to that in STRUCTURE (Pritchard, Stephens, and Donnelly 2000) but differs by accounting for uncertainty in genotypes arising from finite sequence coverage and sequence errors, and by allowing simultaneous estimation of genotypes and admixture proportions. For each species, we fit the model with $k \in \{2…5\}$ source
populations. For each value of k, we ran three MCMC chains, each with 8000 iterations, a burn-in of 5000 iterations and a thinning interval of 3. We used assignments from a discriminant analysis of principal components to initialize the MCMC algorithm; this speeds convergence to the posterior and avoids label switching during MCMC without affecting the posterior probability distribution. We obtained genotype estimates as the posterior mean allele count for each individual and locus across chains and values of k (i.e., this integrates over uncertainty in the number of hypothetical source populations). We summarized patterns of population structure and admixture across the sampled populations and individuals based on these admixture proportions for k=2 and a principal component analysis (PCA) of the genotypic data. We then used the prcomp function (Kassambara 2019) to perform a PCA in R (3.4) on the centered, but unstandardized genotype matrix.

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AUTHOR CONTRIBUTIONS

PN, MM, OO, ZG, and SC designed the study. PN, MM, RR and VS-C collected data. ZG, OO, and SC analyzed the data. SC wrote the manuscript with feedback from all co-authors.

COMPETING INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

DNA sequence data, genome, experimental data and CHC data used in this study are associated with the previously published studies (Gompert, Comeault, et al. 2014; Riesch et al. 2017). The
associated DNA sequence data have been archived on NCBI's SRA (Accession: PRJNA356405 ID: 356405). Computer code are available on 
https://github.com/karwan/Timema_climate_adaptation_genomics. Correspondence for materials (data, scripts, or samples) should be addressed to Samridhi Chaturvedi (samridhi.chaturvedi@gmail.com) or Zachariah Gompert (zach.gompert@usu.edu) or Patrik Nosil (patrik.nosil@cefe.crans.fr).
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Figure 1: Conceptual figure to summarize the analyses conducted in this study. (A) Diagram shows the approach to quantify overlap of top climate-associated SNP windows between a given pair of species (Species 1 and species 2). Here red dots denote climate-associated SNP windows for each species. We then quantify overlap in these windows between the two species (“N”). (B) Parallelism: Diagram shows the approach to quantify excess overlap of top climate-associated SNP windows between a given pair of species (“pairwise comparison”) and for multiple species (“multi-species comparison”). (C) Experimental comparison: Diagram shows two steps to identify excess overlap in climate-associated SNP windows and that changed in an elevation-dependent manner during an experiment. Here, first we identify loci/genomic regions associated with the greatest allele frequency change in an elevational dependent manner in an experiment as those which show exceptional change as compared to a null expectation (denoted in green line, denoted as “X”). Second, we compare if these regions (“X”) show excess overlap with the climate associated SNP windows (“N”). (D) CHC comparison: Diagram shows two steps to identify excess overlap in climate-associated SNP windows and genomic regions associated with CHCs. First we identify loci/genomic regions associated with greatest effect on CHC traits (denoted in green line, denoted as “C”). Second, we compare if these regions (“C”) show excess overlap with the climate associated SNP windows (“N”).
Figure 2: Map of species range and plots for within species variation in climate PC scores. (A) Map of the ranges of the eight species included in the study, where the coloured shapes represent the geographic ranges of each species. (B) Two hypotheses which we use to test for decay of parallelism: First diagram shows our prediction for the “shared ecology” hypothesis where we expect a decay in parallelism with an increase in ecological (i.e., climate) distance. Second diagram shows our prediction for the “shared genetics” hypothesis where we expect a decay in parallelism with an increase in genetic distance. We use these two hypotheses to study the decay of parallelism. (C-E) Box plots of PC variation for the first three principal components (PC1, PC2, PC3) for the eight species included in the study (n = 1420 individuals from 53 localities).
Figure 3: Manhattan plots showing the strength of evidence for association (measured here using the Bayes factor from the software BayPass) between a SNP window and climate (in this case, PC3, see Figures S2 and S3 for analogous results for PC1 and PC2). Results are shown along the 13 linkage groups. Red points denote the SNP windows in the top 10% quantile (i.e., referred to as ‘climate-associated SNP windows’ throughout the main text). In each panel title, the two values in parentheses are the number of SNP windows in the top 10% quantile (“windows”), followed by the number of linkage groups with at least 1 SNP window in the top 10% quantile (“LGs”).

