

EVOLUTION OF AN APHID-PARASITOID INTERACTION: VARIATION IN RESISTANCE TO PARASITISM AMONG APHID POPULATIONS SPECIALIZED ON DIFFERENT PLANTS

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Abstract.—The evolution of associations between herbivorous insects and their parasitoids is likely to be influenced by the relationship between the herbivore and its host plants. If populations of specialized herbivorous insects are structured by their host plants such that populations on different hosts are genetically differentiated, then the traits affecting insect-parasitoid interactions may exhibit an associated structure. The pea aphid (*Acyrtosiphon pisum*) is a herbivorous insect species comprised of genetically distinct groups that are specialized on different host plants (Via 1991a, 1994). Here, we examine how the genetic differentiation of pea aphid populations on different host plants affects their interaction with a parasitoid wasp, *Aphidius ervi*. We performed four experiments. (1) By exposing pea aphids from both alfalfa and clover to parasitoids from both crops, we demonstrate that pea aphid populations that are specialized on alfalfa are successfully parasitized less often than are populations specialized on clover. This difference in parasitism rate does not depend upon whether the wasps were collected from alfalfa or clover fields. (2) When we controlled for potential differences in aphid and parasitoid behavior between the two host plants and ensured that aphids were attacked, we found that pea aphids from alfalfa were still parasitized less often than pea aphids from clover. Thus, the difference in parasitism rates is not due to behavior of either aphids or wasps, but appears to be a physiologically based difference in resistance to parasitism. (3) Replicates of pea aphid clones reared on their own host plant and on a common host plant, fava bean, exhibited the same pattern of resistance as above. Thus, there do not appear to be nutritional or secondary chemical effects on the level of physiological resistance in the aphids due to feeding on clover or alfalfa, and therefore the difference in resistance on the two crops appears to be genetically based. (4) We assayed for genetic variation in resistance among individual pea aphid clones collected from clover fields and found no detectable genetic variation for resistance to parasitism within two populations sampled from clover. This is in contrast to Henter and Via's (1995) report of abundant genetic variation in resistance to this parasitoid within a pea aphid population on alfalfa. Low levels of genetic variation may be one factor that constrains the evolution of resistance to parasitism in the populations of pea aphids from clover, leading them to remain more susceptible than populations of the same species from alfalfa.

Key words.—Coevolution, evolution of resistance, herbivore, parasitoid, pea aphid, population structure, tritrophic interactions.

Received September 16, 1998. Accepted April 20, 1999.

Population structure arises from the genetic differentiation of populations due to processes such as genetic drift, non-random mating, founder effects, and differential selection. The importance of population structure in the evolution (Wright 1940, 1978; reviewed in Barton and Clark 1990) and ecology (Nicholson and Bailey 1935; Huffaker 1958; reviewed in Hastings and Harrison 1994) of species has long been recognized, but only recently has it been incorporated into research on the evolution of species interactions (Thompson 1994). The role of population structure in species interactions can be explicitly examined by evaluating the genetic basis and spatial distribution of the traits that are important in shaping species interactions. Recently, this approach has been taken in research on interactions between plants and pathogens (Parker 1985; Jarosz and Burdon 1991), hosts and parasites (Lively 1989; Ebert 1994), and hosts and parasitoids (Carton and Nappi 1991; Kester and Barbosa 1991; Kraaijeveld and Van Alphen 1995). These studies illustrate that populations are often genetically distinct from one another in ways that affect species interactions. These differences frequently are due to local adaptation in traits directly associated with the interaction such as plant resistance to path-

ogens (Parker 1985; Jarosz and Burdon 1991) or parasite adaptation to hosts (Lively 1989).

The plant-insect literature is replete with examples of herbivorous insect species comprised of populations or demes that are locally adapted to different species of plants (e.g., Via 1991a, 1994; Pellmyr and Thompson 1992; Feder et al. 1998; Itami et al. 1998; reviewed in Thompson 1994; Berenbaum and Zangerl 1998) and even different individual conspecific plants (Karban 1989; Mopper et al. 1995; Stiling and Rossi 1996; references and reviews in Mopper and Strauss 1998). The herbivorous insects described in these studies interact not only with their host plants, but also with competitors, predators, and parasitoids. These different types of interactions occur simultaneously, and herbivore population structure due to host-plant relationships may affect, or be affected by, other interactions. Currently, there is little information on whether and how the population structure of herbivorous insects specialized on different host plants shapes their interactions with other species.

Here, we report on an insect-parasitoid system in which a herbivorous insect, the pea aphid (*Acyrtosiphon pisum*, Hemiptera: Aphididae), is a species comprised of populations that are extremely host-plant specific and that are locally adapted either to red clover (*Trifolium pratense*) or to alfalfa (*Medicago sativa*; Via 1991a,b, 1994; Sandstrom 1994a,b,

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1996). Populations of pea aphids are genetically structured by their host-plant preference and are differentiated with respect to several allozyme loci (Via 1999). Pea aphids on both plants are attacked commonly by the parasitoid wasp *Aphidius ervi* (Hymenoptera: Braconidae). Pea aphids specialized on alfalfa in New York state are genetically variable in resistance to *A. ervi* (Henter and Via 1995). The resistance is physiological: Resistant aphids are attacked by the parasitoid, but the parasitoid egg does not develop successfully (Henter and Via 1995). Thus, the pea aphid is engaged simultaneously in at least two interactions that both have the potential to affect aphid fitness. In this study, we ask how the structure of the aphid populations specialized on different host plants affects the evolution of the aphid-parasitoid association.

