The Genetic Architecture of Ecological Specialization: Correlated Gene Effects on Host Use and Habitat Choice in Pea Aphids

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abstract: Genetic correlations among phenotypic characters result when two traits are influenced by the same genes or sets of genes. By reducing the degree to which traits in two environments can evolve independently (e.g., Lande 1979; Via and Lande 1985), such correlations are likely to play a central role in both the evolution of ecological specialization and in its link to speciation. For example, negative genetic correlations between fitness traits in different environments (i.e., genetic trade-offs) are thought to influence the evolution of specialization, while positive genetic correlations between performance and characters influencing assortative mating can accelerate the evolution of reproductive isolation between ecologically specialized populations. We first discuss how the genetic architecture of a suite of traits may affect the evolutionary role of genetic correlations among them and review how the mechanisms of correlations can be analyzed using quantitative trait locus (QTL) mapping. We then consider the implications of such data for understanding the evolution of specialization and its link to speciation. We illustrate this approach with a QTL analysis of key characters in two races of pea aphids that are highly specialized on different host plants and partially reproductively isolated. Our results suggest that antagonism among QTL effects on performance in the two environments leads to a genetic trade-off in this system. We also found evidence for parallel QTL effects on host-plant acceptance and fecundity on the accepted host, which could produce assortative mating. These results suggest that the genetic architecture of traits associated with host use may have played a central role in the evolution of specialization and reproductive isolation in pea aphids.

Keywords: speciation, evolution, insect-plant interactions.

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A population or species is ecologically specialized if it has characteristics that suit it to a particular habitat or resource and limit its ability to use other environments. Ecological specialization is an important cause of biological diversity in the morphological, physiological, and behavioral traits that affect resource and habitat use. Moreover, ecological specialization may play a key role in speciation (e.g., Bush 1994; Orr and Smith 1998; Schluter 1998; Schemske 2000; Via 2002). Understanding the evolutionary mechanisms that lead to ecological specialization is therefore central to deciphering the causes of both phenotypic diversity and speciation.

Ecological specialization and its potential link to speciation have received considerable attention in recent years (e.g., Futuyma and Moreno 1988; Thompson 1994; Schluter 1996, 1998). The goal of this article is to complement previous studies by addressing the role of genetic architecture in the evolution of specialization. We assert that estimating the effects of individual chromosome segments on key traits can provide new insights into ecological specialization and its role in speciation that have not been provided by traditional quantitative genetics.

Here, we present preliminary results on the genetic architecture of specialization in sympatric, highly specialized races of pea aphids. In order to evaluate the number, location, and directionality of gene effects that distinguish these specialized races and to test the mechanism of genetically based trade-offs, we performed a quantitative trait locus (QTL) mapping analysis. We also used this analysis to consider the link between specialization and speciation by probing the mechanisms of genetic correlations among characters causing specialization and assortative mating.

Trade-offs, Genetic Correlations, and Ecological Specialization

The prevailing context for thought on ecological specialization over the past two decades has been the adage "a jack-of-all-trades is master of none" (MacArthur 1972;

Futuyma and Moreno 1988). This commonsense notion implies that it is impossible to exhibit superior performance in all environments, and evolutionary biologists have argued that genetic trade-offs are a likely cause of specialized resource use (e.g., Futuyma and Moreno 1988; Via 1991*a*). A genetic trade-off can be measured experimentally as a negative genetic correlation between fitness traits in different environments by considering a trait expressed in two environments as two separate characters that are potentially genetically correlated (i.e., "fecundity in environment 1" and "fecundity in environment 2"; Falconer 1952; Via 1984, 1990; Via and Lande 1985).

Causes of Genetic Correlations

A genetic correlation reflects the shared effects of genes on two traits. Genetic correlations may be caused either by pleiotropy (multiple effects of an allele on several traits) or by linkage disequilibrium (nonrandom association of alleles at different loci affecting two traits; Falconer and MacKay 1996). Linkage disequilibrium (LD) is greatly facilitated by physical linkage, but correlations due to LD can occur even between unlinked loci in subdivided populations or under strong selection (Lande 1980; Kondrashov and Mina 1986; Lynch and Walsh 1998).

Because these causes of genetic correlations can differ in their likely evolutionary consequences, it would be useful to distinguish them. For example, LD of unlinked loci requires strong selection to be established or maintained given the eroding effects of recombination. Thus, genetic correlations caused by LD of unlinked loci are unlikely to pose long-term constraints on evolution if patterns of selection change or subdivided populations merge. In contrast, genetic correlations among traits due to pleiotropy persist without selection, although they may be shaped by natural selection acting on new mutations with variable correlational effects (Lande and Arnold 1983). Genetic correlations due to LD of unlinked loci build up during a response to selection when having particular alleles at two loci is advantageous. They are therefore unlikely to influence initial stages of evolutionary divergence although they may affect later stages of the process. In contrast, genetic correlations due to pleiotropy may often facilitate initial responses to selection or cause fundamental constraints on evolution (e.g., Lande 1979; Via and Lande 1985).

There is a third cause of genetic correlations, with intermediate evolutionary consequences: linkage disequilibrium of linked loci. Close linkage of genes that affect two different traits can lead to genetic correlations that build rapidly and may be nearly as stable as those due to pleiotropy. The rate at which LD is established and erodes depends on the rate of recombination (c), which is pro-portional to the genetic map distance (Lynch and Walsh 1998, fig. 14.6). For example, LD between two genes that are 10 centiMorgans (cM) apart (corresponding to approximately $c = 0.075$) decays by only 7.5% in one generation of recombination, in comparison to a 50% decline for unlinked loci ($c = 0.5$; Lynch and Walsh 1998, fig. 5.5 and eq. [7.15c]). After 10 generations of random mating without selection, approximately 45% of the initial LD at loci 10 cM apart remains, while LD between unlinked loci is essentially gone (reduced to 0.1% of the starting level). The conditions for establishing a genetic correlation due to LD at linked loci are correspondingly eased because the frequency of individuals with particular combinations of linked genes may increase by genetic drift or weak selection (e.g., Lande 1980) before becoming separated by recombination. Because genetic correlations due to LD of linked loci may build up rather easily and are somewhat resistant to the eroding effects of recombination, they may play a causative role in evolution more similar to that of pleiotropy than to LD of unlinked loci. Surprisingly, the positive role of physical linkage in causing genetic correlations has been largely ignored (but see Lande 1980; Kondrashov 1983).

