Migration and the Genetic Covariance between Habitat Preference and Performance

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abstract: Studies of the genetic covariance between habitat preference and performance have reported conflicting outcomes ranging from no covariance to strong covariance. The causes of this variability remain unclear. Here we show that variation in the magnitude of genetic covariance can result from variability in migration regimes. Using data from walking stick insects and a mathematical model, we find that genetic covariance within populations between host plant preference and a trait affecting performance on different hosts (cryptic color pattern) varies in magnitude predictably among populations according to migration regimes. Specifically, genetic covariance within populations is high in heterogeneous habitats where migration between populations locally adapted to different host plants generates nonrandom associations (i.e., linkage disequilibrium) between alleles at color pattern and host preference loci. Conversely, genetic covariance is low in homogeneous habitats where a single host exists and migration between hosts does not occur. Our results show that habitat structure and patterns of migration can strongly affect the evolution and variability of genetic covariance within populations.

Keywords: phytophagous insects, quantitative genetics, host preference, crypsis, population genetics.

A fundamental equation in evolutionary genetics states that between-generation evolutionary change in trait means, $\Delta \bar{z}$, is a function of the matrix of genetic variances

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and covariances, **G**, and the vector of selection gradients, ß: $\Delta \bar{z} = \mathbf{G}\beta$ (Lande 1979). Thus, genetic covariance between traits is a central topic in evolutionary biology because it may either constrain or facilitate both adaptation (Lande 1979; Brodie 1989; Arnold 1992; Barton 1995; Schluter 1996; Hodges et al. 2002; Sinervo and Svensson 2002; Otto 2004) and speciation (Felsenstein 1981; Diehl and Bush 1989; Hawthorne and Via 2001; Kirkpatrick and Ravigné 2002; Blows and Higgie 2003; Coyne and Orr 2004; Kawecki 2004).

In contrast to general agreement as to how genetic covariances affect the evolution of trait means, the degree to which genetic covariances themselves evolve and how they evolve remain controversial (Gould 1977; Lande 1979, 1980; Turelli 1988; Arnold 1992; Jernigan et al. 1994; Roff 2000; Begin and Roff 2004). Of particular interest is the evolution of genetic covariance within populations, because covariance among populations can arise simply from natural selection causing correlated change in a suite of traits among populations (Armbruster and Schwaegerle 1996; Sinervo and Svensson 2002). Thus, we focus on the evolution of genetic covariance within populations.

By one mechanism, genetic covariance within populations may simply reflect underlying developmental constraints (i.e., patterns of pleiotropy) that remain relatively unchanged through space and time (Gould 1977; Arnold 1992). Physical linkage might also remain relatively constant and help maintain associations between alleles at different loci (linkage disequilibrium), thereby facilitating constant genetic covariance (Hawthorne and Via 2001). Another mechanism that generates genetic covariance within populations is migration between genetically differentiated populations. Theory clearly demonstrates that migration produces associations between alleles at different loci (linkage disequilibrium), even when they are physically unlinked (Kimura 1956; Nei and Li 1973; Kirkpatrick et al. 2002). Natural selection can play a role in this latter process by causing the required population divergence.

In this article, we test for genetic covariance within populations generated by migration between populations. We predict greater genetic covariance in habitats where mi-

gration between divergent populations occurs. Empirical examples of genetic covariance generated by migration are lacking and are of interest on two levels. In a general sense, we would like to know whether migration plays an important role in determining how covariances evolve or whether pleiotropy is the dominant factor. In a more specific context, we are interested in understanding the causes of variation in the genetic covariance between habitat preference and traits conferring fitness in different habitats. This question has been the focus of substantial empirical work and has led to conflicting conclusions. Thompson (1988) and Jaenike and Holt (1991, tables 2, 6) reviewed these studies and found reports of no covariance, weak covariance, and strong covariance (see also below for more recent references). The causes of this variability are unclear.

Research on genetic covariance between habitat preference and traits linked to habitat-specific performance has focused on two scenarios. One body of work is focused on background color matching via cryptic coloration, a classic example of adaptation via natural selection (Cott 1940; Kettlewell 1973; Endler 1984). Because cryptic organisms are subject to strong selection to prefer backgrounds upon which they are well hidden, covariance is expected between substrate preference and body color. While some studies detect the expected covariance (Kettlewell 1955; Gillis 1982), others do not (e.g., Steward 1985; Grant and Howlett 1988). A second situation involves host preference and traits conferring adaptation to hosts in phytophagous insects (Thompson 1988; Diehl and Bush 1989; Hawthorne and Via 2001; Kawecki 2004). This scenario is well studied because genetic covariance between host preference and performance has implications for speciation via host shifting. Again, some studies have detected the expected covariance between these two traits (e.g., Via 1986; Singer et al. 1988; Hawthorne and Via 2001; Bossart 2003; Forister 2004), while others have not (Futuyma and Moreno 1988; Jaenike and Holt 1991; Fox 1993; Fry 1996; Poore and Steinberg 2001).

Populations of phytophagous insects differ in the ecological and spatial structure of the habitats they occupy. This diversity provides the opportunity to examine whether variation in regimes of migration contributes to variation in genetic covariance. In this study, we document genetic covariance between host plant preference and host plant–specific performance (represented here by cryptic coloration). We show that the magnitude of this genetic covariance within populations varies positively with the opportunity for migration between divergent populations. Thus, genetic covariance is strongly affected by habitat structure. We also estimate genetic variance and show that it does not vary systematically with the opportunity for migration. Thus, the low genetic covariance observed when there is no migration between populations does not

appear to stem solely from a lack of genetic variation. Our findings indicate that patterns of migration can strongly affect the evolution and variability of genetic covariance within populations. This effect may at least partly explain why previous studies of phytophagous insects have sometimes found a correlation between preference and performance and sometimes not.