(A) *T. bartmani* (155 windows, 13 LGs)

(B) *T. podura* (215 windows, 13 LGs)

(C) *T. chumash* (157 windows, 13 LGs)

(D) *T. cristinae* (807 windows, 13 LGs)

(E) *T. knulli* (386 windows, 13 LGs)

(F) *T. popensis* (315 windows, 13 LGs)

(G) *T. landelsensis* (333 windows, 12 LGs)

(H) *T. californicum* (79 windows, 13 LGs)
Figure 4: Tests for parallel climate-associated SNP windows between and among species of *Timema* stick insects (all plots are for the top 10% empirical quantile). In this case, PC3, see Figures S4 and S5 for analogous results for PC1 and PC2. (A) Plot shows observed versus expected number of overlapping climate-associated SNP windows for species pairwise comparison for PC3. Bars denote expected values and solid lines denotes observed values. Open points indicate P-value > 0.05 and filled points indicate P-value ≤ 0.05. (B) Pairwise plot shows x-fold enrichment values for each comparison between pairs of species. Black dot on each box denotes P-value ≤ 0.05. (C) Plot shows x-fold enrichments for number of overlapping climate-associated SNP windows for PC3 for comparisons between multiple species, i.e., beyond pairs of species (e.g., 2 or more species, 3 or more species, 4 or more species). Bars denote observed x-fold value for each multi-species comparison. Black lines on bars show the 95% confidence intervals. N value above each bar indicates the observed number of overlapping climate-associated SNP windows for each comparison. * Indicates x-fold enrichments with P-value ≤ 0.05.
Figure 5: Tests for introgression and “shared ecology” and “shared genetics” hypotheses. (A) Population graph from TREEMIX for all Timema populations used in this study (N = 53), allowing no migration or admixture event (the actual migration edge is not shown due to the proportion of variation explained from the admixture model as shown in Table S9). Terminal nodes are labelled by abbreviations for locations from where samples were collected and coloured according to species. (B) Scatterplot shows the relationship between ecological distance (measured as distance in PC3 scores and is distance in climate variables) and genetic distance (measured as pairwise phylogenetic distance). (C) Scatterplot shows the relationship between x-fold enrichment (measure for parallelism) and ecological distance (measured as distance in PC3 scores) (D) Scatterplot shows the relationship between X-fold enrichment (measure for parallelism) and genetic distance (measured as pairwise phylogenetic distance). (E) Plot shows parameter estimates with standardized coefficients for the full model for PC3. Estimates diverging from zero indicate a positive or negative effect of ecology or genetics on parallelism. Analogous results for (B)- (E) are shown in figure S8 and S9 for PC1 and PC2. A negative or positive estimate which deviates from zero is indicative of effect on parallelism.
Figure 6. Evidence for excess overlap between 100kb windows associated with climate in nature and that changed in an elevation-dependent manner during an experiment. (A) The scatterplot shows the mean correlation between change and elevation during an experiment versus the median Bayes factor measuring SNP-climate (PC3) association in nature for T. cristinae for 100 kb windows. Points denoting windows in the top 10% for change-elevation correlations are shown in orange, those in the top 10% for SNP-climate associations are shown in blue, and those in the top 10% for both are in purple (other windows are shown with gray points). We are interested in the top right corner of the plot, that is the purple points denoting windows were exceptional (top 10%) in the experiment and nature, and we used a randomization test to ask whether more windows fall in this category than expected by chance. Panels (B), (C) and (D) show null expectations for the number of windows in the top 10% for the experiment and nature based on climate PCs 1, 2 and 3, respectively. The null distribution from the constrained randomization test in each case is denoted by the gray density plot, whereas the observed value is shown with a vertical purple line. The P-value for the null hypothesis of no association between SNP-climate and change-elevation correlations is reported in each panel as well.
Table S1: Locality information and sample sizes for the eight species and 53 localities for which the GBS data has been included in this study. The GBS data associated with these populations and individuals was first presented in Riesch et al. 2017.

| Species            | No. of populations | No. of individuals |
|--------------------|--------------------|--------------------|
| T. bartmani       | 6                  | 195                |
| T. californicum   | 3                  | 77                 |
| T. chumash        | 12                 | 358                |
| T. cristinae      | 6                  | 205                |
| T. knulli         | 5                  | 89                 |
| T. landelsensis   | 4                  | 125                |
| T. podura         | 12                 | 255                |
| T. poppensis      | 5                  | 116                |
Table S2: Details of climate variables included in this study and their principal component scores for first three PCs (Total proportion of variation explained by each PC: PC1 = 51.7%, PC2 = 24.4% and PC3 = 16.1%).