In sum, one can think of genetic correlations caused by pleiotropy as "fundamental" genetic relationships among traits that can dramatically influence the course of evolution by natural selection (e.g., Lande 1979). Genetic correlations due to closely linked genes may be nearly as important evolutionarily. However, genetic correlations due to linkage disequilibrium between unlinked loci are more appropriately considered to be a product of evolution than a primary determinant of its rate and direction. The ability to distinguish these causes of correlation will add depth to our understanding of phenotypic evolution.

Quantitative trait locus mapping (e.g., Lander and Botstein 1989) may prove useful in understanding the mechanisms of genetic correlations because it can readily separate loci on different linkage groups from those that map to the same linkage group or chromosomal block. Quantitative trait loci can often be localized to 10–20-cM intervals (Tanksley 1993; Beavis 1997), indicating either relatively close linkage or pleiotropy, which both facilitate genetic correlations. Fine mapping can then be used to further dissect the nature of the QTL affecting multiple characters if required to test particular hypotheses.

Trade-offs and the Evolution of Specialization

Many estimates have been made of genetic correlations across environments (or their analog, genotype × environment interaction; e.g., Via 1987) to test for genetic trade-offs in specialized populations. This issue has been pursued with particular intensity in herbivorous insects,

and yet, most measured cross-environment genetic correlations have been either zero or weakly positive, suggesting no fundamental genetic constraint on generalism (review in Joshi and Thompson 1995). This surprising result has prompted various explanations and alternative hypotheses for the evolution of specialization (Bernays and Graham 1988; Rausher 1988; Jaenike 1990; Houle 1991; Fry 1993; Bjorkman et al. 1997). Recently, theoretical work suggests that genetic trade-offs are not an absolute requirement for the evolution of specialization (Fry 1996; Kawecki 1996, 1997; Whitlock 1996). However, there is little question that if fundamental genetic trade-offs in performance in different environments were present, the evolution of specialization would be facilitated. We believe that more targeted effort should be made to dissect gene effects on fitness in different environments, both to determine the mechanisms of genetic trade-offs, when present, and to reveal alternatives to trade-offs at the genetic level (e.g., genes with negative effects on fitness in seldom used environments; Kawecki 1997).

The Link between Specialization under Divergent Selection and Speciation

When do highly specialized populations go on to become separate species? Speciation can occur only between sympatric populations on different resources, when individuals preferentially mate with individuals that selected by the same resource or habitat (e.g., mate assortatively; Felsenstein 1981; Rice 1984, 1987; Diehl and Bush 1989; Kirkpatrick and Ravigné 2002). Habitat choice can be a powerful cause of assortative mating and can greatly facilitate speciation under divergent selection in different habitats (Maynard Smith 1966; Diehl and Bush 1989). However, the evolution of assortative mating, even by habitat choice, is complicated when recombination reduces the association between the choice allele and alleles for performance in each environment (Futuyma and Mayer 1980; Felsenstein 1981; Kirkpatrick and Ravigné 2002).

The negative effects of recombination on the evolution of assortative mating are aggravated by two assumptions made in most models. First, it is generally assumed that no selection occurs directly on the assortative mating or habitat choice loci (Felsenstein 1981; Diehl and Bush 1989; Johnson and Gullberg 1998). Instead, selection is assumed to act indirectly through poor performance of hybrid progeny, and it is argued that this is rarely strong enough to prevent recombination between mating loci and performance loci and the consequent loss of assortative mating (Felsenstein 1981). Second, these models assume that genes for assortative mating (or habitat choice) are independent of those for performance. Clearly, pleiotropy or close linkage of the alleles affecting assortative mating

and performance would facilitate the evolution of reproductive isolation by reducing recombination (e.g., Kondrashov and Mina 1986).

Recent work in speciation has explored the relaxation of these assumptions. For example, Rice (1984, 1987) and Rice and Salt (1990) showed that sympatric speciation is greatly facilitated when disruptive selection acts either directly on habitat choice or on another character that leads to assortative mating as a correlated effect. When the same characters that cause ecological specialization are also used as cues for mating, divergent selection can facilitate the evolution of reproductive isolation between specialized populations (Schluter 1996, 1998). Examples that appear to fit this model include stickleback fishes in postglacial lakes (feeding specialization and mating are both determined by body size and shape) and Darwin's finches (seed use is determined by bill depth, which also serves as a cue for assortative mating; reviewed in Schluter 1998; see also Podos 2001). It is likely that even a modest genetic correlation between these traits could provide some synergistic effect and facilitate the evolution of reproductive isolation.

Empirical Analysis of the Genetic Architecture of Correlations

Quantitative trait locus mapping permits study of the genetic mechanisms of trade-offs at a higher resolution than that provided by traditional composite estimates of genetic correlations or biometrical analyses of genetic architecture (e.g., Kearsey and Pooni 1996). Because the divergent pea aphid races on which we have worked have been cited as one of the few examples of trade-offs across environments (e.g., Agrawal 2000), they provide good material for an analysis of the genetic architecture of specialization.