We begin by describing the biology of the walking stick populations studied here. We then present a simple model for the evolution of the genetic covariance due to migration, which is based on the biology of our system but also applies more generally. Next, we test the predictions of the model using both field data and a quantitative genetic experiment. Finally, we discuss the implications of our results for explaining the evolution of genetic covariance between preferences and performance.

Study System

Timema cristinae walking sticks feed on one of two host plant species, *Ceanothus spinosus* or *Adenostoma fasciculatum*. A population is defined as all the insects collected within a patch of a single host species (as in Nosil et al. 2002, 2003; Nosil 2004; Nosil and Crespi 2004). We focus here on nine populations from two distinct types of study sites that are separated from all other sites by regions without suitable hosts (that is, a site is a contiguous area of one or both hosts). Homogeneous sites contained only one of the host plant species and thus only one population of walking sticks. Heterogeneous sites contained both hosts and thus two populations of walking sticks, one feeding on each host species.

We studied walking sticks in three homogeneous and three heterogeneous sites. Homogeneous sites 1 and 3 contained only *Ceanothus*, while homogeneous site 2 contained only *Adenostoma*. In heterogeneous sites 4–6, the frequency of *Ceanothus* varied from 5% to 70% (table 1). Our study focuses on genetic covariance within populations. Host patches are sufficiently large and distinct relative to the mobility of these insects that each population at a heterogeneous site receives only limited migration from the other population using the alternate host (Sandoval 1993; Nosil et al. 2003; see below for details).

Genetic covariance was assessed between two traits that are involved in adaptation and reproductive isolation. The first trait is cryptic color pattern. This trait is determined by a single diallelic locus, with the allele for an unstriped morph dominant over the allele for a striped morph (Sandoval 1993). Both morphs occur within populations on each host, but the unstriped morph is more common on *Ceanothus* (mean frequency = 81%), while the striped morph is more common on *Adenostoma* (mean frequency = 72%; Sandoval 1994*b*; Nosil et al. 2002; Nosil

Table 1: Estimated genetic covariance (G_{CH}) between color pattern and host preference in homogeneous versus heterogeneous sites

Sites	Estimated G_{CH}	Predicted G_{CH}		C at site (%)	Prefer C (%)	n	Estimated G_H	Unstriped (%)	n	Estimated G_C
		Low m	High m							
Homogeneous:										
1	.03	0	0	100	93	149	.60	82	501	.09
2	.01	0	0	0	63	139	.27	14	608	.12
3	.00	0	0	100	100	33	.08	60	154	.19
Heterogeneous:										
4C	.10	.002	.009	5	87	52	.12	23	190	.17
4A	.07	.002	.010	5	60	68	.37	8	99	.07
5C	...	.002	.011	39	81	16	...	45	29	.21
5A	.15	.003	.014	39	62	52	.41	16	174	.13
6C	.26	.004	.024	70	90	70	1.11	61	444	.18
6A	.12	.006	.031	70	44	57	.26	35	343	.20

Note: Also shown are the covariances predicted by equation (1) assuming free recombination with low ($m = 0.043$) and high ($m = 0.232$) migration rates (these values are the mean and maximum estimates of m from Nosil et al. 2003), the percent of the site occupied by *Ceanothus* (C), the percent of individuals preferring to rest on C, the estimated genetic variance in host preference (G_H), the percent of unstriped individuals, and the estimated genetic variance in color pattern (G_C). n = sample sizes. No data were available for genetic covariance in population 5C.

2004). Population divergence has occurred via differential visual predation: the unstriped color pattern confers high survival on *Ceanothus* but low survival on *Adenostoma* and vice versa for the striped pattern (Sandoval 1994*a*, 1994*b*; Nosil 2004). Although studies of phytophagous insects generally consider physiological traits when referring to performance, divergent selection on color also results in performance trade-offs.

The second trait is host preference, which has also diverged between populations. Populations feeding on *Ceanothus* exhibit a stronger preference for that host than do populations feeding on *Adenostoma* (Nosil et al. 2005). Population divergence in host preference has a genetic basis and occurs between adjacent populations that use alternative hosts and between geographically separated populations that each use single but different hosts. The latter observation indicates that host preference is under selection even in homogeneous sites (those with a single host), perhaps because of search and efficiency costs acting directly on host preference (Bernays and Wcislo 1994). Because these insects mate exclusively on their hosts, divergent host preferences confer partial premating isolation. A detailed study of divergence in mean host preference between populations has shown that mean host preference evolves via a balance between selection and gene flow and will appear elsewhere (Nosil et al. 2005); here we focus on genetic covariance. Less is known about the genetic determination of host preference than that of color pattern, but additive gene action is suggested by the fact that crosses between populations yield offspring with intermediate preferences (Nosil et al. 2005).