There is a large body of research on the tritrophic interactions between plants, herbivores, and natural enemies of herbivores that provides a background for this study (reviewed in Price et al. 1980; Price 1986, 1991; Godfray 1993). This research has demonstrated that the success of a natural enemy can be affected by the plant a herbivore is feeding on in both direct and indirect ways. Direct influences include natural enemies cueing into plant volatiles to find herbivores (e.g., Read et al. 1970; reviewed in Vinson 1981; Vet and Dicke 1992) and searching and parasitism behaviors being more or less effective on plants with different growth forms (e.g., Grevstad and Klepetka 1992; Eigenbrode et al. 1995; Feder 1995; Clark and Messina 1998; reviewed in Obrycki 1986). Indirect effects are mediated through the herbivore; insects feeding on plants with different chemical or nutritional composition may be more or less available and suitable as hosts or prey (e.g., Feeny 1976, 1992; Stubblebine and Langenheim 1977; Thorpe and Barbosa 1986; Loader and Damman 1991; reviewed in Duffy et al. 1986). Thus, there are important direct and herbivore-mediated interactions between plants and natural enemies that can lead to variability in successful attack rates, and perhaps to enemy-free space (Jeffries and Lawton 1984; Bernays and Graham 1988; Berdegue et al. 1996; reviewed in Fryer 1986).

With the focus of research on the ecology of tritrophic interactions, little attention has been aimed at the potential for there to be intrinsic differences among populations of herbivores in their susceptibility to attack by natural enemies. When herbivorous insect populations are structured by their host-plant use, genetically based traits affecting other interactions (e.g., the physiological resistance to parasitism known to exist in some populations of pea aphids) may exhibit a parallel structure. Thus, we can think of the different insect populations as representing different environments for the wasp.

We describe experiments in which we assess whether pea aphids specialized on alfalfa or on red clover differ in their response to parasitism by *A. ervi*. We examine whether differences in rates of parasitism on the two plants are due to differences in behavior or physiology, and then we explore whether physiological differences in susceptibility to parasitism are induced by feeding on different host plants or are due to differences in the average levels of the genetically based resistance trait. Finally, as a first step toward examining constraints on the evolution of resistance to parasitism, we

assay genetic variation for resistance among individual pea aphid clones collected from clover fields.

MATERIALS AND METHODS

Study System

The pea aphid is a pest of herbaceous legumes. In North America, red clover and alfalfa (grown by dairy farmers for hay) are two of its main host plants. Populations of pea aphids specialized on these two crops are morphologically indistinguishable and are considered to be one species, despite their extreme host-plant specificity. Although migration is restricted between the specialized populations, there appears to be sufficient gene flow to allow neutral and advantageous traits to pass between them (Via 1999). Thus, these host-specific pea aphids are akin to the host races of *Rhagoletis* flies (e.g., Feder et al. 1998).

Pea aphids are cyclically parthenogenetic: They reproduce asexually during the summer, and sexual forms develop in the fall to produce eggs that overwinter on the host plant (Lamb and Pointing 1972). The asexual reproduction maintained by long day length makes it possible to replicate experiments with genetically identical clonal individuals. Pea aphids are native to Europe and Asia and invaded North America in the 1870s (Angalet and Fuester 1977), probably with immigrant European dairy farmers. Despite this recent introduction, there is significant genetic variation in both quantitative traits and in allozyme loci in North American populations of pea aphids (Via 1991a, 1994, 1999; Henter and Via 1995; Via and Shaw 1996).

Parasitoids are insects that are parasites of arthropods; they kill their host and have a free-living adult stage (Godfray 1993). Endoparasitoids, which develop within their hosts, tend to be relatively specialized because of the intimate physical and biochemical association with their hosts and their vulnerability to host immune responses. Insects have a simple yet effective immune system that responds to disease agents or foreign bodies such as parasitoid eggs through coagulation of hemolymph, inducible and constitutive humoral responses, and cellular responses such as phagocytosis and encapsulation (Vinson 1990). Because successful parasitoid development kills the host and host immune responses can prevent parasitoid development, hosts and their parasitoids can exert strong selection on each other. This makes them good models for studying the evolution of species interactions (Price et al. 1980; Thompson 1994; Kraaijeveld et al. 1998).

Five steps are recognized as important for parasitoid success: (1) location of the host's habitat; (2) location of individual hosts within the habitat; (3) acceptance of the host, which is influenced by size, color and chemical stimuli; (4) host suitability for development, which can be affected by environmental factors such as the nutritional and chemical attributes of the host's food source; and (5) successful physiological regulation or avoidance of host immune responses (Doutt 1959; Vinson and Iwantsch 1980). Overcoming host immunity has been called virulence in the literature on host-parasitoid coevolution (e.g., Carton and Nappi 1991; Henter 1995; Holt and Hochberg 1997; Jervis 1997; Kraaijeveld et al. 1998). Our experiments cover steps 2–5, and we discuss

them mainly from the perspective of the aphid in terms of avoiding attack and physiological resistance to parasitism.

Aphidius ervi is a solitary endoparasitoid of the pea aphid. The female wasp lays a single egg inside an aphid, and if the egg hatches, the larva feeds on the aphid's internal tissues, eventually killing it. The parasitoid pupates inside the dried exoskeleton of the dead aphid, or "mummy." *Aphidius ervi* was introduced into the northeastern United States in 1959 by the USDA from collections made from alfalfa in France to control the pea aphid (Halfhill et al. 1972; Mackauer and Campbell 1972; Angalet and Fuester 1977). Since its release, *A. ervi* has spread rapidly to become the dominant parasitoid of pea aphids in North America (Angalet and Fuester 1977) and is generally considered to be a successful control agent (Gonzalez et al. 1995).

Although the pea aphid-*A. ervi* system is introduced, it is an ecologically complex system. Members of five insect families of generalist aphid predators and numerous species of spiders are found in clover and alfalfa fields, some of them also introduced from Europe. There are other parasitoid species in the genus *Praon* that attack pea aphids. Although these parasitoids are found in most fields, rates of parasitism by these species are low (R. A. Hufbauer, unpubl.). There is also a cosmopolitan fungal pathogen of pea aphids, *Pandora neoaphidis*, which is common in both alfalfa and clover. Thus, although the introduction history may affect the current interaction between pea aphids and *A. ervi*, the study system is not biologically depauperate and it provides a good model for understanding insect-parasitoid interactions.