In QTL mapping, the genetic causes of phenotypic differences between divergent individuals are localized to small chromosome segments of 10–20 cM (reviewed in Tanksley 1993; Paterson 1997). Concerning trade-offs, QTL mapping can be used to test a very clear hypothesis: Is the negative correlation in fitness across environments seen within and between pea aphid populations (Via 1991a) entirely due to linkage disequilibrium of unlinked loci? If this is true, then early fecundity in alfalfa and clover will map to different chromosomal blocks and no intervals will be found with QTL that affect fitness in both environments. In contrast, identifying chromosome segments containing QTL that affect fitness in the two environments in opposite ways provides evidence that trade-offs are due to pleiotropy or close linkage. By dissecting the genetic basis of observed genetic correlations in this way, we may be able to distinguish the fundamental causes (pleiotropy/ close linkage) from the more derived causes (linkage dis-
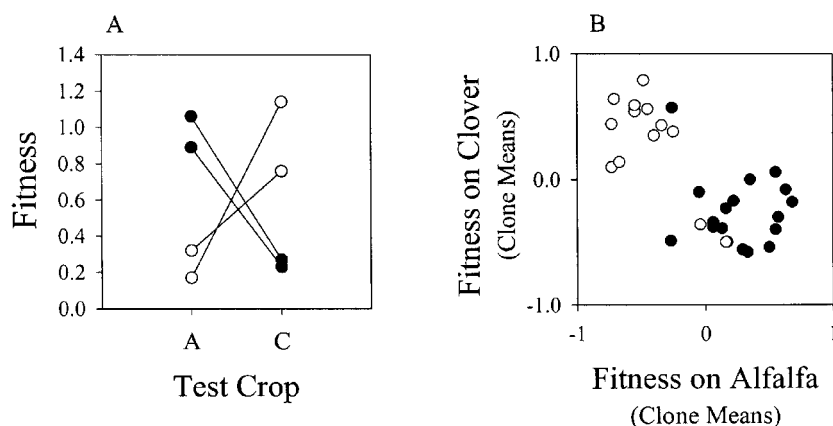
Figure 1: Performance of pea aphids collected from alfalfa and clover and field tested in a replicated reciprocal transplant. *A*, Reaction norms for two populations from each host. The same clones were tested in replicate in both environments, and the population means are the means of 10 clonal means for each population. *B*, Scatterplot of the mean fitness of each of the clones when tested on alfalfa and clover. (Modified with permission from Via 1991*a*.)

equilibrium generated by selection or population subdivision).

The model of ecological speciation under divergent selection (e.g., Schluter 1998) suggests a hypothesis about the evolution of assortative mating that can be tested using QTL mapping. If a positive correlation between habitat acceptance and performance within each habitat is caused by pleiotropic effects of the same alleles or by close linkage, then a mapping analysis would place QTL for both traits at a similar location and the QTL would be expected to affect the two traits in the same direction. This type of genetic architecture should facilitate the evolution of reproductive isolation by causing assortative mating (habitat choice) to increase as a correlated response to selection for improved performance within environments. In contrast, if QTL that influence performance and habitat choice map to entirely different locations on the genome, the genetic architecture would be less favorable for the evolution of reproductive isolation because free recombination between the loci influencing these key traits would stymie the evolution of a genetic correlation and preclude favorable correlated responses to selection.

Our hypotheses about trade-offs and the link between specialization and speciation can realistically be tested using QTL mapping techniques because they rely only on quantities that can be estimated with experiments of moderate size (general QTL location and the directionality of QTL effects). In contrast, hypotheses that rest on the number of QTL influencing a given trait or the exact magnitude of QTL effects are influenced by biases in estimating these quantities, given sample sizes that are reasonable for most

evolutionary biologists (Beavis 1997; review in Via and Hawthorne 1998).

The System

Pea aphids (*Acyrthosiphon pisum* Harris, Homoptera: Aphididae) are cyclically parthenogenetic herbivorous insects. They are known to feed on a number of legumes (Eastop 1973), with alfalfa and red clover being primary hosts in the northern tier of the United States. Pea aphids are commonly found in a dense patchwork of fields in dairy-farming areas where there are no physical barriers to movement among the hosts.

Evidence for Trade-offs

We have a long history of work on pea aphid populations in both Iowa and New York (Via 1989, 1991*a*, 1991*b*, 1994, 1999; Caillaud and Via 2000; Via et al. 2000; Hawthorne and Via 2001). This work has clearly established that closely adjacent pea aphid populations on alfalfa and clover are genetically differentiated and highly specialized on one of the two crops (fig. 1*A*). Within populations, there is generally some genetic variation among clonal genotypes in the relative use of the two plants over and above the differences between population means (e.g., fig. 1*B*).

The crossing pattern of the reaction norms for fitness in figure 1*A* is consistent with the interpretation that there is a trade-off in these populations for performance on alfalfa and red clover. The overall correlation of genetic values shown in figure 1*B* is highly negative ($r = -0.70$;

Via 1991*a*), but much of the negative correlation is due to divergence between population means and so may not have been caused by a fundamental trade-off. Instead, this between-populations component could be a result of LD caused by selection within environments and the subdivision caused by restricted gene flow. This could have been augmented by deterioration of adaptation to the alternate host if mutations expressed only in the less utilized environment have accumulated (e.g., Kawecki 1997).

A truly fundamental genetic trade-off, caused by antagonistic pleiotropy or very close physical linkage, would be seen both within populations (Futuyma and Moreno 1988) and among F_2 progeny of a cross between divergent races. Although estimates of within population genetic correlations in fitness across environments are significantly negative for these aphid populations, they are modest ($r = -0.38$ and $P < .01$ for the data in fig. 1*B*). The correlation in fitness across environments among F_2 progeny from a cross between two specialist parents has approximately the same value ($r = -0.41$, $P < .01$; Hawthorne and Via 2001). The genetic correlation within the segregating F_2 generation could be due either to pleiotropy or to linkage disequilibrium that remains after one generation of recombination. Are these negatively correlated gene effects on fitness in the two environments due to antagonistic pleiotropy at a single locus, to close linkage between loci with different effects in each environment, or to linkage disequilibrium between unlinked loci? We have used QTL mapping to address these questions.