We compared the genetic covariance between color pattern and host preference within populations at heteroge-

neous sites with the covariance at homogeneous sites. Walking sticks have relatively low motility (Sandoval 1993), allowing selection to cause divergence between populations within a heterogeneous site (Sandoval 1994*b*; Nosil et al. 2003; Nosil 2004). Morphological and molecular data indicate, however, that some migration between populations on different hosts does occur in heterogeneous sites (Sandoval 1994*b*; Nosil et al. 2003; Nosil and Crespi 2004). For example, divergence in trait means and in DNA is consistently lower between adjacent populations at the same site than between geographically separated populations, indicative of migration. The migration rate into populations at heterogeneous sites (defined as the mean proportion of individuals that are immigrants arriving from the other host species in that generation) is estimated as 0.043 (maximum = 0.232). These estimates are derived using the coalescent-based methods of Beerli and Felsenstein (2001) but are rough because they consider only a single locus (mitochondrial DNA; Nosil et al. 2003). In homogeneous sites, by contrast, migration between populations does not occur because only one population on a single host exists. Thus, genetic covariance between color pattern and host preference caused by migration is expected only within populations from heterogeneous sites.

Thus, the main prediction we test is that the genetic covariance between color pattern and host preference will be higher within populations from heterogeneous sites. We then go on to ask how a quantitative measure of habitat heterogeneity (the relative abundance of the two hosts) varies with the genetic covariance. Genetic covariance is expected to increase as the two hosts become more similar in relative abundance because greater population divergence occurs under such scenarios (Sandoval 1994*b*; Nosil

et al. 2003; Nosil and Crespi 2004; Nosil 2004), and covariance generated by migration is proportional to the degree of population divergence. Greater population divergence itself is observed as relative host abundances become similar because migration rates between pairs of populations at the same site become more symmetric but not necessarily lower overall (for details, see Nosil et al. 2003). Increased symmetry in migration promotes divergence because one population does not incur such disproportionately high levels of homogenizing migration that swamping occurs. This illustrates how migration can have opposing effects: increased migration causes greater covariance for a given level of population divergence but can decrease the amount of divergence between the populations. To motivate the predictions more precisely, we will now develop a simple mathematical model, based on the ecology and genetics of the walking sticks, that shows how the genetic covariance depends on migration.

Mathematical Model

We are interested in predicting the genetic covariance, which we denote G_{CH}, that develops between color pattern and host preference as the result of migration between two populations. We assume the two traits are controlled by separate sets of autosomal loci (no pleiotropy) that are expressed equally in males and females. In order to quantify the color pattern phenotypes, we give striped individuals a score of $c = 0$ and unstriped individuals a score of $c = 1$; the frequency of striped individuals in the population is denoted f_s. Consistent with the data from *Timema cristinae*, we assume color pattern is controlled by a single locus C, with allele 1 for unstriped coloration dominant over the allele 0 for striped. Note that the additive genetic variance is well defined even though color pattern is controlled by a single locus with a dominant allele (Lynch and Walsh 1998, chap. 21).

We assume genetic variation in host preference is caused by an arbitrary number of loci that show no dominance or epistasis. Each of these loci has two alleles, also denoted 0 and 1, and different loci may make unequal contributions to host preference. An individual's host preference, denoted h, is defined as the probability that an individual will chose *Ceanothus*, and the frequency that host is chosen across all individuals in the population is written $\bar{h}$. For simplicity, we assume that individuals within a population mate randomly. Although there is some evidence for assortative mating by color pattern within populations, it does not vary according to site heterogeneity such that it could confound our test for an association between genetic covariance and site heterogeneity (Nosil et al. 2002). Note that Nosil et al. (2003) examined assortative mating between (rather than within) populations and that their findings do not apply to estimating covariance within populations.

The appendix derives the dynamic equation and equilibrium for the genetic covariance between color pattern and host preference under these fairly general assumptions. The results simplify under several scenarios. If there is only a single host preference locus, or if all host preference loci have equal effects and equal allele frequencies, we find that the genetic covariance at equilibrium is

$$G_{CH} \approx \frac{m\left(\sqrt{f_s^M f_s} - f_s\right)(\bar{h} - \bar{h}^M)}{\tilde{r}}, \tag{1}$$

where m is the migration rate between populations, f_s is the frequency of striped individuals, $\bar{h}$ is the frequency of individuals preferring *Ceanothus* within the focal population, superscripts M denote corresponding values among arriving migrants, and $\tilde{r}$ is the harmonic mean recombination rate between the color pattern and host preference loci. Equation (1) is an approximation that assumes that migration is weak relative to recombination ($m \ll \tilde{r}$).

As we expect intuitively, the covariance increases with the migration rate and with the genetic differences between the populations in the two traits. The covariance decreases with larger recombination rates between the color pattern and host preference loci.

An alternative scenario also leads to a simple expression for the covariance. If the color pattern locus is unlinked to any of the host preference loci, even if preference loci have unequal effects and allele frequencies, then equation (1) also applies if $\tilde{r}$ is replaced by 1/2. With this model as motivation, we now present the methods used in our empirical study.

Methods

Population Divergence

For migration to generate genetic covariance between color pattern and host preference, populations must differ in both traits. Morph frequencies were obtained by randomly sampling individuals from the nine populations at the six study sites (total $n = 2,542$ individuals; table 1 for population-specific sample sizes). Mean host preference for each population was estimated with randomly sampled individuals from the nine populations, using protocols described below for the quantitative genetic experiment (total $n = 636$ individuals; table 1). Differences among populations were assessed using χ^2 tests. Seven of our populations were used in previous studies (Nosil et al. 2002, 2003; Nosil 2004; Nosil and Crespi 2004). Sites 1–5 of this study correspond, respectively, to sites P, LA, PE,

MBOXC and MBOXA, and MC and MA of the earlier work.