Pea aphids can escape attack by natural enemies by kicking and dropping from plants (Dixon 1985; Losey and Denno 1998). Another means of defense against parasitism by *A. ervi* in particular is through a genetically based physiological resistance that inhibits development of parasitoid eggs. This was documented by Henter and Via (1995) among pea aphids specialized on alfalfa. The exact mechanism of this resistance trait is unknown, and there are many possibilities (reviewed in Vinson and Iwantsch 1980; Vinson 1990; Strand and Pech 1995). Parasitoid eggs in resistant aphids were not encapsulated by host hemocytes (Henter and Via 1995), as seen in other systems (e.g., Carver and Woolcock 1985; Carton and Nappi 1991; Davies and Siva-Jothy 1991). Thus, a humoral response may be involved.

Aphidius ervi follows host plant volatiles to find pea aphids (Powel and Wright 1992; Du et al. 1996), and at close range, visual cues are used in recognition and acceptance of pea aphids as hosts (Battaglia et al. 1995). *Aphidius ervi* populations in upstate New York are genetically variable in virulence, the ability to successfully overpower pea aphid resistance (Henter 1995). Heritable variation in resistance and virulence makes coevolution of the pea aphid-parasitoid interaction possible. The presence in both the aphid and the wasp of behavioral and physiological traits that affect parasitism rates led us to ask how the extreme specialization of pea aphids on different host plants might have affected the evolution of the pea aphid-*A. ervi* interaction.

Insect Collections for Experiments

Aphids used in the experiments described below were clonally produced offspring of parthenogenetic females collected

from fields in Tompkins County, New York, in June 1995. Collections were made from two clover fields (C1, C2) and two alfalfa fields (A1, A2). The fields were paired so that C1 and A1 were within a 0.5 km of each other, and were separated from C2 and A2 by about 15 km. C2 and A2 were also within a 0.5 km of each other. A single parthenogenetic female was collected roughly every 40 paces within each field, for a total of about 30 aphids per field. Sampling from widely spaced intervals relatively early in the season (and therefore shortly after sexual reproduction) when aphid densities are low decreased the chance that individuals of a single clonal genotype would be sampled more than once. We initiated 15 clonal lineages from the individuals collected from each field, for a total of 60 separately maintained aphid clones. Pea aphids collected from clover were maintained on potted red clover plants, and those from alfalfa were maintained on potted alfalfa plants at approximately 21°C and 16:8 L:D. Cages for the clonal lineages were made from tightly sealing transparent, two-gallon plastic containers, which are modified to allow ventilation. Rigorous protocols for maintenance of the colonies were followed to ensure that no cross-contamination of the clones occurred.

Aphidius ervi were collected in July 1995 both as pupae (in mummies) and as larvae (in parasitized immature aphids). As with the aphids, the wasps were collected roughly every 40 paces to ensure adequate representation of the genetic variability within each field. Due to unusually hot and dry weather in 1995 and high rates of hyperparasitism by the cosmopolitan ectoparasitoid *Dendrocerus carpenteri* (Hymenoptera: Megaspilidae), which attack *A. ervi* in the pupal stage, *A. ervi* were rare in the aphid source fields when we were collecting, making obtaining parasitoids for experiments extremely difficult. Despite searching exhaustively, we were not able to acquire parasitoids from A2. In the first experiment described, parasitoids from both clover fields (C1 and C2) and one alfalfa field (A1) were used. Because we found that wasp collection site did not affect rate of successful parasitism (results below), parasitoids were subsequently maintained in a mixed colony of at least 200 individuals that was initiated with 10–15 wasps from each of those three fields. The wasp colony was maintained on two aphid clones, one clover specialist and one alfalfa specialist. These clones were not used in experiments. All experiments using wasps from the colony took place within six months (roughly 12 generations).

Experimental Design and Analysis

We subjected pea aphids to parasitism in four experiments to answer the following questions: (1) Do pea aphids from clover and alfalfa differ in response to parasitism? (2) Are differences in parasitism rate due to differences in aphid or wasp behavior on the two host plants or to differences in the physiology of aphids from different crops? (3) Are differences in physiological resistance due to host-plant-induced effects on aphid physiology or to differences in average levels of the genetically based resistance trait? (4) Might low levels of genetic variation for resistance in pea aphids from clover fields contribute to the susceptibility of clover populations?

In all four experiments, we assayed parasitism rates by

forming experimental populations of the aphids from clover and alfalfa and then subjecting them to parasitism in laboratory trials. The composition of the experimental populations was somewhat different for each experiment and is described below. Aphids used in the experiments were late second- to early third-instar nymphs. When mummies formed, approximately 10 days after parasitism, the aphids were scored as either living, dead, or successfully parasitized. Experimental cages were identical to those used for clone maintenance. Assays were performed under a combination of wide-spectrum grow lights and daylight fluorescent lamps, to approximate natural light.

Wasps used in the experiments were two to four days old and were maintained with a supply of honey and water. Each naive wasp was exposed to aphids in a petri dish prior to the experiments to gain experience with parasitization. Only after a wasp had attacked at least one aphid was it included in a trial.

For all experiments, the proportion of aphids successfully parasitized was calculated as (number of mummies)/(number of mummies + living aphids). Adult wasps emerged from almost all mummies, and no differences in emergence rates from aphids collected from the two host-plant species were observed. A natural-log transformation of the proportion parasitized was employed to improve the normality of the residuals.