Reproductive Isolation in Pea Aphids: The Role of Habitat Choice

Previous work has shown that the pea aphid races on alfalfa and red clover are highly reproductively isolated (Via 1999). Much of this reproductive isolation is due to significant habitat choice: approximately 90% of the migrants from each crop migrate to the same host (Via 1999). This leads to assortative mating when sexual forms are produced each autumn because mating occurs on the host. We have also shown that there is strong divergent selection on winged adults favoring accurate habitat choice (Via et al. 2000). With selection on both fitness and habitat choice and a genetic correlation between them, the evolution of reproductive isolation between these specialized races could have been facilitated through positive correlated responses to both sources of selection (see also Hawthorne and Via 2001).

Among populations, the positive genetic correlation between habitat acceptance under no-choice conditions and fecundity on the host is very high on both hosts ($r = 0.99$ on alfalfa, and $r = 0.88$ on clover; see fig. 5 in Via 1999). Within our F_2 mapping populations, the genetic

correlations between host acceptance and performance on each crop are more modest: $r = 0.24$ on alfalfa (Bonferroni-corrected $P < .008$), and $r = 0.32$ ($P < .0009$) on clover (Hawthorne and Via 2001). As before, the genetic correlation among the F_2 is the result of either pleiotropy or residual linkage disequilibrium that remains after one generation of recombination. The much higher genetic correlation between populations is again likely to be highly influenced by linkage disequilibrium between unlinked loci due to divergent selection on the partially reproductively isolated populations.

The QTL Mapping Methods

Quantitative trait locus mapping rests on the estimation of a statistical association between phenotypic variation and possession of particular chromosomal segments from one of two divergent parents (e.g., Tanksley 1993; Zeng 1995; Paterson 1997). Meiosis and crossing over within the F_1 from such a cross leads to an F_2 mapping population in which all progeny have hybrid chromosomes with potentially different combinations of the divergent parental genomes. Given a linkage map for each parental genome (or a consolidated one if possible), molecular markers are used to tag chromosome blocks from each of the parents. Genotyping the F_2 offspring for each marker reveals from which parent a given chromosome block was inherited. Variation in phenotypic traits of interest can then be statistically associated with possession of particular chromosome blocks. When such an association is found, a QTL is mapped onto that interval on the linkage map. Mapping a QTL means that individuals possessing that portion of the genome from a given parent are likely to have a phenotypic value that is significantly more like one parent than the average F_2.

Making the Cross between Specialists

In our QTL analysis of genetic architecture in pea aphids, we have taken advantage of two benefits of cyclical parthenogenesis. First, many copies of males and females may be produced from a given genotype, which permits a single pair of genotypes to be reciprocally crossed. Second, the sexually produced progeny are parthenogenetic and can be kept in clonal culture indefinitely, as can the parental genotypes. For this study, we made a reciprocal cross between two specialist genotypes. Two different F_1 genotypes from this cross were mated to form the F_2 (see Via et al. 2000 for information about genetic variation within the F_1). By making clonal replicates of the sexual forms for each F_1 genotype, we could perform replicate crosses, generating more F_2 than could be obtained from any pair of individual F_1 (194 F_2 were used here).

Sexually produced F_2 progeny were maintained as clonal lineages in the laboratory and then phenotyped with the parental genotypes. Replicated phenotypic testing increases power in the QTL analyses as approximately $\sqrt{n}$, where n is the number of replicates. We measured four replicates of each F_2 progeny, which made the power of our analysis close to the number recommended by Beavis (1997) for acceptably low bias and resolution of QTL with relatively small effects.

For each of the four replicate tests of fecundity on one of the hosts, a single individual of a given F_2 clone was confined in a small cage containing one potted plant. The next day, the adult was removed, leaving progeny that had been larviposited during the 24-h interval. These offspring (genetically identical to each other and to the adult) were checked on day 5, and one was transferred into a second cage. The age of first reproduction and fecundity during the first 9 d of adult life (fec9) of this individual were recorded. We calculated the best linear unbiased predictors (BLUPs) for early fecundity of each F_2 clone in each environment (Proc Mixed, SAS; Littel et al. 1996) and used these BLUPS in the QTL analyses as our measures of fitness.

We phenotyped the same 194 F_2 progeny genotypes for host-acceptance behavior, again in replicated trials. In each trial, four winged individuals (2 d old) of a given genotype were placed in a small enclosure with either an alfalfa or a clover plant. We observed that these individuals would fly or walk on and off the plant during the first 48 h within the enclosure, as they do in larger and more realistic flight cages (Via 1999). Previous experiments also showed that winged individuals begin to reproduce on the accepted host by about 72 h (Via 1999). We used this information to score acceptance as the percentage of individuals that were on the plant with offspring at 72 h (data were arcsine square root transformed). Again, BLUPs were calculated for the QTL analyses.

Genotyping and Construction of the Linkage Maps

The parental clones, the two F_1, and the 194 F_2 were each genotyped for 173 informative amplified fragment length polymorphism (AFLP) markers (Vos et al. 1995). The AFLP are dominant markers, which have good power for measuring recombination when linked in coupling phase but low power when linked in repulsion (Sall and Nilsson 1994). To map coupling phase markers only, we scored segregating markers in which a band is absent in one parent (e.g., homozygous recessive) and in which a band is seen in the other parent and in both F_1 as expected if the other parent is homozygous dominant. The coupling phase markers from each parent were used to make separate linkage maps (MAPMAKER/Exp. 3.0; Lander et al. 1987). Linkage was determined at LOD > 3.0 (LOD = log-odds), and the order of markers along the linkage groups was determined at LOD > 2.0. Karyotype analysis has identified four chromosomes in pea aphids (Sun and Robinson 1966), and our maps resulted in four linkage groups, suggesting that each linkage group corresponds to a chromosome.