| Code | Description                                                   | PC1  | PC2  | PC3  |
|------|---------------------------------------------------------------|------|------|------|
| BIO1 | Annual Mean Temperature                                       | -0.24| 0.21 | 0.15 |
| BIO2 | Mean Diurnal Range (Mean of monthly (max temp - min temp))   | 0.17 | 0.19 | 0.03 |
| BIO3 | Isothermality (BIO2/BIO7) (×100)                              | -0.22| -0.12| -0.24|
| BIO4 | Temperature Seasonality (standard deviation ×100)             | 0.25 | 0.16 | 0.19 |
| BIO5 | Max Temperature of Warmest Month                             | 0.06 | 0.33 | 0.31 |
| BIO6 | Min Temperature of Coldest Month                             | -0.29| 0.03 | 0.04 |
| BIO7 | Temperature Annual Range (BIO5-BIO6)                         | 0.25 | 0.19 | 0.17 |
| BIO8 | Mean Temperature of Wettest Quarter                          | -0.29| 0.08 | 0.03 |
| BIO9 | Mean Temperature of Driest Quarter                           | -0.1 | 0.29 | 0.34 |
| BIO10| Mean Temperature of Warmest Quarter                          | -0.02| 0.34 | 0.33 |
| BIO11| Mean Temperature of Coldest Quarter                          | -0.29| 0.06 | 0.01 |
| BIO12| Annual Precipitation                                         | 0.09 | -0.32| 0.31 |
| BIO13| Precipitation of Wettest Month                               | 0.02 | -0.32| 0.36 |
| BIO14| Precipitation of Driest Month                                | 0.26 | -0.14| -0.04|
| BIO15| Precipitation Seasonality (Coefficient of Variation)         | -0.25| -0.01| 0.18 |
| BIO16| Precipitation of Wettest Quarter                             | 0.04 | -0.31| 0.36 |
| BIO17| Precipitation of Driest Quarter                              | 0.27 | -0.06| -0.11|
| BIO18| Precipitation of Warmest Quarter                             | 0.28 | -0.05| -0.07|
| BIO19| Precipitation of Coldest Quarter                             | 0.04 | -0.32| 0.34 |
| Elev | Elevation                                                    | 0.29 | 0    | -0.02|
| Lat  | Latitude                                                      | -0.19| -0.25| 0.08 |
| Long | Longitude                                                    | 0.25 | 0.19 | -0.02|
Table S3. Summary of model posterior predictive performance as approximated by the deviance information criterion (DIC) for models predicting parallelism as a function of genes and ecology. The full model in each case (for each PC) includes genes and ecology, and the null model includes only an intercept term. D gives the mean deviance and pD denotes the effective number of parameters. Lower DIC values denote better models.

| PC  | Model | D   | pD  | DIC |
|-----|-------|-----|-----|-----|
| PC1 | Full  | 51.03 | 12.17 | 63.2 |
|     | Genes | 52.04 | 11.03 | 63.1 |
|     | Ecology | 69.55 | 10.27 | 79.8 |
|     | Null  | 76.1  | 5    | 81.1 |
| PC2 | Full  | 81.38 | 4.95 | 86.3 |
|     | Genes | 80.59 | 3.89 | 84.5 |
|     | Ecology | 84.18 | 3.98 | 85.2 |
|     | Null  | 80.48 | 2.85 | 83.3 |
| PC3 | Full  | 68.32 | 5.64 | 74   |
|     | Genes | 74.95 | 4.51 | 79.5 |
|     | Ecology | 78.03 | 3.88 | 81.9 |
|     | Null  | 80.2  | 2.9  | 83.1 |
Table S4. Excess overlap between top climate-associations windows and those where change was mostly strongly correlated with elevation in the release-recapture experiment. Results are shown for different top quantiles. Here 0.90 indicates the top 10% of windows, which corresponds to the results in the main text. We report the observed number of windows in the top quantiles for both change and climate association, the x-fold enrichment relative to null expectations, and the corresponding P-value for each PC climate variable. Results are shown for null distributions where all windows were permuted or randomized (“Full randomization”) and where randomizations were limited to windows with similar numbers of SNPs (“Constrained randomization”). P-values ≤ .05 are highlighted in bold.