We analyzed the data using the mixed linear model (PROC MIXED, SAS Institute 1997), which is designed for analysis of both fixed and random effects with unbalanced data. In the mixed linear model, random effects, like the random error, are assumed to be normally distributed. The significance of a random effect is tested using a likelihood-ratio test comparing full and reduced models (a χ^2 test with one degree of freedom). The χ^2 probabilities are divided by two to obtain *P*-values because variances cannot be negative, thus the tests are one-sided (Littell et al. 1996). The significance of fixed effects is tested with approximate *F*-tests using the Satterwaite method. Thus, a consequence of using PROC MIXED is that no complex mean-square denominators are employed or reported and degrees of freedom for *F*-tests may not be integers. Means of random effects are estimated with best linear unbiased predictors (BLUPs). Means of fixed effects are estimated with estimated generalized least squares (Littell et al. 1996; SAS Institute 1997).

Experiment 1: Do Aphids from Alfalfa and Clover Differ in Response to Parasitism?

We assayed the response to parasitism by making experimental populations with the aphids from each source field and subjecting them to parasitism with wasps from both crops. The experimental aphid populations were comprised of two nymphs from each of the 15 clones from a given source field placed together in a cage on a potted plant, for a total of 30 aphids in each test cage. Because of the host-plant specificity of the aphids, the plant type used was always the same as the crop from which that set of aphid genotypes were collected. After the nymphs had settled on the test plant for 24 h, a single parasitoid was introduced into each cage, allowed to search and parasitize for 24 h, and then removed.

Wasps from fields C1, C2, and A1 were used in this experiment. When the aphids matured, parasitism was scored as described above. This assay tests for effects of aphid or wasp physiology and aphid or wasp behavior on parasitism rate. Differences in physiology or behavior could be associated with either the host-plant environment or the aphid or wasp populations.

This experiment was performed in two temporal blocks in which four to six replicates of each aphid source field were subjected to wasps from A1, and another four to six replicates were subjected to wasps from either C1 or C2. In the analysis, the crop from which the aphids were collected (aphid crop), and the crop from which the wasps were collected (wasp crop) were considered fixed effects, whereas block, aphid source field and wasp source field were considered to be random effects. Aphid source field was nested within aphid crop, and wasp source field was nested within wasp crop.

Experiment 2: Are Differences in Rates of Parasitism Due to Differences in Aphid and Wasp Behavior on the Two Host Plants?

Wasp searching behaviors and aphid behaviors such as kicking at parasitoids and dropping from the plant when attacked may differ on clover and alfalfa. We controlled for potential behavioral differences by removing the aphids from their host plants and observing the wasps attack each aphid in a petri dish. To set up the bioassay, we again formed replicated experimental populations of aphids in the laboratory to represent each source field. Each experimental population was comprised of a *single* nymph from each of the 15 clones of a source field, for a total of 15 aphids in each test group. Five aphids at a time were placed in a petri dish with a parasitoid until each was individually observed to have been attacked one or two times by the wasp. No differences in the behavior of the wasps towards the aphids or in the aphids from the two crops were observed. Previous experiments in which pea aphids were dissected after being attacked indicate that when *A. ervi* are observed to oviposit in pea aphids, they reliably insert eggs (Henter and Via 1995). A single parasitoid was used for each replicate experimental population of 15 aphids. Because the source of the wasps did not affect rates of parasitism in the first experiment (see results below), wasps for this and subsequent experiments were progeny of individuals chosen haphazardly from the mixed colony of wasps from alfalfa and clover. After aphids were parasitized, they were placed on their appropriate host plant in a cage with the rest of their test group to mature and then scored for parasitism.

Eight replicates of each of the four aphid source fields were tested in a single run of the experiment. Aphid crop was considered a fixed effect; aphid source field was considered random and was nested within aphid crop. Because wasps were taken from the mixed colony, wasp source field and wasp crop were not included in the model.

Experiment 3: Are Differences in Physiological Resistance Host-Plant Induced or Due to Differences in Average Levels of the Genetically Based Resistance Trait?

Simply feeding on a particular host plant may affect aphid physiology due to some nutritional or chemical factor in the

TABLE 1. Mixed-model ANOVA for proportion of pea aphids from alfalfa and from clover successfully parasitized in a laboratory assay for resistance to the wasp *Aphidius ervi*. Aphids were collected from two fields of alfalfa and two fields of clover to initiate clonal lineages for this experiment. Replicate experimental populations were constructed from these clones as described in the text. Wasps were collected from one alfalfa field and two clover fields. Random effects are tested with likelihood-ratio tests distributed as χ^2 with one degree of freedom, and fixed effects are evaluated with approximate *F*-tests (Littell et al. 1996).

Source	Test statistic		<i>P</i>
Fixed effects	Type III <i>F</i>	df ₁ , df ₂	
Aphid crop	20.80	1, 2.22	0.036
Wasp crop	1.29	1, 36	0.264
Aphid crop*wasp crop	0.02	1, 36	0.884
Random effects	Likelihood Ratio	df	
Block	0	1	> 0.50
Aphid field(aphid crop)	8.21	1	0.002
Wasp field(wasp crop)	0	1	> 0.50
Wasp field(crop)*aphid field(crop)	0	1	> 0.50

plants that could either increase or decrease physiological resistance. We controlled for host-plant-induced effects on aphid physiology by rearing aphids on a common host prior to exposure to parasitoids.

Because the pea aphids are so host-plant specialized, we could not raise aphids collected from clover on alfalfa and vice versa (Via 1991b). However, the aphids collected from both crops fed and grew successfully on fava bean (*Vicia fabae*). For this experiment, a subset of five pea aphid clones was chosen randomly from the 15 clones of each of the four source fields, for a total of 20 clonal lines. These clonal lines were reared individually on fava beans for five generations to remove effects of feeding on clover or alfalfa from their system. Replicates of these 20 clones were maintained simultaneously on the crop from which they had been collected. Experimental populations were comprised of 15 aphids: three second-instar nymphs from each of the five subsampled clones from a given source field. The aphids were exposed to parasitism in a petri dish, as described above, thus also controlling for potential behavioral differences associated with the three plant species. One parasitoid from the mixed wasp colony was used for each replicate experimental population of 15 aphids. After the aphids had been attacked, they were returned to their rearing plant (clover, alfalfa, or fava) to mature.