To determine homology among linkage groups in the two separate maps, seven codominant markers were developed from cloned AFLP bands as follows. The cloned AFLP fragments were sequenced, and primers were designed to amplify the cloned fragment from genomic DNA. The resulting amplified fragments were evaluated for size polymorphism (using agarose or denaturing polyacrylamide gel electrophoresis) or sequence polymorphism (using double-stranded conformational polymorphism; Atkinson and Adams 1997). If segregating appropriately, they were then placed on the replicate linkage maps using the "try" command in MAPMAKER. Linkage groups in the two maps that share a codominant marker are considered homologous. In this way, three of the four linkage groups could be aligned and homologues of all four chromosomes identified (figs. 2–4).

The QTL Mapping Analysis of Fecundity on the Two Hosts and Acceptance of Either Host

The number and location of QTL affecting the difference between the specialized parents in fecundity in each of the two hosts and the acceptance behavior of each host were estimated using composite interval mapping in QTL Cartographer (Basten et al. 1999). Here, we treat the correlations involved in trade-offs and reproductive isolation separately in a preliminary analysis. For an integrated analysis and the complete linkage map, see Hawthorne and Via (2001).

A separate QTL analysis was done for each of four traits: fecundity in the first 9 d of adult life on alfalfa (fec9A), 9-d fecundity on clover (fec9C), acceptance of alfalfa (accA), and acceptance of clover (accC). A QTL is detected at a given location when there is a significant difference between the phenotype of individuals homozygous for that marker and that of individuals with alleles from the other parent at that location. Only those QTL with likelihood ratios significant by experimentwise permutation tests at $P < .05$ (Churchill and Doerge 1994) are considered here. Directionality of QTL effects was determined from the sign of the additive effect estimated in QTL Cartographer (Basten et al. 1999).
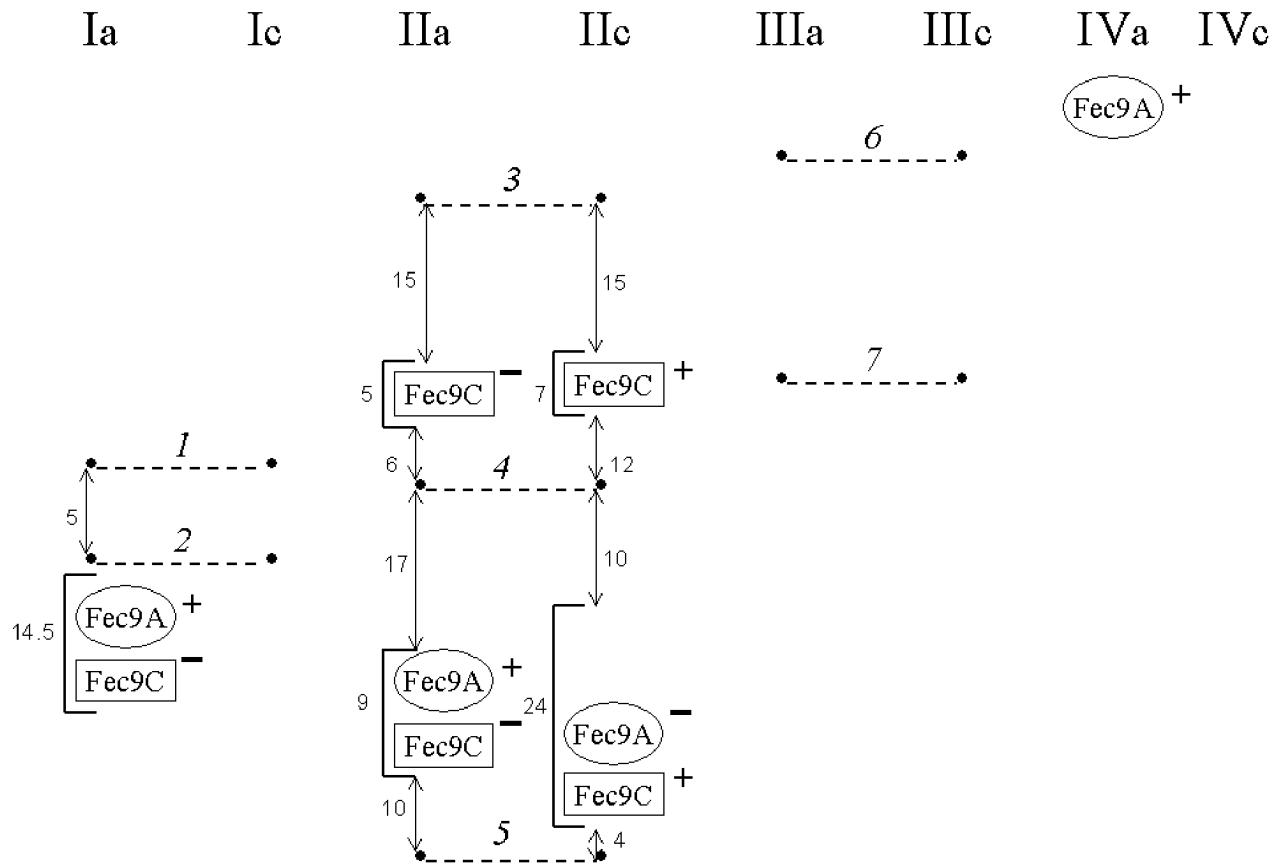
Figure 2: Partial map of pea aphid genome showing portions containing QTL for fecundity in the first 9 d of adulthood on alfalfa (*Fec9A*, *ovals*) or 9-d fecundity on clover (*Fec9C*, *rectangles*) relative to seven codominant markers. Four linkage groups from each specialist genome were mapped using dominant AFLP markers, and three of four could be aligned using the codominant markers. The race from which a given homologue was derived is indicated with a subscript. Dotted lines connect the codominant markers on each homologue. Intervals between the codominant markers containing QTL and the map distances shown were defined on linkage maps containing the full set of dominant markers that were informative (see Hawthorne and Via 2001). Symbols indicate placement of QTL that were significant at $P < .05$ by permutation test. The directionality of the effect of each QTL is indicated as a superscript plus or minus near each symbol.