| PC1               | Full randomization | Constrained randomization |
|------------------|--------------------|--------------------------|
| Quantile         | Observed           | X-fold | P-value | X-fold | P-value | X-fold | P-value |
| 0.9              | 108                | 1.40   | <0.001 | 1.24   | 0.005   |        |         |
| 0.91             | 86                 | 1.39   | <0.001 | 1.19   | 0.040   |        |         |
| 0.92             | 75                 | 1.53   | <0.001 | 1.29   | 0.014   |        |         |
| 0.93             | 59                 | 1.56   | <0.001 | 1.29   | 0.013   |        |         |
| 0.94             | 48                 | 1.72   | <0.001 | 1.36   | 0.014   |        |         |
| 0.95             | 43                 | 2.21   | <0.001 | 1.65   | <0.001  |        |         |
| 0.96             | 33                 | 2.68   | <0.001 | 1.84   | 0.001   |        |         |
| 0.97             | 25                 | 3.58   | <0.001 | 2.29   | <0.001  |        |         |
| 0.98             | 15                 | 4.83   | <0.001 | 2.63   | 0.001   |        |         |
| 0.99             | 6                  | 7.47   | <0.001 | 3.07   | 0.14    |        |         |

| PC2               | Full randomization | Constrained randomization |
|------------------|--------------------|--------------------------|
| Quantile         | Observed           | X-fold | P-value | X-fold | P-value | X-fold | P-value |
| 0.9              | 101                | 1.32   | 0.003   | 1.21   | 0.015   |        |         |
| 0.91             | 77                 | 1.24   | 0.034   | 1.12   | 0.138   |        |         |
| 0.92             | 67                 | 1.37   | 0.005   | 1.21   | 0.062   |        |         |
| 0.93             | 53                 | 1.39   | 0.010   | 1.22   | 0.064   |        |         |
| 0.94             | 44                 | 1.59   | 0.001   | 1.32   | 0.039   |        |         |
| 0.95             | 36                 | 1.86   | <0.001  | 1.45   | 0.014   |        |         |
| 0.96             | 28                 | 2.29   | <0.001  | 1.66   | 0.003   |        |         |
| Quantile | Observed | X-fold | P-value | X-fold | P-value |
|---------|----------|--------|---------|--------|---------|
| 0.9     | 105      | 1.37   | <0.001  | 1.21   | 0.021   |
| 0.91    | 91       | 1.46   | <0.001  | 1.27   | 0.005   |
| 0.92    | 73       | 1.48   | 0.001   | 1.27   | 0.012   |
| 0.93    | 50       | 1.32   | 0.019   | 1.11   | 0.232   |
| 0.94    | 40       | 1.45   | 0.008   | 1.17   | 0.157   |
| 0.95    | 26       | 1.33   | 0.068   | 1.04   | 0.438   |
| 0.96    | 20       | 1.60   | 0.027   | 1.22   | 0.188   |
| 0.97    | 12       | 1.72   | 0.049   | 1.25   | 0.264   |
| 0.98    | 5        | 1.68   | 0.188   | 1.04   | 0.516   |
| 0.99    | 3        | 3.95   | 0.028   | 2.50   | 0.103   |
Table S5. Bayesian estimates of the percent of CHC variation explained by sequenced SNPs.
Estimates are from the polygenic GWA in gemma. The posterior median gives the point estimate of the percent of CHC variation explained by the SNPs; the 95% equal-tail probability intervals (ETPIs) are also given.

| Trait         | Posterior median | 95% ETPI    |
|---------------|------------------|-------------|
| Female pentacosanes | 89.7             | 35.8-99.9   |
| Female heptacosanes   | 52.5             | 4.9-98.9    |
| Female nonacosanes    | 80.2             | 15.5-99.8   |
| Male pentacosanes    | 53.2             | 8.3-97.2    |
| Male heptacosanes    | 52.4             | 10.3-96.5   |
| Male nonacosanes     | 50.8             | 7.8-95.6    |
Table S6: X-fold enrichments and associated $P$-values for number of overlapping SNP windows for PC1 for comparison with CHC experiment. Observed value gives the mean posterior inclusions probability (i.e., probability of a genotype-phenotype association) across all SNPs in 100 kb windows for each of the six CHC traits. $P$-values $\leq .05$ are highlighted in bold.