Four replicates of aphids from each field were tested on both their home plant and on fava beans in a single block. The crop from which the aphids originated (aphid crop), and whether the aphids were feeding on their natural host or on fava bean (home-away) were considered to be fixed effects; aphid source field was treated as random and was nested within aphid crop. The interaction between aphid crop and home-away was tested to determine if aphids exhibited differential susceptibility to parasitoids when feeding on fava versus their natural host.

Experiment 4: Could Low Levels of Genetic Variation for Resistance in Pea Aphids from Clover Contribute to Their Susceptibility to Parasitism?

Two results motivated this experiment: (1) in experiments 1–3 we found that aphids from clover were very susceptible to parasitism relative to aphids from alfalfa (see Results below); and (2) previous experiments had revealed significant

genetic variation for physiological resistance to parasitism within pea aphids from alfalfa (Henter and Via 1995). Thus, low levels of genetic variation for resistance among clones from clover could help explain the susceptibility of the clover populations. We assayed each of the 30 pea aphid clones established from the two clover fields for resistance in order to quantify clonal variation in resistance. Unlike the previous experiments, in this assay each experimental group of aphids represented an individual clone, rather than a genetically variable population. The experimental groups were comprised of 30 second instars from a *single* clonal lineage. Nymphs were placed on a clover plant and allowed to settle for 24 h, a parasitoid from the mixed wasp colony was introduced and allowed to search for 24 h, and then the aphids were reared to maturity.

Four replicates of each aphid clone were tested in four temporal blocks. All effects (block, aphid source field, and aphid clone nested within aphid source field) were treated as random.

RESULTS

In Experiment 1, the assay of the response to parasitism of aphids from alfalfa and clover, the crop from which we collected the aphids dramatically affected parasitism rates. Mean parasitism levels of aphids from alfalfa were less than half that of aphids from clover (mean for alfalfa = 0.36, mean for clover = 0.90; Table 1, Fig. 1). There was also a significant difference in parasitism levels between the aphid from the two alfalfa fields ($F_{1,33.4} = 14.16$, $P = 0.0006$, Fig. 1a). However the source of the wasps from different fields and crops did not significantly affect parasitism rates (Table 1, Fig. 1b), suggesting that these populations do not differ on average with respect to the virulence documented by Henter (1995). There were no significant interactions between wasp crop and aphid crop or between wasp field and aphid field, indicating that rates of parasitism are not affected by the particular combination of aphids and wasps used (Table 1). Thus, in this experiment, there is not evidence of local adaptation of the wasps to the aphids from their field of origin.

The lower rates of parasitism on aphids from alfalfa could have several causes in this experiment. It may be due to the wasps searching less effectively on alfalfa or to the aphids

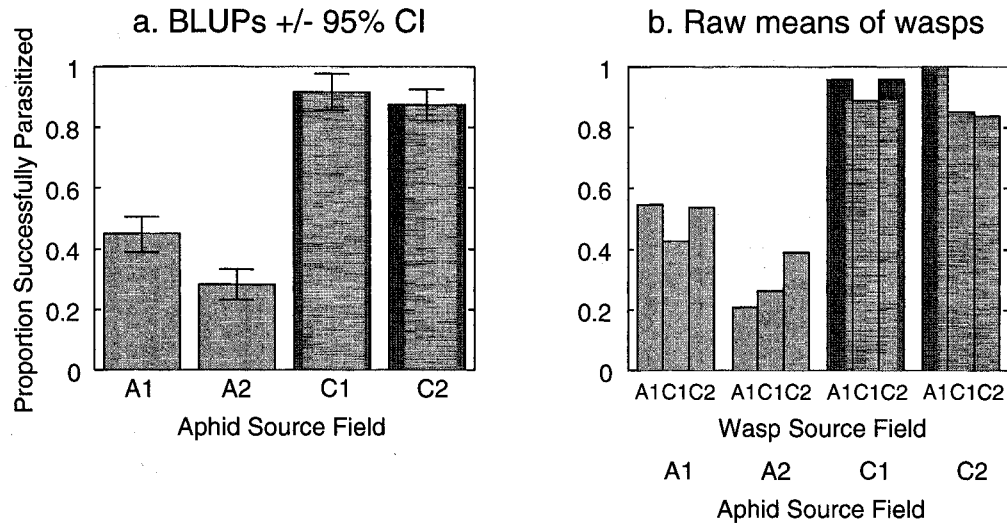


FIG. 1. Rate of successful parasitism in experimental populations of aphids from two alfalfa and two clover fields. This assay tests for resistance that could be due to behavior or physiology and that could be host-plant induced or genetically based. (a) Bars are best linear unbiased predictors (BLUPs) and 95% confidence intervals for each aphid source pooling all wasps. (b) Bars represent means from raw data for each separate wasp source. No differences among wasp sources within an aphid source are significant (Table 1).

from alfalfa being better able to escape parasitism or being more physiologically resistant to parasitism than aphids from clover.

In experiment 2, when aphids were prevented from escaping and parasitoid attack was ensured, aphids collected from alfalfa were still parasitized significantly less often than aphids from clover (Table 2, Fig. 2). The patterns in Figures 1 and 2 are remarkably similar, both in the relative differences in parasitism rates between alfalfa and clover and in the absolute levels of parasitism. This experiment indicates that the difference in parasitism rate observed in pea aphids from alfalfa and clover cannot be explained by differences in behavior of either aphids or wasps on the two host plants. Thus, the difference in parasitism rate between aphids from the two crops appears to be based on a difference in the physiology of the aphids specialized on clover and alfalfa. This difference in physiology may be due simply to feeding on the different host plants or it may be a difference in levels of the genetically based resistance trait documented by Henter and Via (1995).