Results

Genetic Architecture of Specialization

The composite interval mapping analysis revealed QTL that affect the difference between the specialists in fec9A and/or fec9C on three of the four linkage groups (fig. 2). Recall that separate maps were made for the genome of each specialist parent using these dominant markers, and QTL were mapped separately onto each genome. Here, we show the positions of QTL relative to the seven codominant markers that we used to align the two maps (numbers *1–7* correspond to the codominant markers shown on the full map in Hawthorne and Via 2001). On linkage group I*a* ("*a*" indicates the homologue from the alfalfa specialist parent), we found a pair of QTL within a 14.5-

cM interval near codominant marker *2*, one of which increases fec9A and the other of which decreases fec9C.

Linkage group II is particularly interesting because we detected pairs of QTL on both homologues that map to nearly the same position and have opposite effects on fecundity in the two environments. For example, if an individual has the section of linkage group II*a* in a 9-cM interval between codominant markers *4* and *5*, it is likely to have a pair of closely linked QTL (or one pleiotropic QTL) that increase fecundity on alfalfa and decrease fecundity on clover. Similarly, if an individual has the chromosomal block on linkage group II*c* (genome of the clover specialist) between codominant markers *4* and *5*, it is likely to have one or a pair of closely linked QTL that increase performance on clover and decrease it on alfalfa. Note that
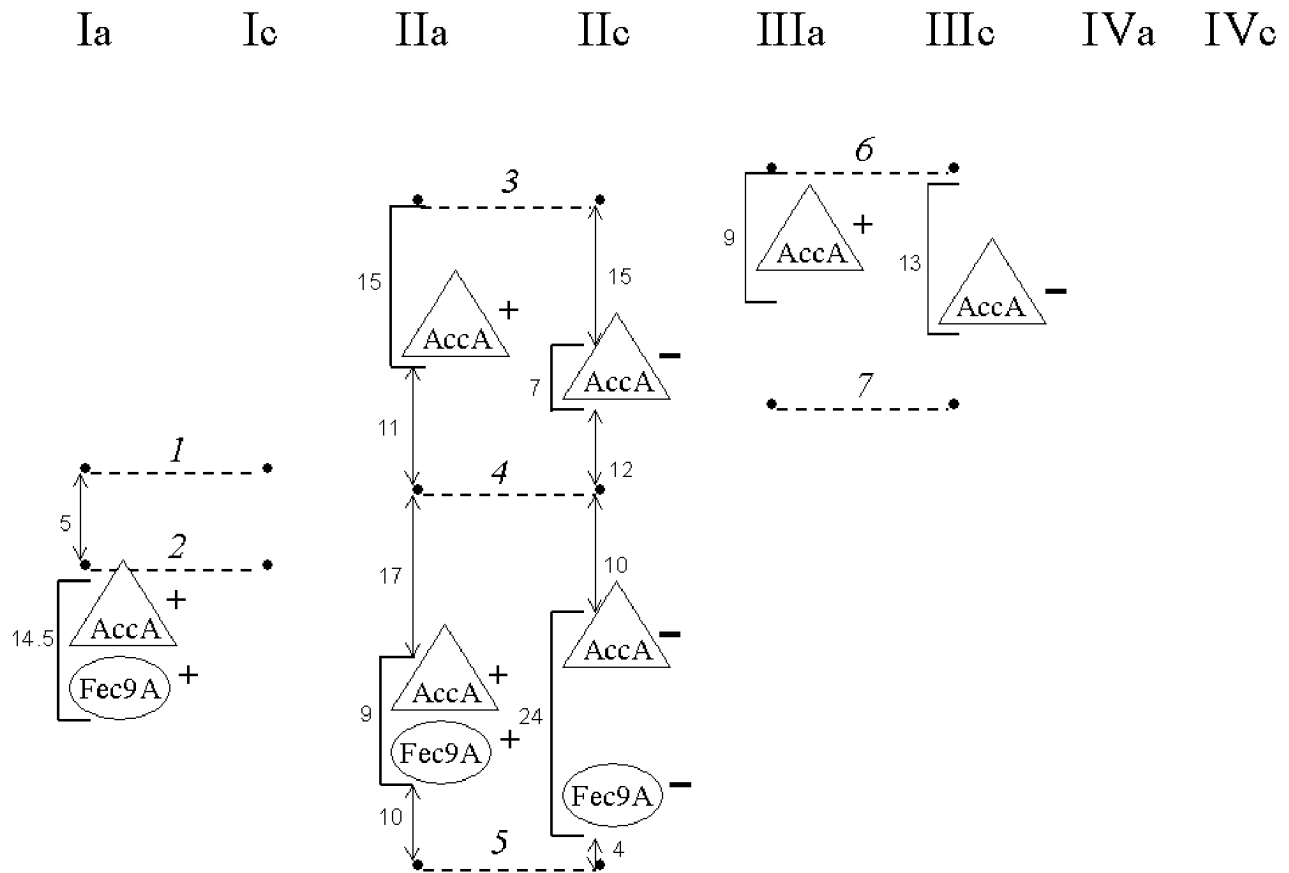
Figure 3: Map of QTL for early fecundity on alfalfa (*Fec9A*, *ovals*) and behavioral acceptance of alfalfa as host (*AccA*, *triangles*). The partial linkage map is the same as in figure 1, and a superscript plus or minus indicates the directionality of the QTL effects.

the antagonistic effects of the QTL are in opposite directions in the two genomes. Although we cannot say whether the QTL on the alfalfa and clover map are homologous, the data clearly suggest that there is a gene or a complex of genes in similar locations on linkage group II in both aphid genomes that have antagonistic effects on fitness in alfalfa and red clover. These nearby QTL with antagonistic effects on fitness in two environments are the raw material for a genetic trade-off.

We also detected several QTL on both genomes that appear to affect fecundity in only one of the environments. For example, on linkage group II*a*, we detected a QTL between codominant markers *3* and *4* that decreases fecundity on clover, but there is no QTL with an effect on fecundity on alfalfa. Linkage group II*c* contains a QTL in a similar location that increases fecundity on clover, again with no nearby QTL affecting fecundity on alfalfa. We also mapped a QTL near one end of linkage group IV*a* that increases fecundity on alfalfa but saw no corresponding QTL that affects fecundity on clover. Note that all of the

QTL that we found have positive effects on fecundity in the native environment and, when present, negative effects in the alternate environment.