Genetic Covariance

To estimate genetic covariance, crosses between individuals from within eight of the nine populations were carried out in 2003. Sexually immature instars were captured in the field using sweep nets. The sexes were reared separately on *Ceanothus* cuttings in glass jars. Within a few days of sexual maturity, a single virgin male and a single virgin female were housed together in a petri dish until copulation was observed. In order to maximize the probability that we would detect genetic covariance between color pattern and host preference, individuals were paired assortatively by color. Thus, all matings were between individuals of the same color pattern morph, with approximately equal numbers of pairs of each morph; this allowed a reasonable number of crosses to be conducted using the rarer morph within a population. Each pair was maintained on *Ceanothus* cuttings until the female died (females lay eggs singly and daily).

Offspring were scored for host preference within a few days of emergence. In total, 428 offspring were scored from 64 families, with 9, 12, 8, 10, 13, and 12 families for sites 1–6, respectively. The number of crosses from the *Ceanothus* population was 6, 0, and 9 in heterogeneous sites 4–6, respectively. Between 39 and 108 nymphs per population and between 3 and 21 per family were tested.

Individual offspring were placed in the bottom of a 500-mL plastic cup (height, 15 cm) with one 12-cm cutting from each host species in the cup. The bottom end of each cutting was in a water-filled aqua pick that held the cutting upright and kept it fresh. Containers were covered with wire netting secured by elastic bands. Assays were initiated in the evening and left in darkness overnight. In the morning, the host on which each individual was resting was recorded. For assays where the test individual did not choose a host, the container was left overnight until a host was chosen (for up to two nights). Each individual was used only once. All scoring was done blind to parental population and morph by P. Nosil. Color pattern was not scored because of difficulties rearing the nymphs until it was distinguishable.

The genetic covariance between color pattern and preference was estimated as twice the phenotypic covariance between the mean parental color pattern and the mean offspring host preference (Lynch and Walsh 1998). The genetic covariances were estimated using full-sib families and thus represent broad-sense genetic measures. Additionally, because the parents used in the genetic crosses were initially captured in the field, we cannot explicitly rule out maternal effects. However, the fact that all parents were reared to maturity in a common environment, along with several other lines of reasoning, indicates that covariance has a strong genetic basis (see "Discussion").

The assortative mating used in the breeding design increases the covariance between parents and offspring, leading to upwardly biased estimates of the genetic covariance. In principle, it is possible to apply a correction for this bias, but when there is genetic dominance (as with color pattern in these insects), the correction involves genetic quantities that we cannot estimate directly. Our primary goal is to test whether the covariance is correlated positively with site heterogeneity rather than to make precise estimates of the covariance. The assortative mating in the breeding design would only confound our results if the bias also varied positively with site heterogeneity. In contrast, if a bias does exist, we expect it to work in the opposite direction; homogeneous sites have more extreme color pattern frequencies and preferences, so the nonrandom mating used in the breeding design will inflate the parent-offspring covariance more in these crosses than in those from heterogeneous sites. In brief, the quantitative genetic experiment will give upwardly biased estimates of the covariance for all populations, but it is conservative with respect to the correlation with habitat structure that we seek to test.

The difference in host preference between offspring derived from striped versus unstriped parents is indicative of genetic covariance between host preference and color pattern. We tested for this difference using ANOVA analyses on family means. The analysis included parental morph (striped or unstriped), parental population, and parental habitat type (heterogeneous or homogeneous) as factors as well as the interaction between parental morph and habitat type. We are primarily interested in the interaction term, which tests whether genetic covariance differs between habitat types.

We tested for an association between the magnitude of genetic covariance and site heterogeneity using a quantitative index of host plant diversity. Our measure of site heterogeneity is the Simpson species diversity index, which for our case of two host species is equal to $2p_i(1 - p_i)$, where p_i is the proportion of the patch occupied by *Ceanothus* (thus, maximal values are achieved when both species are equally abundant). This proportion was estimated using aerial photographs and ground proofing (as in Sandoval 1994*b*; Nosil et al. 2003).

Phenotypic Covariance

In addition to our study of the genetic covariance, we also looked for a positive relationship between site heterogeneity and the phenotypic covariance of color pattern and host preference within natural populations. This associa-

tion was estimated by comparing the difference in host preference between color pattern morphs that were captured in the field. Laboratory choice tests were conducted on individuals collected in seven populations from six sites in 1992 (two sites were homogeneous). We offered insects one 30-cm-high branch of each host species (branches were kept 10 cm apart and out of contact in a Styrofoam sheet floating in a container with water, thereby keeping the plant fresh and preventing insects from escaping). Approximately 10 insects from the same population and of the same morph were placed on the Styrofoam, midway between the two branches, and left overnight (this is considered one replicate). The following morning, the number of insects on each branch was counted by C. P. Sandoval. The choice test was replicated for each site and morph type on the basis of insect availability (total number of individuals = 604, total number of replicates = 79, mean number of replicates per morph type per population = 5.6). Each insect was used only once. Mean preference from each replicate (percent of individuals choosing *Ceanothus*) was used as a single data point when calculating the mean preference of each morph within each population.

Genetic Variances

In addition to its effects on genetic covariance, migration may influence the genetic variance of each trait. We therefore estimated the genetic variances of host preference and color pattern within the study populations. Our main focus was to compare genetic covariance between populations, and logistical constraints limited the experimental designs we were able to use. Our estimates for the genetic variances in host preference should be interpreted with caution (and thus we prefer not to report genetic correlations explicitly).