Experiment 3 tested for effects of the nutritional or chemical composition of alfalfa and clover plants on the physiological ability of aphids to resist parasitism. The difference in parasitism rates between alfalfa and clover populations of

pea aphids seen in the first two experiments was unaffected by whether the aphids were reared on the plant they were collected from or on fava bean (Table 3, Fig. 3). Aphids collected from alfalfa remained significantly more resistant to parasitism than did aphids from clover whether they were raised on alfalfa or on fava beans, and aphids collected from clover remained significantly less resistant to parasitism whether they were raised on clover or on fava bean (Fig. 3). Thus, the greater physiological resistance of aphids from alfalfa appears to be due to genetic differences between the aphid populations in levels of resistance, rather than to environmentally induced nutritional or chemical effects of the host plant.

Controlling for Behavior

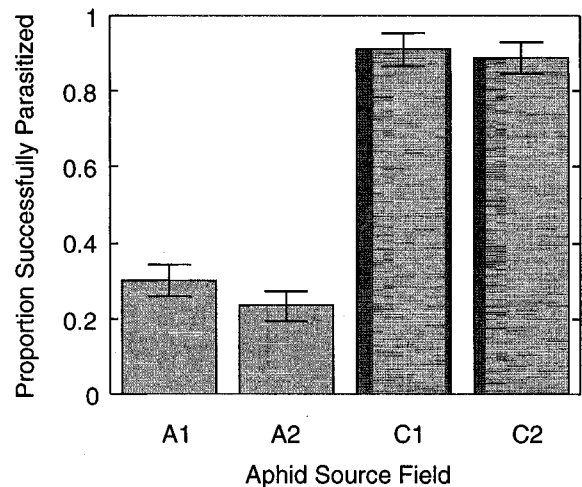


FIG. 2. Rate of successful parasitism among experimental aphid populations in an assay controlling pea aphid and parasitoid behavior and ensuring parasitoid attack. Bars represent best linear unbiased predictors (BLUPs) and 95% confidence intervals.

TABLE 2. Mixed-model ANOVA for proportion of pea aphids from alfalfa and from clover successfully parasitized by *Aphidius ervi* in a laboratory assay that prevented aphid escape behaviors and ensured wasp attack. Wasps were taken from a laboratory colony initiated from individuals collected in both alfalfa and clover fields.

Source	Effect type	Type III F (df ₁ , df ₂) or likelihood ratio (df)	P
Aphid crop	fixed	108.32 (1, 2.04)	0.008
Aphid field(aphid crop)	random	1.85 (1)	0.087

TABLE 3. Mixed-model ANOVA for proportion of pea aphids from alfalfa and from clover that were successfully parasitized by *Aphidius ervi* in a laboratory assay that controlled for host-plant-induced effects on aphid resistance by rearing aphids on a common plant (fava bean). "Home-away" indicates whether the aphids were reared on their natural host (home) or on fava bean (away) for the experiment.

Source	Test statistic		P
	Type III F	df ₁ , df ₂	
Fixed effects			
Home-away	0.36	1, 2	0.554
Aphid crop	67.89	1, 26	0.014
Aphid crop*home-away	0.14	1, 26	0.712
Random effects			
Aphid field(aphid crop)	Likelihood ratio	df	
Aphid field(aphid crop)*home-away	0.31	1	0.290
	0	1	> 0.50

In experiment 4, we evaluated levels of genetic variation for resistance among the pea aphid clones from clover. The assay of aphid clones from clover for resistance to parasitism revealed mean parasitism rates ranging from 0.60 to 0.95 with no statistically significant variance among clones (Table 4, Fig. 4a). This narrow range of parasitism rates is in sharp contrast with that found by Henter and Via (1995) for alfalfa clones, in which parasitism ranges from near zero to 0.9 among clones (Fig. 4b). Thus, at least in this experiment, there was no measurable genetic variation for resistance among aphid clones from clover populations.

Our ability to detect clonal variation in resistance depends on the magnitude of the true clonal variation relative to the error variance. It is therefore possible that we did not detect the levels of variation observed in pea aphid clones from alfalfa by Henter and Via (1995) among our clones from clover, despite having performed the same number of replicates, simply because the error variance hid true differences among clones. To assess our power to detect differences among clones, we calculated the ratio of clonal variance to error variance necessary to detect differences in resistance at least 80% of the time they actually exist (Neter et al. 1990). We found that the true clonal variation would need to be only 33% of the of the error variance in our design to detect significant differences between clones. If there had actually been clover clones with mean parasitism rates ranging from near zero to 0.9, as observed in Henter and Via (1995), the variance due to clone would have been much greater than 33% of the error variance, and we should have been able to detect the differences. Thus, levels of genetic variation for resistance in clover may be substantially lower than levels of genetic variation for resistance in alfalfa.

DISCUSSION

We have shown that pea aphids collected from alfalfa and from clover have significantly different responses to parasitism by *A. ervi*: rates of parasitism on aphids from alfalfa are about half that of aphids from clover. Parasitism rate was not affected by whether the wasps were collected from clover or alfalfa fields, suggesting that wasps from the three fields do not differ in their ability to successfully parasitize pea aphids from alfalfa and clover. Controlling for aphid defen-