Genetic Architecture of Acceptance and Fecundity on Alfalfa

The QTL for acceptance of alfalfa were found on three of the four linkage groups (fig. 3). Three of the seven QTL for acceptance of alfalfa mapped to the same interval as one for early fecundity on alfalfa (linkage group I*a*, near codominant marker *2*, and linkage groups II*a* and II*c*, between codominant markers *4* and *5*). These three QTL pairs are in the same complexes that appear to be involved in a trade-off in performance across environments (cf. locations to fig. 2). We also found two pairs of possibly homologous QTL that affect acceptance of alfalfa but do not map close to a QTL for fecundity on alfalfa (fig. 3; linkage groups II*a* and II*c*, between markers *3* and *4*, and III*a* and III*c*). Notably, all of the QTL on the alfalfa genome

increase acceptance of alfalfa, while QTL found on the clover genome decrease it. In the three cases where one or several QTL influence both acceptance and fecundity, their effects are in the same direction, which would contribute to a positive genetic correlation between these traits.

Genetic Architecture of Acceptance and Fecundity on Clover

Only one QTL was found for a difference between the specialists in behavioral acceptance of clover. This QTL both maps to the same interval as a QTL for early fecundity on clover and has the same directionality of effect (fig. 4), which would contribute to a positive genetic correlation between these traits. A fundamental positive correlation between performance and a trait leading directly to assortative mating would lead to positive feedback in correlated responses that would speed the divergence and isolation of alfalfa and clover populations (as suggested

generally by Kondrashov and Mina 1986; Rice 1987; Schluter 1998).

Discussion

Distinguishing pleiotropy or close linkage from linkage disequilibrium of unlinked loci as causes of genetic correlations may illuminate the course of past evolution as well as suggest its future trajectory under divergent selection. Here, we consider genetic correlations between traits associated with ecological specialization and those that lead to assortative mating. The partially reproductively isolated races of pea aphids on alfalfa and red clover in Iowa and New York show trade-offs across environments at the population level (Via 1991a, 1999), which makes them a good system for the investigation of the mechanisms of genetic correlation between these key characters. Using the techniques of QTL mapping, we localized genetic factors contributing to differences between the specialized parents in
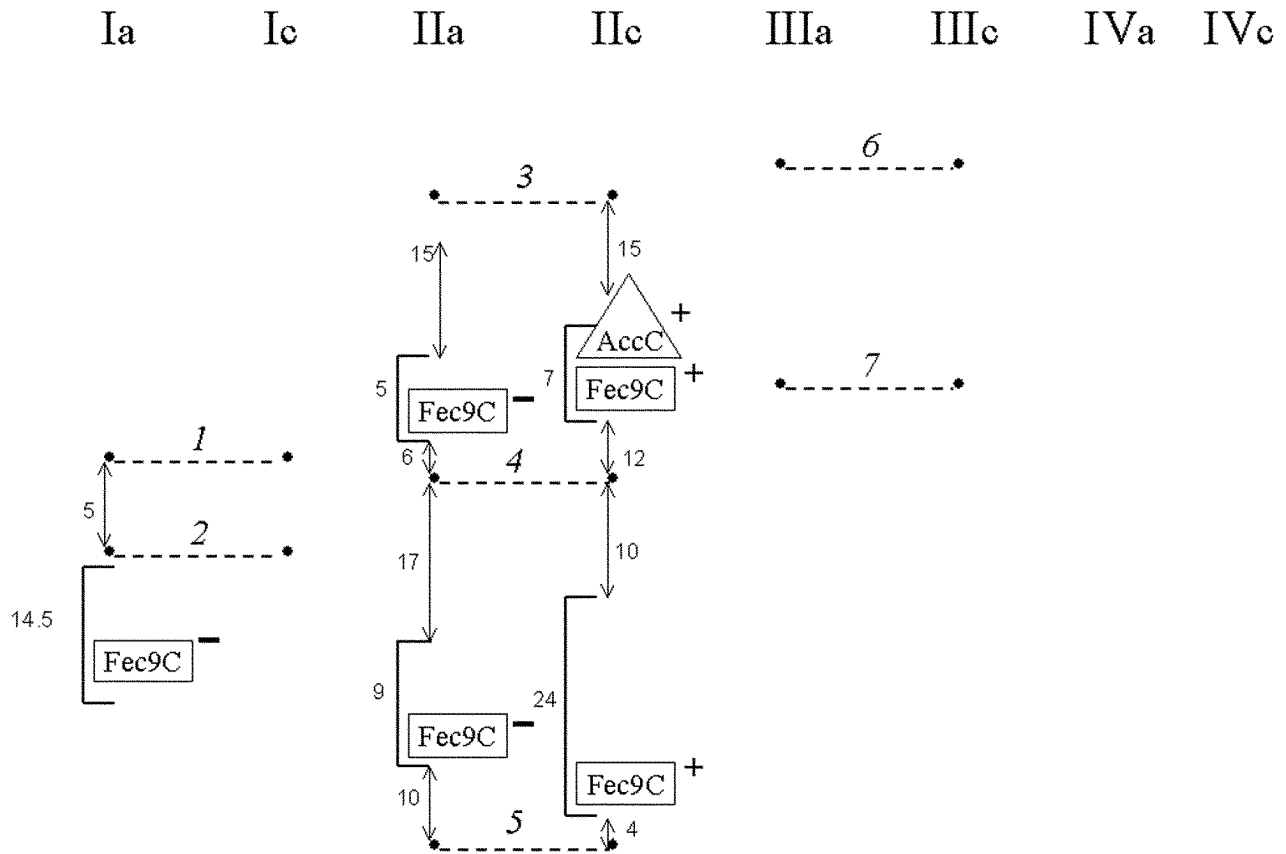

Figure 4: Map of QTL for early fecundity on clover (*Fec9C, rectangles*) and behavioral acceptance of clover as host (*AccC, triangles*). The partial linkage map is the same as in figure 1, and a superscript plus or minus indicates the directionality of the QTL effects.

these traits to chromosomal blocks of average size 9 cM. We also determined the directionality of these QTL effects.

