

## PEA APHID–PARASITOID INTERACTIONS: HAVE PARASITOIDS ADAPTED TO DIFFERENTIAL RESISTANCE?

RUTH A. HUFBAUER<sup>1</sup>

Department of Entomology and Department of Ecology and Evolutionary Biology, Corson Hall,  
Cornell University, Ithaca, New York 14853 USA

**Abstract.** In insect host–parasitoid systems, successful immune responses of the host kill the parasitoid, and successful development of the parasitoid will kill the host. Thus, hosts and their parasitoids can exert strong selection on each other that may lead to the coevolution of host defense traits and parasitoid counter-defenses. In upstate New York, pea aphids (*Acyrtosiphon pisum*) specialized on alfalfa are, on average, two times more physiologically resistant to parasitism by *Aphidius ervi* parasitoids than pea aphids specialized on clover. There is genetic variation within populations of *Aphidius ervi* in the ability to overcome pea aphid resistance; thus an evolutionary response to the differences in aphid resistance is possible. Here, I document that pea aphid populations that are more resistant to parasitism in laboratory assays exhibit lower rates of parasitism in the field, suggesting that the genetically based differences in aphid resistance may influence rates of successful parasitism. I found that the difference in resistance between pea aphids specialized on alfalfa or on clover in New York is present in additional populations of pea aphids assayed over several years and is present in Maryland as well as New York. However, despite the potentially large difference in selective regimes due to differences in aphid resistance, parasitoids collected from alfalfa fields and clover fields do not differ in their ability to overcome pea aphid resistance. There is no evidence that they are adapted to aphids from their home crop, or locally adapted to aphids from their home field. The mobility of this parasitoid, and its relatively recent introduction to North America as a biological control agent, may contribute to the lack of adaptation.

**Key words:** *Acyrtosiphon pisum*; *Aphidius ervi*; *Braconidae*; *coevolution*; *local adaptation*; *parasitoid*; *pea aphid*; *resistance to parasitism*; *tritrophic interactions*; *virulence*.

### INTRODUCTION

Coevolution is reciprocal evolutionary change in interacting species (Janzen 1980, Thompson 1994). The potential for evolution to be reciprocal can vary among populations of interacting species because selective regimes in different locations may favor different traits. Furthermore, the degree to which populations are connected can affect the evolution and spread of traits important in species interactions (Thompson 1988, 1994, 1999, Travis 1996). Because of these complex dynamics, Thompson (1994) asserts that the coevolutionary process is likely to be more spatially and temporally variable than is apparent from either the study of individual populations or the distribution of characters found in phylogenetic trees. Thus, a more complete understanding of the evolution of species interactions in general, and coevolution in particular, must include a focus on intraspecific variation among the species involved.

Here, I explored the interaction between the pea

aphid, *Acyrtosiphon pisum* (Hemiptera: Aphididae), and its parasitoid *Aphidius ervi* (Hymenoptera: Braconidae). In this system, there is genetic variation in the physiological ability of pea aphids to resist parasitism (Henter and Via 1995), and in the ability of parasitoids to overcome that resistance (Henter 1995), fulfilling the fundamental prerequisites for coevolution. I investigated patterns of aphid resistance and parasitoid ability to overcome resistance in order to assess whether parasitoids have adapted to populations of aphids differing in resistance.

Insect host–parasitoid systems are particularly well suited to research on the evolution of species interactions (Price et al. 1980, Thompson 1994, Kraaijeveld et al. 1998). Parasitoids are parasites of other arthropods that kill their host, and have a free-living adult stage (Godfray 1993). Endoparasitoids develop within their host, and must overcome or evade the host's cellular and humoral immune responses (Vinson 1990). Because host immune responses can kill the parasitoid, and successful parasitoid development kills the host, hosts and their parasitoids can exert strong selection on each other. However, as with other host–parasite systems, selection may be asymmetrical because every parasitoid must overcome host defenses, while not all hosts will be attacked. For host–parasitoid interactions to coevolve, there must be genetic variation in hosts

Manuscript received 23 March 1999; revised 17 January 2000; accepted 25 January 2000; final version received 6 March 2000.