Controlling for Host Plant and Behavior

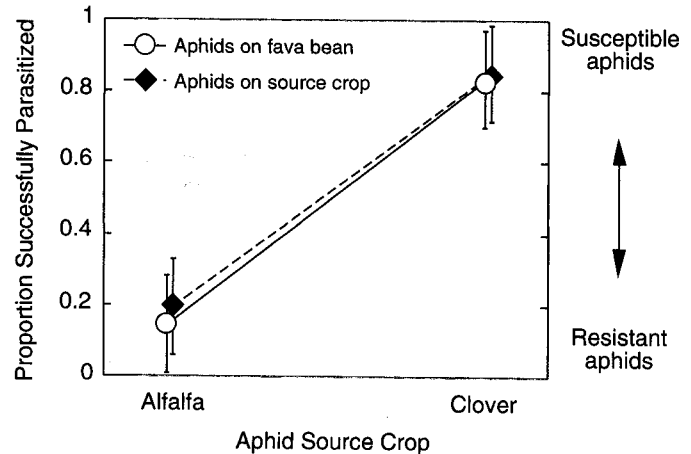


FIG. 3. Rate of successful parasitism among experimental aphid populations in an assay examining effects of feeding on different host plants. The aphid source crop represents the plant from which the aphids were collected, either alfalfa or clover. Replicate experimental populations were reared on either their source crop or on fava and then subjected to parasitism, as described in the text. Bars are least-squared means and 95% confidence intervals.

sive behaviors such as kicking and dropping from the plant when attacked and for differences in parasitoid behavior on plants of differing architecture did not alter the dramatic difference in parasitism rate documented here. Aphids from alfalfa are successfully parasitized less often than are aphids from clover regardless of their ability to exhibit defensive behaviors. This result does not imply that aphid escape behaviors are not important, but simply that such behaviors do not explain the difference in parasitism rate observed in our trials. Thus, physiological differences in resistance between aphids specialized on alfalfa and clover appear to be responsible for the lower rate of successful parasitism of pea aphids from alfalfa. Controlling for potential differences in host-plant chemistry and nutrition by rearing aphids on a common host plant (fava bean) also did not alter the difference in physiological resistance between aphids collected from alfalfa and clover. Thus, the difference in physiological resistance between aphids from alfalfa and clover is not induced by the host plant, but instead appears to be a difference in the average level of the genetically based resistance trait previously documented by Henter and Via (1995). However, it is possible that pea aphids specialized on alfalfa have evolved the ability to utilize secondary plant metabolites from alfalfa to defend themselves from wasps, and that this

TABLE 4. Mixed-model ANOVA for the proportion of individuals from 30 different pea aphid clones from clover that were successfully parasitized in a laboratory assay. Fifteen clones from each of two clover fields were tested in four temporal blocks.

Source	Effect type	Likelihood ratio (df)	P
Block	random	0.48 (1)	0.244
Aphid field	random	0 (0)	> 0.5
Clone(aphid field)	random	0 (1)	> 0.5

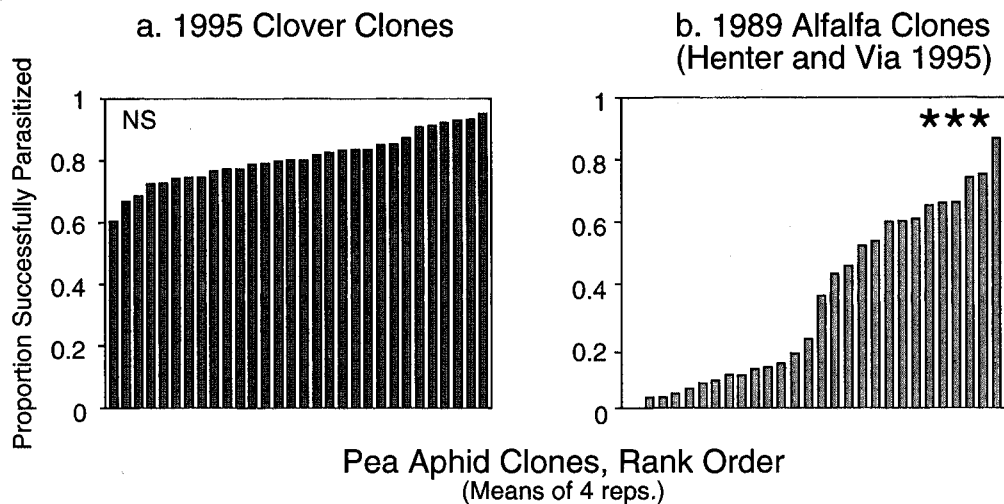


FIG. 4. Rate of successful parasitism among aphid clones. (a) Thirty clones from clover graphed in rank order. Means are from raw data (the mixed procedure cannot calculate best linear unbiased predictors (BLUPs) for random effects that are not statistically significant). See Table 4 for statistical analysis. (b) For comparison, 30 clones from alfalfa graphed in rank order. Data are least-squared means from Henter and Via (1995, reproduced with permission). NS, not significant; *** $P < 0.001$.

ability can be used when feeding on fava bean, whereas pea aphids specialized on clover lack that ability. In this way, host-plant chemistry could interact with genetics and thereby still play a role in determining resistance levels. Similarly, the ability of pea aphid clones to feed on clover may be somehow physiologically or genetically correlated with susceptibility to parasitism.

Most research in tritrophic systems has focused on ecological effects of the host plant on the third trophic level. However, in our system, differences in rates of parasitism are due not to insect behavior or interactions between the plants and the parasitoids, but to genetic differences in resistance to parasitism among the herbivore populations. Thus, when differences in rates of parasitism are observed on different host plants, they cannot necessarily be attributed to plant-parasitoid interactions, either direct or mediated through the herbivore. More research on the genetic basis and mechanisms of resistance traits and their distribution among populations will indicate how often genetic differences among herbivore populations play a role in shaping the ecology and evolution of herbivore-parasitoid interactions.

The Evolution of the Aphid-Parasitoid Association: The Aphids

We have shown here that, on average, pea aphids from clover are much less resistant to parasitoids than are pea aphids from alfalfa. Although many factors might play a role in the evolution of resistance, one fundamental prerequisite to any evolutionary response to selection is the presence of genetic variation. Bradshaw (1991) argues persuasively that evolution is commonly limited by a lack of appropriate genetic variation. Thus, very low levels of genetic variation for resistance could slow the evolutionary response of the clover populations to selection for increased resistance.