The Genetic Architecture of Ecological Specialization in Pea Aphids

Is the ecological specialization seen in pea aphids on alfalfa and clover the result of a fundamental genetic trade-off in performance in the two environments caused by pleiotropy or close linkage? Alternatively, are entirely separate and unlinked loci responsible for the differences between the parents in fecundity on alfalfa and clover?

We found QTL for differences between the specialists in early fecundity on three of the four linkage groups, with a particularly interesting complex of correlated QTL on linkage group II (fig. 2). In this complex, QTL that have opposite effects on fecundity in the two environments were found on homologues from both parents in similar locations relative to the codominant markers. This is the type of genetic architecture that would lead to a fundamental genetic trade-off in the use of different resources, facilitating the evolution of specialization.

We also found QTL that affect differences in fecundity in only one environment (fig. 2). These QTL contribute independent (uncorrelated) genetic variation to each trait, reducing the negative genetic correlation in fitness across environments. All of the QTL that we identified have positive effects on fecundity on the native host or negative effects on fecundity in the alternate environment (i.e., QTL in chromosomal blocks from the clover genome affect fecundity on clover positively or affect fecundity on alfalfa negatively).

Our results therefore suggest a mixed mechanism for the intermediate value of the negative genetic correlation that we have observed in performance across crops within natural and F_2 populations. First, complexes of one or several closely linked genes on linkage groups I and II have antagonistic effects on early fecundity on the two crops. Then, these gene effects combine with uncorrelated variation contributed from additional loci that are expressed in only one of the two environments to moderate the trade-off across environments produced by the QTL with antagonistic fitness effects. There is no hint in our data that antagonistic gene effects across hosts are canceled out by positively correlated gene effects at other closely adjacent loci.

This mixture of correlated and uncorrelated variation across environments is consistent with results of a biometrical analysis of segregating variation for fecundity in the two environments within this F_2 population (S. Via, unpublished data). That analysis revealed a significantly negative genetic correlation of $r = -0.41$ ($P < .001$) among F_2 genotypes, which suggested that the overall correlation was a combination of negatively pleiotropic effects at one or more loci and uncorrelated variation from loci that affect fitness in only a single environment.

In light of the modest correlations within populations resulting from this mixed genetic architecture, various mechanisms could have contributed to the much higher negative genetic correlation for fecundity across the two environments that is seen between races (e.g., fig. 1*B*; Via 1991*a*). First, divergent selection to increase fitness within each environment would be expected to produce some correlated decline in mean performance in the other environment. Second, within populations, mutations might spread that increase performance in the home environment and have no effects elsewhere (Kawecki 1996). Also, as gene flow declines between races, other mutations that cause degradation of performance in the alternate environment could accumulate because they are not regularly exposed to selection (e.g., Kawecki 1997). These effects of new mutations would increase the population-level linkage disequilibrium that contributes to the trade-off between populations.

The detailed decomposition of genetic correlations within and between specialist populations into portions that have different causes may help us to formulate the next generation of hypotheses about the mechanisms that lead to the evolution of ecological specialization. For example, is there a different time course of evolutionary change at different loci? Might loci that contribute to antagonistic correlations be involved in early responses to divergent selection, with evolution of specialization refined in later stages by new genetic changes at loci expressed in only a single environment? Or perhaps uncorrelated variation is more likely to contribute early on, leaving loci with antagonistic effects to contribute later in the response to divergent selection. The identification of loci with different contributions to genetic correlations will be the first step in testing this type of hypothesis.

Evolution of Assortative Mating through Habitat Choice

Assortative mating both facilitates the evolution of specialization and causes reproductive isolation. Because pea aphids mate on their host plant, a correlation between host choice and performance on the host leads to de facto assortative mating. Within our segregating F_2 population, we found a modest positive genetic correlation between performance and our measure of host acceptance on both hosts. Our analysis provides evidence that a positive genetic correlation between host acceptance and early fecundity could be a fundamental correlation generated by QTL that either have pleiotropic effects on both traits or are linked (within 10–15 cM). The effects that such correlations could have on facilitating the evolution of re-

productive isolation have been known for some years (e.g., Kondrashov and Mina 1986; Rice 1987). However, this is the first study that has gone beyond the simple estimation of genetic correlations to the study of mechanisms by identifying chromosome segments that may be involved in the joint evolution of specialization and reproductive isolation.

Conclusions

Quantitative geneticists have often been frustrated by modest values of genetic correlations because they are not entirely consistent with simplistic evolutionary hypotheses in which characters are either perfectly positively or negatively correlated or completely independent. Although polygenic models often assume that all loci have similar effects, it may be productive to recognize that modest positive or negative genetic correlations may represent a combination of underlying mechanisms. Some loci may show pleiotropic effects, others may be closely linked and thus easily drawn into linkage disequilibrium, and still others may have completely independent effects on the characters of interest.

It will be very interesting to dissect other ecologically important genetic correlations using QTL mapping strategies in order to evaluate the generality of this mixed picture. Even more interesting would be to ask whether mixtures of loci with different kinds of gene effects and different contributions to genetic correlations open any new evolutionary possibilities that have not been considered in the simpler models with symmetrical gene effects. For example, it would be fascinating to obtain information about the time course of evolution at these different types of loci under divergent selection.

Though the data for analyses of genetic architecture are a challenge to gather, this level of detail is likely to provide truly new insights into the evolutionary mechanisms and dynamics not only of ecological specialization and speciation but of many of the biological world's most extravagant adaptations as well.

Acknowledgments

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