Because of its simple genetic determinism, we can directly estimate the additive genetic variance for color pattern in each site from morph frequencies. Color pattern is controlled by a single locus, with the allele for unstriped coloration dominant over the striped allele (Sandoval 1993). The additive genetic variance for color pattern is therefore $G_C = 2p^3 q$, where p is the frequency of the striped allele (Falconer and MacKay 1996, eq. [8.6]). We assume the color pattern locus is in approximate Hardy-Weinberg equilibrium, and we estimated the allele frequency as $p = (\bar{c})^{1/2}$, where $\bar{c}$ is the frequency of the striped morph.

We estimated genetic variance in host preference from the genetic experiment described above by comparing variation within families to variation between families (i.e., a full-sib analysis). We used two methods, both based on one-way ANOVA. First, we estimated broad-sense genetic

variance and heritability using all the data with a boot-strapping method developed by Phillips and Arnold (1999). We ran 10,000 bootstrap replicates using their program H2boot to assess whether these parameters were significantly different from 0. Second, we used a full-sib analysis to estimate the genetic variance in host preference (G_H) for each population separately. Our estimator is $2(MS_T - MS_W)$, where MS_T is the total mean square and MS_W is the mean square within full-sib families (Falconer and MacKay 1996, p. 167). This estimate is biased upward by genetic dominance and common environmental effects (including maternal effects). Further, the estimates have large errors because only a small number of families was measured. For these two reasons, we interpret the results with great caution.
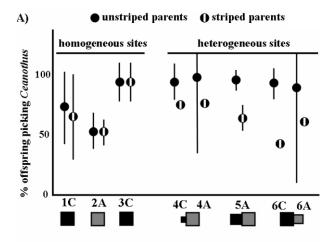
Results

Population Divergence

Population divergence in color pattern and host preference is required for migration between populations to generate genetic covariance between these traits. This precondition is fulfilled. Morph frequencies (percent of striped individuals) differ among populations using different hosts ($\chi^2 = 489.27$, $P < .001$, data from populations of the same host pooled). They also differ between adjacent pairs of populations on different hosts at the same site ($\chi^2 = 9.48$, 13.50, 54.64 for sites 4–6, respectively; all $P < .01$; see table 1 for frequencies). Likewise, host preferences (percent preferring *Ceanothus*) differ among populations using different hosts ($\chi^2 = 91.45$, $P < .001$) and between adjacent pairs of populations on different hosts at the same site ($\chi^2 = 10.21$, 14.62, 77.30; $P = .07$, .006, <.001 for sites 4–6, respectively; table 1).

Genetic Covariances

The genetic covariance between color pattern and host preference is greater within populations from heterogeneous sites than within populations from homogeneous sites. Three lines of statistical argument support this conclusion.

First, estimates for the genetic covariance in the five populations at heterogeneous sites are all larger than those from the three populations at homogeneous sites (table 1; fig. 1). The probability that this pattern would arise by chance is $(5!)(3!)/(8!) = 1/56 < 0.05$, and so we conclude that the association between site heterogeneity and genetic covariance is statistically significant. We stress that the individual estimates for the covariance have low precision because of the small number of families tested from each single population. Nevertheless, among populations, the
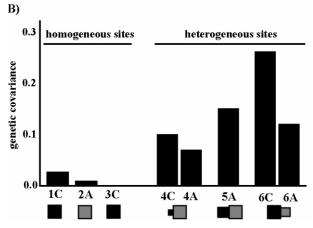
A)

B)

Figure 1: Results of the genetic experiment showing greater genetic covariance between color pattern and host preference in heterogeneous than in homogeneous sites. Populations are labeled below the *Y*-axis (where *A* and light boxes denote *Adenostoma* populations and *C* and dark boxes denote *Ceanothus* populations). *A*, Mean percent of offspring preferring *Ceanothus* (±95% confidence interval; the mean of the family means is depicted) for offspring derived from each morph for each population. The difference in preference between offspring derived from striped versus unstriped parents is proportional to the genetic covariance between color pattern and host preference. This difference varies among habitat types such that it is greater in heterogeneous habitats ($P < .001$; ANOVA; see "Results"). *B*, Estimated genetic covariance between color pattern and host preference within each population. The probability that covariance would be greater in all five heterogeneous populations than in any of the three homogeneous populations is <.05 (see "Results" for details).

predicted pattern of larger covariance at heterogeneous sites is strongly supported.

Second, the difference in host preference between offspring derived from striped versus unstriped parents varied significantly between the two types of sample sites, such that this difference was greater in populations from heterogeneous sites (parental morph × sample site type in-

teraction, $F = 15.32$, df = 1, 64, $P < .001$). These data are shown in figure 1.

Third, when all eight populations are considered, the genetic covariance is positively correlated with our quantitative measure of site heterogeneity (Spearman nonparametric correlation $\rho = 0.952$, $P < .001$, one-tailed probabilities due to the a priori expectation of a positive association). The pattern is shown in figure 2*A*. This association is suggestive but not significant when only the five populations from heterogeneous sites are considered ($\rho = 0.791$, $P = .056$ using only these five populations). The same association with the quantitative index of site heterogeneity was observed for the phenotypic covariance between color pattern and host preference. Consistent with greater phenotypic covariance in more heterogeneous sites, the difference between morphs in host preference was positively correlated our quantitative measure of site heterogeneity ($\rho = 0.67$, $P < .05$ for all seven populations; $\rho = 0.87$, $P < .05$ using only the five populations from heterogeneous sites). These associations are depicted in figure 2*B*.

Although the qualitative agreement between the data and the theory is good, the quantitative agreement is not. The estimates for the genetic covariance are all much larger than our model predicts (table 1). We return to this discrepancy in "Discussion."