| T. bartmani | T. podura | T. chumash | T. cristinae | T. knulli | T. poppensis | T. landelsensis | T. californicum |
|-------------|-----------|------------|--------------|-----------|--------------|----------------|----------------|
| **CHC**     | **X-fold**| **Observed**| **P-value**  | **CHC**   | **X-fold**   | **Observed**  | **P-value**    |
| F-penta     | 1.06      | 0.000439   | 0.128        | F-penta   | 1.07         | 0.000425      | 0.059          |
| F-hepta     | 1.02      | 0.000301   | 0.271        | F-hepta   | 1.01         | 0.0002928     | 0.457          |
| F-nona      | 0.99      | 0.000264   | 0.526        | F-nona    | 0.94         | 0.00024994    | 0.909          |
| M-penta     | 0.96      | 0.000345   | 0.781        | M-penta   | 0.99         | 0.00035676    | 0.551          |
| M-hepta     | 1.07      | 0.000508   | 0.072        | M-hepta   | 1.03         | 0.00048354    | 0.265          |
| M-nona      | 0.9       | 0.000325   | 0.576        | M-nona    | 0.98         | 0.00032352    | 0.641          |
| **CHC**     | **X-fold**| **Observed**| **P-value**  | **CHC**   | **X-fold**   | **Observed**  | **P-value**    |
| F-penta     | 0.89      | 0.00036706 | 0.983        | F-penta   | 1.01         | 0.00041936    | 0.217          |
| F-hepta     | 0.94      | 0.00027501 | 0.898        | F-hepta   | 1.01         | 0.00029503    | 0.266          |
| F-nona      | 0.98      | 0.00025862 | 0.674        | F-nona    | 0.94         | 0.00024953    | 0.997          |
| M-penta     | 0.99      | 0.00035754 | 0.512        | M-penta   | 0.99         | 0.0003586     | 0.509          |
| M-hepta     | 0.97      | 0.00045792 | 0.712        | M-hepta   | 0.95         | 0.00044654    | 0.992          |
| M-nona      | 1.06      | 0.00034639 | 0.122        | M-nona    | 0.97         | 0.00031857    | 0.942          |
| **CHC**     | **X-fold**| **Observed**| **P-value**  | **CHC**   | **X-fold**   | **Observed**  | **P-value**    |
| F-penta     | 0.96      | 0.00039798 | 0.859        | F-penta   | 0.98         | 0.00040417    | 0.689          |
| F-hepta     | 1.02      | 0.00029934 | 0.201        | F-hepta   | 0.99         | 0.00029192    | 0.521          |
| F-nona      | 1.03      | 0.00027378 | 0.132        | F-nona    | 0.96         | 0.00025463    | 0.856          |
| M-penta     | 1.02      | 0.00036701 | 0.232        | M-penta   | 1           | 0.00036056    | 0.429          |
| M-hepta     | 0.99      | 0.00046794 | 0.554        | M-hepta   | 1.03         | 0.00048384    | 0.208          |
| M-nona      | 1         | 0.00033049 | 0.465        | M-nona    | 1.07         | 0.00035128    | **0.024**      |
| **CHC**     | **X-fold**| **Observed**| **P-value**  | **CHC**   | **X-fold**   | **Observed**  | **P-value**    |
| F-penta     | 0.94      | 0.00039035 | 0.951        | F-penta   | 0.97         | 0.00040217    | 0.753          |
| F-hepta     | 0.98      | 0.00028747 | 0.684        | F-hepta   | 0.99         | 0.00028878    | 0.601          |
| F-nona      | 0.97      | 0.00025692 | 0.819        | F-nona    | 0.98         | 0.00026206    | 0.625          |
| M-penta     | 0.98      | 0.00035169 | 0.726        | M-penta   | 0.96         | 0.00034453    | 0.892          |
| M-hepta     | 1.03      | 0.00048427 | 0.201        | M-hepta   | 1.05         | 0.00049524    | 0.071          |
| M-nona      | 1.02      | 0.00033471 | 0.304        | M-nona    | 1.01         | 0.00033312    | 0.333          |
Table S7: X-fold enrichments and associated $P$-values for number of overlapping SNP windows for PC2 for comparison with CHC experiment. Observed value gives the mean posterior inclusions probability (i.e., probability of a genotype-phenotype association) across all SNPs in 100 kb windows for each of the six CHC traits. $P$-values $\leq .05$ are highlighted in bold.

| T. bartmani   | T. podura   |
|---------------|-------------|
| CHC           | X-fold | Observed | $P$-value | CHC           | X-fold | Observed | $P$-value |
| F-penta       | 0.97   | 0.000403 | 0.681     | F-penta       | 0.99   | 0.00041351 | 0.486     |
| F-hepta       | 1.05   | 0.000306 | 0.143     | F-hepta       | 0.92   | 0.00027053 | 0.978     |
| F-nona        | 1      | 0.000265 | 0.494     | F-nona        | 1.06   | 0.00028006 | 0.089     |
| M-penta       | 0.96   | 0.000345 | 0.773     | M-penta       | 0.97   | 0.00035019 | 0.709     |
| M-hepta       | 0.96   | 0.000455 | 0.734     | M-hepta       | 1.05   | 0.00049492 | 0.113     |
| M-nona        | 0.99   | 0.000326 | 0.573     | M-nona        | 0.89   | 0.0002953  | 0.994     |