Our assay of parasitism rates in 30 clones collected from clover failed to reveal significant genetic variation for resistance. However, Henter and Via (1995) did find significant

genetic variation for resistance among 30 pea aphid clones collected from alfalfa fields. Power tests indicate that we would have been able to detect differences of the magnitude observed in alfalfa had they existed in clover. Thus, our data suggest that there is less genetic variation for resistance in aphids specialized on clover than aphids specialized on alfalfa. However, despite using similar protocols and levels of replication, there were differences between the two experiments that could not be avoided, such as the individuals performing the trials. Another difference that might account for the difference in our results is the fact that we collected our parasitoids six years after Henter and Via. Thus, the parasitoids in our experimental colony may have been more virulent than those in Henter and Via's colony, leading to our higher rates of parasitism overall. The fact that our parasitoids were not particularly virulent on the pea aphids from alfalfa in the other experimental trials argues against this; nonetheless, any comparison of our results with Henter and Via's (1995) results must be made with caution. Pea aphid clones from both clover and alfalfa need to be assayed simultaneously to test conclusively the hypothesis that there is less variation for resistance among clones from clover. However, our data strongly suggest that lower levels of genetic variation for resistance among pea aphids from clover could be one factor that constrains the evolution of increased resistance to parasitism, causing pea aphids from clover to remain more susceptible than are populations of pea aphids from alfalfa.

With respect to other quantitative genetic traits (e.g., body size and host-plant preference; Via 1991a; Via and Shaw 1996) and variation in allozyme loci (Via 1999), populations of pea aphids from clover are as variable as populations from alfalfa. Thus, low levels of variation for resistance is not associated with low levels of genetic variation among clover clones in general. Furthermore, although migration of aphids between crops is quite restricted, enough gene flow appears to occur among the aphid populations for genes that are ad-

vantageous in both environments to be exchanged (Via 1999). Thus, it seems possible that the resistance found in alfalfa might introgress into clover populations and provide the necessary genetic variation upon which selection could act. Given this, it is somewhat baffling that resistance does not appear to have moved more freely from populations in alfalfa to populations in clover. However, the likelihood of resistance introgressing to clover will depend on whether the loci affecting resistance to parasitism segregate independently from the loci affecting both host-plant preference and performance on the two host plants. Preference and performance are correlated in this system and are under strong divergent selection between alfalfa and clover (Via 1991a); thus, a negative genetic correlation between the ability to feed on clover and resistance would help explain the observed pattern.

*The Evolution of the Aphid-Parasitoid Association:
The Wasps*

The large difference in resistance to parasitism by *A. ervi* between aphids from alfalfa and clover means that the success rate of wasps attacking aphids from alfalfa is low relative to wasps attacking aphids from clover in our assays. In the field, parasitism rates are also lower in alfalfa than in clover (R. A. Hufbauer, unpubl.). Thus, there is the potential for strong selection against wasps attacking the more resistant aphids from alfalfa. Such selection might be expected to lead to the evolution of increased ability to overcome aphid resistance (i.e., virulence) among wasps on aphids from alfalfa or of increased preference for aphids from clover. Henter (1995) documented significant genetic variation in virulence on pea aphids among *A. ervi* from a single alfalfa field. This genetic variation makes the evolution of increased virulence toward aphids from alfalfa theoretically possible. However, our data on the wasps from the first experiment that included wasps from both alfalfa and clover indicate that the populations do not differ in their average ability to successfully parasitize pea aphids. These results are in accordance with subsequent experiments in which wasps from more populations were sampled (R. A. Hufbauer, unpubl.). Thus, if virulence has increased, it has not occurred in populations on alfalfa exclusive to any increases in clover. The wasps are very mobile, as indicated by their rapid spread across northeastern North America following their introduction (Angalet and Fuester 1977). Thus, the response to selection for increased virulence may be slowed by gene flow from clover fields, where selection appears to be less intense (e.g., Storfer and Sih 1998). The evolution of virulence also may be constrained by physiological costs or trade-offs with other selectively important traits not examined here, such as parasitoid resistance to hyperparasitoids.

Parasitoid wasps are known to use chemical cues from plants to find their host's habitat and their hosts (reviewed in Vinson 1981; Vet and Dicke 1992). Selection may favor individual wasps that choose clover habitats and therefore attack the more susceptible pea aphids found there. However, in our study area, pea aphid populations tend to be smaller in clover fields, and perhaps because of their susceptibility, a greater proportion of the population is parasitized (R. A. Hufbauer, unpubl.). Thus, the benefits of attacking more sus-

ceptible aphids may be countered by the greater difficulty of finding unparasitized hosts in clover.

Conclusions

We have documented significant genetic variation among populations of pea aphids specialized on different host plants in resistance to parasitism. In this tritrophic system, the genetic constitution of the herbivore has a greater effect on the probability of successful parasitism than does the host plant. Thus, a direct interaction between plant and parasitoid may not always be the cause of differences in parasitism rates on different host plants in tritrophic systems. Populations of other herbivorous insect species should be examined for genetically based differences in resistance to natural enemies to assess whether differences of the magnitude seen here are common in tritrophic systems.

ACKNOWLEDGMENTS

We thank R. B. Root, T. E. Dawson, A. P. Norton, and S. K. Remold for their useful discussions throughout the project and for improving the manuscript. The manuscript also benefited from helpful comments by C. M. Lively and two anonymous reviewers. S. D. Van Nouhuys kept our thirsty plants watered while doing her own research in the greenhouse, H. Henter provided advice on wasp rearing and protocols, and A. Bouck kept the laboratory running smoothly. Financial support for this research was provided by grants to RAH from the Andrew W. Mellon Student Research Fund, the W. Arthur and Alma D. Rawlins Graduate Student Endowment, Sigma Xi, and a National Science Foundation Graduate Fellowship and by National Science Foundation grant DEB-9207573 to SV.

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Corresponding Editor: C. Lively