Genetic Variances

We detected significant genetic variation in host preference. Combining data across sites, the bootstrap method estimates heritability as $h^2 = 0.20$ (SE = 0.07) and the genetic variance as 0.041 (SE = 0.013); these estimates are significantly >0 ($P < .002$). Likewise, color pattern is genetically variable, although this was already known from data on phenotypic variation and the simple inheritance of this trait.

Estimates of the genetic variances for both traits for each individual population are shown in table 1. In contrast to the results for the genetic covariance, there is no evidence for a systematic association between habitat heterogeneity and the additive genetic variance for either host preference or color pattern.

Discussion

We detected broad-sense genetic covariance between color pattern and host preference within populations of walking stick insects. This genetic covariance was greater in habitats where migration occurs between genetically divergent populations. Moreover, this pattern does not appear to solely reflect levels of genetic variation, because genetic variance did not vary systematically with habitat heterogeneity.
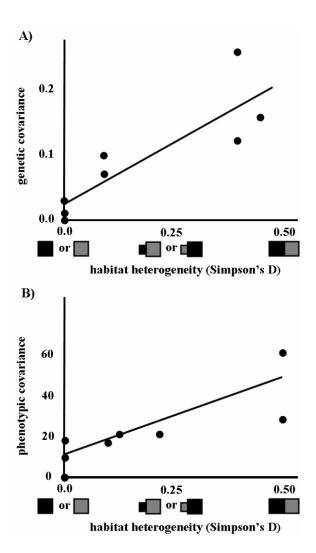
Figure 2: A positive association was detected between site heterogeneity and the covariance between color pattern and host preference. Site heterogeneity was estimated using Simpson's D, where maximal heterogeneity is achieved when each of the two host species occurs at roughly equal proportions (depicted by boxes below the Y-axis). *A*, Genetic covariance estimated from the quantitative genetic experiment. *B*, Phenotypic covariance, depicted here as the difference in host preference between morphs collected in the wild (unstriped minus striped preference for *Ceanothus*).

Even if migration did affect covariance by affecting levels of genetic variance, variability in covariance is nonetheless explained by migration regimes. These findings suggest that genetic covariance can evolve in response to migration and is therefore not only determined by underlying developmental mechanisms.

Genetic covariance caused by linkage disequilibrium between physically unlinked loci is often considered to be transitory, because recombination can erode it. Thus, genetic covariance between traits affected by unlinked loci might have a weaker effect on future evolution than genetic covariance facilitated by pleiotropy or physical linkage (Hawthorne and Via 2001). However, genetic covariance can persist even between traits affected by physically unlinked loci, so long as migration between genetically differentiated populations occurs (Nei and Li 1973; model used in this study). The degree to which migration and pleiotropy are mutually exclusive mechanisms for the generation of genetic covariance is an interesting remaining question, because it might influence the longer-term evolutionary consequences of genetic covariance generated by migration. Could covariance generated by migration affect whether genetic covariance due to other causes arises? We do not claim that migration is the only factor explaining variable covariance, and our results do not preclude a role for other factors, such as variation in pleiotropy and physical linkage (Hawthorne and Via 2001). Rather, our results show that migration itself can play an important role in the evolution of genetic covariance.

Population divergence is required for migration to generate genetic covariance. Our results also demonstrate that divergent selection can play an integral role in the generation of genetic covariance by causing the required population divergence. For example, both the traits examined in the current study diverge between populations due to selection (Sandoval 1994a, 1994b; Nosil 2004). Moreover, the greatest covariance was observed when both hosts were equally common. This indicates that greater genetic covariance was generated when selection could best overcome the homogenizing effects of asymmetric gene flow to cause greater population divergence.

Irrespective of its underlying cause, an important consequence of the increased genetic covariance is that it will cause a greater correlated response to selection in heterogeneous than in homogeneous habitats. This is because the change in the mean of an indirectly selected trait is the product of the selection gradient on the directly selected trait and the genetic covariance between the two (Lande 1979). Thus, direct selection on color pattern, for example, is expected to cause a greater change in the mean habitat preference when genetic covariance is high (as in heterogeneous sites) than when it is low (as in homogeneous sites). Thus, our findings have implications for understanding the evolution of host preference in this system: host preferences can evolve as a correlated response to selection acting on color pattern in heterogeneous habitats but not in homogeneous sites (for detailed consideration of divergence in mean host preference among populations, see Nosil et al. 2005).

The host preferences detected in this study were atypical in that they were relative, not absolute. When offspring derived from different morphs differ in preference (i.e., in heterogeneous sites), offspring from unstriped individuals

strongly prefer to rest on *Ceanothus*, whereas offspring from striped individuals tend to exhibit no preference. Consideration of the biology of this system, in particular the relative fitness of the four different combinations of color pattern and preference, yields a potential explanation. The striped morph has higher survival on *Adenostoma*, and the unstriped morph has higher survival on *Ceanothus* (Sandoval 1994a, 1994b; Nosil 2004), but both morphs have higher fecundity on *Ceanothus* (Sandoval and Nosil 2005). Thus, the genotypic combination of unstriped with preference for *Ceanothus* will have particularly high fitness because it will accrue both high survival and high fecundity. Conversely, the combination of unstriped with preference for *Adenostoma* will suffer both low survival and low fecundity. Thus, the former genotypic combination should be much more highly represented in nature such that most unstriped individuals prefer *Ceanothus* (as observed). The picture is somewhat different for the host preference of striped individuals, where both genotypic combinations might accrue similar fitness; striped with preference for *Ceanothus* will gain high fecundity, whereas striped with preference for *Adenostoma* will gain high survival. This similarity in "total" fitness could result in both genotypic combinations being maintained in populations from heterogeneous sites such that only half of striped individuals prefer *Adenostoma* (as observed).