| T. chumash    | T. cristinae |
|---------------|--------------|
| CHC           | X-fold | Observed | $P$-value | CHC           | X-fold | Observed | $P$-value |
| F-penta       | 1.01   | 0.00041693 | 0.417    | F-penta       | 1.05   | 0.0004352  | **0.009** |
| F-hepta       | 0.95   | 0.00027777 | 0.863    | F-hepta       | 0.98   | 0.00028726 | 0.805     |
| F-nona        | 1.04   | 0.00027621 | 0.191    | F-nona        | 1.01   | 0.00026784 | 0.286     |
| M-penta       | 1.05   | 0.00037559 | 0.172    | M-penta       | 1.01   | 0.00036299 | 0.286     |
| M-hepta       | 0.98   | 0.00046092 | 0.628    | M-hepta       | 0.96   | 0.00045164 | 0.976     |
| M-nona        | 0.92   | 0.00030343 | 0.955    | M-nona        | 0.99   | 0.00032555 | 0.681     |

| T. knulli     | T. poppensis |
|---------------|--------------|
| CHC           | X-fold | Observed | $P$-value | CHC           | X-fold | Observed | $P$-value |
| F-penta       | 1.02   | 0.00041785 | 0.295    | F-penta       | 1.03   | 0.00042699 | 0.171     |
| F-hepta       | 1.02   | 0.00029805 | 0.221    | F-hepta       | 1.04   | 0.0003051  | 0.087     |
| F-nona        | 0.98   | 0.00025978 | 0.738    | F-nona        | 0.97   | 0.00025664 | 0.811     |
| M-penta       | 0.97   | 0.00034943 | 0.806    | M-penta       | 1.06   | 0.0003824  | **0.042** |
| M-hepta       | 0.99   | 0.00046472 | 0.612    | M-hepta       | 1.05   | 0.00049262 | 0.093     |
| M-nona        | 1      | 0.00032921 | 0.471    | M-nona        | 0.97   | 0.00032084 | 0.772     |

| T. landelsensis | T. californicum |
|-----------------|-----------------|
| CHC             | X-fold | Observed | $P$-value | CHC             | X-fold | Observed | $P$-value |
| F-penta         | 0.92   | 0.00038096 | 0.987    | F-penta         | 0.95   | 0.00039456 | 0.895     |
| F-hepta         | 0.97   | 0.0002817  | 0.864    | F-hepta         | 0.93   | 0.00027181 | 0.99      |
| F-nona          | 1.01   | 0.00026628 | 0.43     | F-nona          | 1.01   | 0.00026813 | 0.346     |
| M-penta         | 0.99   | 0.00035724 | 0.517    | M-penta         | 1.05   | 0.00037699 | 0.062     |
| M-hepta         | 1.01   | 0.00047379 | 0.437    | M-hepta         | 1.01   | 0.00047356 | 0.395     |
| M-nona          | 0.99   | 0.00032686 | 0.561    | M-nona          | 0.96   | 0.00031934 | 0.831     |
Table S8: X-fold enrichments and associated $P$-values for number of overlapping SNP windows for PC3 for comparison with CHC experiment. Observed value gives the mean posterior inclusions probability (i.e., probability of a genotype-phenotype association) across all SNPs in 100 kb windows for each of the six CHC traits. $P$-values ≤ .05 are highlighted in bold.

| CHC       | X-fold | Observed | $P$-value | CHC       | X-fold | Observed | $P$-value |
|-----------|--------|----------|-----------|-----------|--------|----------|-----------|
| F-penta   | 1.06   | 0.000439 | 0.128     | F-penta   | 1.03   | 0.00042715 | 0.222     |
| F-hepta   | 1.02   | 0.000301 | 0.271     | F-hepta   | 1.04   | 0.00030464 | 0.132     |
| F-nona    | 0.99   | 0.000264 | 0.526     | F-nona    | 0.93   | 0.00024736 | 0.948     |
| M-penta   | 0.96   | 0.000345 | 0.781     | M-penta   | 1.08   | 0.00038877 | 0.029     |
| M-hepta   | 1.07   | 0.000508 | 0.072     | M-hepta   | 1.09   | 0.00051268 | 0.028     |
| M-nona    | 0.9    | 0.000325 | 0.576     | M-nona    | 0.99   | 0.00032619 | 0.531     |

| CHC       | X-fold | Observed | $P$-value | CHC       | X-fold | Observed | $P$-value |
|-----------|--------|----------|-----------|-----------|--------|----------|-----------|
| F-penta   | 1.07   | 0.0004442 | 0.088    | F-penta   | 1.05   | 0.00043482 | 0.012     |
| F-hepta   | 0.99   | 0.0002889 | 0.569    | F-hepta   | 1.01   | 0.00029312 | 0.393     |
| F-nona    | 0.94   | 0.00024939 | 0.889    | F-nona    | 1.03   | 0.00027351 | 0.051     |
| M-penta   | 1.03   | 0.00037132 | 0.216    | M-penta   | 0.99   | 0.00035579 | 0.666     |
| M-hepta   | 1.03   | 0.00048255 | 0.281    | M-hepta   | 0.98   | 0.00046005 | 0.832     |
| M-nona    | 1.05   | 0.00034602 | 0.164    | M-nona    | 0.98   | 0.00032394 | 0.791     |

| CHC       | X-fold | Observed | $P$-value | CHC       | X-fold | Observed | $P$-value |
|-----------|--------|----------|-----------|-----------|--------|----------|-----------|
| F-penta   | 1.05   | 0.00043564 | 0.054    | F-penta   | 1.06   | 0.00043928 | 0.034     |
| F-hepta   | 0.99   | 0.0002911 | 0.547     | F-hepta   | 0.97   | 0.00028301 | 0.832     |
| F-nona    | 0.97   | 0.00025644 | 0.842    | F-nona    | 0.96   | 0.00025389 | 0.894     |
| M-penta   | 1.03   | 0.00036955 | 0.141    | M-penta   | 0.93   | 0.00033533 | 0.981     |
| M-hepta   | 0.99   | 0.00046841 | 0.563    | M-hepta   | 0.98   | 0.0004604 | 0.723     |
| M-nona    | 0.99   | 0.00032395 | 0.676    | M-nona    | 0.96   | 0.00031526 | 0.903     |

| CHC       | X-fold | Observed | $P$-value | CHC       | X-fold | Observed | $P$-value |
|-----------|--------|----------|-----------|-----------|--------|----------|-----------|
| F-penta   | 1.03   | 0.0004247 | 0.236    | F-penta   | 1.02   | 0.0004232 | 0.268     |
| F-hepta   | 1.03   | 0.00030062 | 0.175    | F-hepta   | 0.95   | 0.00027931 | 0.913     |
| F-nona    | 0.99   | 0.00026403 | 0.532    | F-nona    | 0.97   | 0.00026    | 0.732     |
| M-penta   | 0.97   | 0.00034978 | 0.769    | M-penta   | 0.98   | 0.00035164 | 0.713     |
| M-hepta   | 0.95   | 0.00044452 | 0.939    | M-hepta   | 1.05   | 0.00049509 | 0.068     |
| M-nona    | 1.05   | 0.00034642 | 0.056    | M-nona    | 0.99   | 0.00032631 | 0.594     |
Table S9: Proportion of variation explained by the TREEMIX population graph with different numbers of migration edges.

| Number of migration edges | Proportion of variation explained |
|---------------------------|----------------------------------|
| 0                         | 0.997                            |
| 1                         | 0.998                            |
| 2                         | 0.998                            |
| 3                         | 0.998                            |
| 4                         | 0.999                            |
| 5                         | 0.999                            |
| 6                         | 0.999                            |
| 7                         | 0.999                            |
| 8                         | 0.999                            |
| 9                         | 0.999                            |
Figure S1: Ordination of climate variation (22 variables, see Table S2 for code descriptions) via principal component analysis (PCA). Points denote the study populations, colour-coded by species.
Figure S2: Manhattan plots showing the strength of evidence for association (measured here using the Bayes factor from the software BayPass) between a SNP window and climate for PC1. Results are shown along the 13 linkage groups. Red points denote the SNP windows in the top 10% quantile (i.e., referred to as ‘climate-associated SNP windows’ throughout the main text). In each panel title, the two values in parentheses are the number of SNP windows in the top 10% quantile (“windows”), followed by the number of linkage groups with at least 1 SNP window in the top 10% quantile (“LG”).