What maintains genetic variance in homogeneous patches, where ongoing migration cannot help maintain variation? Several hypotheses can be proposed. For color pattern, visual predation may be frequency dependent such that it maintains variation (Bond and Kamil 2002). Additionally, color (95% of individuals are green) and stripe are controlled by separate loci such that the stripe allele is hidden from selection when found in the red/gray individuals (which occur at about 5% and do not express the stripe; Sandoval 1993). For host preference, evolution away from an ancestral preference for *Ceanothus* (the likely ancestral host) may be ongoing (Crespi and Sandoval 2000). Thus, allopatric populations of *Ceanothus* may have simply retained the ancestral preference, whereas allopatric *Adenostoma* populations are in the process of evolving away from it (and thus do not exhibit a strong preference for their native host). Finally, rare or episodic gene flow between currently allopatric populations could introduce variation in both traits.

One additional consideration is maternal effects, because the parents used in the genetic crosses were initially captured in the field. For example, offspring derived from unstriped parents might be more likely to prefer *Ceanothus* than offspring from striped parents because unstriped parents were more likely to have experienced *Ceanothus* (with this environmental effect then influencing offspring preference). Several lines of reasoning indicate that such maternal effects are extremely unlikely to account for our results: first, all the parents were reared from first instar to sexual maturity in a common environment; second, in heterogeneous sites, the differences in preference among offspring from different parental morphs occurred when the different morphs originated from the same host species; and third, a separate reciprocal-rearing experiment has shown that rearing environment (host reared upon from first instar to sexual maturity) has no effect on host preference, whereas genotype (host or population of origin) has a strong effect (Nosil et al. 2005). Thus, the covariance detected in this study most likely has a strong genetic basis.

Our estimates of the genetic covariance between color pattern and habitat preference are all substantially larger than the values predicted by our model (table 1). There are three obvious explanations for this discrepancy. First, and perhaps most important, the breeding experiment used assortative mating based on color pattern (this method was required to generate enough crosses), which upwardly biases the estimates (Lynch and Walsh 1998). Because of dominance at the color pattern locus, we cannot correct for this bias. But we expect this bias to work against the association between habitat heterogeneity and the size of genetic covariance (see "Methods"). Consequently, this bias is conservative with respect to the hypothesis we are testing. A second factor that would cause the covariance to be larger than the predicted values shown in table 1 is physical linkage between the color pattern and host preference loci. A third possibility is assortative mating by color pattern within the natural populations from which the insects used in the genetic experiment were derived (Nosil et al. 2002).

Genetic covariance will not evolve by the selection/migration mechanism described in our study when divergent selection does not cause population divergence and when the geographic arrangement of hosts or substrates results in low opportunity for migration between genetically divergent populations. We note that a Web of Science search using the terms "migration," "genetic," and "covariance" did not recover any studies examining both migration regimes and the genetic covariance between preference and performance. Likewise, a Web of Science search using "migration" and "genetic correlation" did not recover any such studies. Our results clearly suggest that future studies should consider the effects of habitat structure and migration on variation in genetic covariance.

Two factors make it difficult to assess systematically whether variation in migration has contributed to the discordant results from previous studies of genetic covariance between preferences and performance. First, most previous studies examined only a single population or pooled estimates among populations or subpopulations (Via 1986;

Singer et al. 1988; Thompson 1988); thus, variation within studies cannot be evaluated. Second, estimates of covariance in populations that use only a single host are almost completely lacking, precluding even an among-study comparison of covariance in studies where migration between hosts does versus does not occur (i.e., only the former tend to exist). As documented here, habitat heterogeneity and levels of migration can still differ among populations that are in geographic contact, although again this has not been examined previously.

Via (1986) and Singer et al. (1988) provided the first demonstrations of strong genetic covariance between preference and performance. They studied areas where migration between hosts could occur, and so migration may have contributed to the covariance they found. In both studies, there was the potential for variation in migration rates at different sites. Via (1986) examined covariance in aphids sampled from tomato and pea fields that were close together and in an isolated field. Singer et al. (1988) noted the presence of both pure and mixed patches of the two host plants for their butterflies. In both studies, results from these different kinds of sites are pooled. It would be of interest to reanalyze the data to look for the association between migration and genetic covariance that we report here.

Two studies of butterflies found variation in genetic covariance that are suggestive of effects of selection and migration on genetic covariance. Bossart (2003) detected greater covariance in some instances in polyphagous populations than in monophagous populations. Forister (2004) reported variation in covariance between preference and performance among multiple populations of butterflies. Although a clear association with the opportunity for migration (i.e., geographical allopatry vs. sympatry) was not evident, preference/performance correlations did appear to reflect different levels of local adaptation. For example, groups having undergone recent host shifts exhibited weaker associations.

Variable genetic covariances also have several implications for understanding speciation. This is particularly the case for traits such as performance and habitat preference, which can cause reproductive isolation (Diehl and Bush 1989; Funk 1998; Hawthorne and Via 2001; Funk et al. 2002; Coyne and Orr 2004). Although speciation is generally thought to occur between geographically separated populations (Coyne and Orr 2004), a general feature of models of speciation without geographic isolation is the evolution of genetic covariance between traits under selection and traits conferring reproductive isolation (Felsenstein 1981; Kirkpatrick et al. 2002; Kawecki 2004). Thus, migration can have dual effects. Although its homogenizing effects may generally prevent population differentiation and thus speciation (Slatkin 1987; Coyne and

Orr 2004), migration might affect speciation and evolutionary divergence by generating genetic covariance.