(A) *T. bartmani* (155 windows, 13 LG)

(B) *T. podura* (215 windows, 13 LG)

(C) *T. chumash* (157 windows, 13 LG)

(D) *T. cristinae* (807 windows, 13 LG)

(E) *T. knulli* (386 windows, 13 LG)

(F) *T. poppensis* (315 windows, 13 LG)

(G) *T. landelsensis* (333 windows, 12 LG)

(H) *T. californicum* (342 windows, 13 LG)
Figure S3: Manhattan plots showing the strength of evidence for association (measured here using the Bayes factor from the software BayPass) between a SNP window and climate for PC2. Results are shown along the 13 linkage groups. Red points denote the SNP windows in the top 10% quantile (i.e., referred to as ‘climate-associated SNP windows’ throughout the main text). In each panel title, the two values in parentheses are the number of SNP windows in the top 10% quantile (“windows”), followed by the number of linkage groups with at least 1 SNP window in the top 10% quantile (“LG”).
FIGURE S4: Tests for parallel climate-associated SNP windows between and among species of *Timema* stick insects (all plots are for the top 10% empirical quantile) for PC1. (A) Plot shows observed versus expected number of overlapping climate-associated SNP windows for species pairwise comparison for PC1. Bars denote expected values and dotted lines denote observed values. Open points indicate $P$-value $> 0.05$ and filled points indicate $P$-value $\leq 0.05$. (B) Pairwise plot shows x-fold enrichment values for each comparison between pairs of species. Black dot on each box denotes $P$-value $\leq 0.05$. (C) Plot shows x-fold enrichments for number of overlapping climate-associated SNP windows for PC1 for comparisons between multiple species, i.e., beyond pairs of species (e.g., 2 or more species, 3 or more species, 4 or more species). Bars denote observed x-fold value for each multi-species comparison. Black lines on bars show the 95% confidence intervals. N value above each bar indicates the observed number of overlapping climate-associated SNP windows for each comparison. * Indicates x-fold enrichments with $P$-value $\leq 0.05$. 

(A) Observed vs. expected parallelism

(B) Pairwise comparisons

(C) Multi-species comparisons
FIGURE S5: Tests for parallel climate-associated SNP windows between and among species of *Timema* stick insects (all plots are for the top 10% empirical quantile) for PC2. (A) Plot shows observed versus expected number of overlapping climate-associated SNP windows for species pairwise comparison for PC2. Bars denote expected values and dotted lines denotes observed values. Open points indicate P-value > 0.05 and filled points indicate P-value ≤ 0.05. (B) Pairwise plot shows x-fold enrichment values for each comparison between pairs of species. Black dot on each box denotes P-value ≤ 0.05. (C) Plot shows x-fold enrichments for number of overlapping climate-associated SNP windows for PC2 for comparisons between multiple species, i.e., beyond pairs of species (e.g., 2 or more species, 3 or more species, 4 or more species). Bars denote observed x-fold value for each multi-species comparison. Black lines on bars show the 95% confidence intervals. N value above each bar indicates the observed number of overlapping climate-associated SNP windows for each comparison. * Indicates x-fold enrichments with P-value ≤ 0.05.
FIGURE S6: Test results of the “shared ecology” versus “shared genetics” hypotheses. (A) Scatterplot shows the relationship between X-fold enrichment (measure for parallelism) and ecological distance (measured as distance in PC1 scores) (B) Scatterplot shows the relationship between X-fold enrichment (measure for parallelism) and genetic distance (measured as pairwise phylogenetic distance). (C) Scatterplot shows the relationship between ecological distance (measured as distance in PC1 scores and is distance in climate variables) and genetic distance (measured as pairwise phylogenetic distance) (D) Plot shows parameter estimates with standardized coefficients for the full model for PC1. A negative or positive estimate which deviates from zero is indicative of effect on parallelism.
FIGURE S7: Test results of the “shared ecology” versus “shared genetics” hypotheses. (A) Scatterplot shows the relationship between X-fold enrichment (measure for parallelism) and ecological distance (measured as distance in PC2 scores) (B) Scatterplot shows the relationship between X-fold enrichment (measure for parallelism) and genetic distance (measured as pairwise phylogenetic distance). (C) Scatterplot shows the relationship between ecological distance (measured as distance in PC2 scores and is distance in climate variables) and genetic distance (measured as pairwise phylogenetic distance) (D) Plot shows parameter estimates with standardized coefficients for the full model only for PC2. A negative or positive estimate which deviates from zero is indicative of effect on parallelism.

(A) Parallelism vs. ecological distance

(B) Parallelism vs. genetic distance

(C) Ecological distance vs. genetic distance

(D) Ecology vs. genetics (model)
FIGURE S8, S9, S10: Plots show summaries of population structure based on principal component analysis for 8 species included in this study for PC1 vs. PC2 (Figure 19), PC1 vs. PC3 (Figure 20), and PC2 vs. PC3 (Figure 21). Abbreviations indicate populations corresponding to TABLE S1.