In conclusion, predicting evolutionary change requires understanding not only how selection acts on traits but also how other evolutionary forces affect the genetic properties of populations (Turelli 1988; Arnold 1992; Roff 2000). Habitat structure may be integral to how these genetic properties evolve.

APPENDIX

Supplemental Equations

Our goal is to calculate the genetic covariance G_{CH} that develops between color pattern and host preference as the result of migration between two populations. We assume these two traits are controlled by separate sets of diploid autosomal loci (there is no pleiotropy) that are expressed equally in males and females. We assign striped individuals a phenotypic score of $c = 0$ and unstriped individuals a score of $c = 1$. The frequency of unstriped individuals in the population is denoted $\bar{c}$. We assume coloration is controlled by a single locus C, with allele 1 for unstriped coloration dominant over the allele 0 for striped. Genetic variation in host preference is caused by an arbitrary number of loci; we assume they show no dominance or epistasis. Each of these loci has two alleles, also denoted 0 and 1, and different loci may make unequal contributions to host preference. An individual's host preference, denoted h, is defined as the probability that an individual will chose *Ceanothus*, and the frequency that host is chosen across all individuals in the population is written $\bar{h}$.

Assuming that departures from Hardy-Weinberg in zygotes can be neglected (which implies that drift is weak and mating nearly random), the genetic covariance between color and host preference is

$$G_{CH} = 2b_c \sum_i b_i D_{ci}. \tag{A1}$$

With allele 1 fully dominant to allele 0 at the color

pattern locus, the additive effect b_c is equal to q_c, the frequency of allele 0 at that locus (Fisher 1918); thus, we have $b_c = q_c = (f_s)^{1/2}$, where f_s is the frequency of striped individuals. Under our assumption of no dominance at the host preference loci, the additive effect b_i is simply equal to the difference in the average host preference of heterozygotes and homozygotes at locus i.

We next find an expression for the linkage disequilibrium D_{ci}. We assume that this disequilibrium evolves to an equilibrium under migration and recombination (which implies that correlational selection acting within each host is weak or absent). Following migration, which occurs at rate m, the disequilibria between alleles at color locus C and host preference locus i that were inherited from different parents are

$$D'_{ci} = (1 - m)D_{ci} + mD^{M}_{ci}$$
$$+ m(1 - m)(p_c - p_c^{M})(p_i - p_i^{M}), \qquad (A2)$$

where superscripts M denote values among arriving migrants (eq. [36] in Kirkpatrick et al. 2002). This expression applies to both the cases where the alleles were inherited from different parents and where they were inherited from the same parent. When applied to alleles inherited from different parents, however, the two disequilibria in zygotes that appear on the right-hand side (D_{ci} and D^{M}_{ci}) are 0. When considering alleles inherited from the same parent, equation (A2) requires a value for D^{M}_{ci}, the disequilibria among immigrants. For simplicity, we will assume that it equals D_{ci}, the association among zygotes in the focal population. This would be the case, for example, at an equilibrium between two symmetric populations (i.e., of similar size and exchanging equal numbers of migrants). The within-gamete disequilibrium among zygotes at the start of the next generation is then

$$D''_{ci} = (1 - r_{ci})[D_{ci} + m(1 - m)(p_c - p_c^{M})(p_i - p_i^{M})]$$
$$+ r_{ci}m(1 - m)(p_c - p_c^{M})(p_i - p_i^{M}), \qquad (A3)$$

where r_{ci} is the recombination rate between the two loci.

At an equilibrium, the differences in allele frequencies between the populations are maintained by divergent selection pressures acting on these loci. Equation (A3) ignores the effects that selection has on the disequilibrium. These effects, however, will be negligible if migration is weak relative to recombination, $m \ll r_{ci}$ (the "quasi linkage disequilibrium" approximation; see Kirkpatrick et al. 2002). Proceeding under this assumption, the equilibrium is found by setting D''_{ci} equal to D_{ci} and solving, which gives

$$\hat{D}_{ci} \approx \frac{m(p_c - p_c^{M})(p_i - p_i^{M})}{r_{ci}}. \qquad (A4)$$

Substituting into equation (A1) produces

$$G_{CH} \approx 2m\left(\sqrt{f_s^{M}f_s} - f_s\right)\sum_i \frac{1}{r_{ci}}b_i(p_i - p_i^{M}). \qquad (A5)$$

As we expect intuitively, the genetic covariance is inversely proportional to the recombination rates between the color pattern and host preference loci.

This result simplifies under several scenarios. If the color pattern locus is not linked to any of the host preference loci ($r_{ci} = 1/2$),

$$G_{CH} \approx 2m\left(\sqrt{f_s^{M}f_s} - f_s\right)(\bar{h} - \bar{h}^{M}). \qquad (A6)$$

If there is only a single host preference locus, or if all the host preference loci have equal effects and equal allele frequencies,

$$G_{CH} \approx \frac{m\left(\sqrt{f_s^{M}f_s} - f_s\right)(\bar{h} - \bar{h}^{M})}{\tilde{r}}, \qquad (A7)$$

where $\tilde{r}$ is the harmonic mean recombination rate between the color pattern and host preference loci. This is equation (1) in the text.

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The striped morph of *Timema cristinae* resting on the host plant on which it is less cryptic (*Ceanothus spinosus*).