<sup>1</sup> Present address: Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, Colorado, 80523 USA.  
E-mail: hufbauer@lamar.colostate.edu

in their ability to escape parasitism behaviorally or physiologically, and in parasitoids in their ability to overcome host defenses.

The ability of a parasitoid to overcome physiological host defenses is called virulence in the literature on insect–parasitoid coevolutionary interactions (Carton and Nappi 1991, Godfray 1993, Henter 1995, Holt and Hochberg 1997, Jarvis 1997, Karban and English-Loeb 1997, Kawecki 1998, Kraaijeveld et al. 1998, Althoff and Thompson 1999, Hufbauer and Via 1999, Kraaijeveld and Godfray 1999, Midori and Bonsall 1999, Sasaki and Godfray 1999). This usage is somewhat problematic because a parasitoid cannot be avirulent in the sense that other types of parasites can, as the death of the host is a necessary consequence of successful parasitoid development (but see Karban and English-Loeb [1997] for a possible exception). Here, I discuss virulence mainly in terms of the ability of parasitoids to overcome host resistance.

The pea aphid (*Acyrtosiphon pisum*) feeds on herbaceous legumes, and is a pest of alfalfa (*Medicago sativa*) and red clover (*Trifolium praetense*), which are crops grown for hay by dairy farmers. The pea aphid is native to Europe and Asia, and was introduced to North America in the 1800s (Angalet and Fuester 1977). Populations of pea aphids on alfalfa and clover in Iowa and New York are specialized on one or the other crop, and are locally adapted to their preferred crop; aphids collected from one crop do not feed or reproduce successfully on the other crop (Via 1991a, b, 1994), and there is little gene flow across crops (Via 1999). This host-plant specialization has also been observed in Sweden (Sandstrom 1994).

Host-plant specialized populations of pea aphids in upstate New York differ in their susceptibility to parasitism by *A. ervi* (Hufbauer and Via 1999). In laboratory assays of pea aphids from two alfalfa and two clover fields, the pea aphids specialized on alfalfa succumbed to parasitism by *A. ervi* only 36% of the time, while those from clover were successfully parasitized 90% of the time (Hufbauer and Via 1999). This dramatic difference in parasitism rate is due to a difference in the average ability of the alfalfa and clover specialists to resist parasitism physiologically (Hufbauer and Via 1999). Although aphid and parasitoid behaviors can affect parasitism rates (e.g., Chau and Mackauer 1997, Ives et al. 1999), the observed difference in the resistance of pea aphids from alfalfa and clover is apparent even when monitoring for potential differences in the escape behavior of aphids, and in the attack behaviors of *A. ervi* on the two host plants (Hufbauer and Via 1999). *Aphidius ervi* attacks and lays eggs in resistant pea aphid genotypes, but the eggs do not develop successfully (Henter and Via 1995). The difference in physiological resistance to *A. ervi* between pea aphids specialized on alfalfa and pea aphids specialized on clover appears to have a genetic basis, and it is not

affected by the host plant upon which the aphids feed (Hufbauer and Via 1999).

While Hufbauer and Via (1999) documented that pea aphid populations from alfalfa have higher levels of physiological, genetically based resistance to parasitism by *A. ervi* than pea aphids from clover, it is unknown whether *A. ervi* populations in the two crops have evolved to differ in their ability to overcome aphid resistance. Wasps attacking resistant aphid populations in alfalfa may experience stronger selection to overcome aphid resistance than wasps attacking susceptible populations, because the variance in the success rate of wasps should be higher within populations in which aphids are more resistant. The difference in resistance between aphid populations might therefore lead to the evolution of differences among wasp populations in their ability to overcome aphid resistance. Wasps could become adapted to one or the other host-plant-specialized aphid type; for example, wasps from alfalfa fields may evolve more effective counter-defenses against the resistance found in aphids on alfalfa than wasps from clover fields. Also, wasps could become locally adapted to their particular local population of aphid hosts.

*Aphidius ervi* was introduced to North America in 1959 for biological control, and it spread rapidly across North America (Angalet and Fuester 1977). Thus, although there are no data on gene flow among wasp populations, their rapid spread suggests that they are quite mobile. High levels of gene flow could constrain the evolution of adaptation to the different populations of pea aphids. However, local adaptation can evolve even in mobile organisms if selection is strong and consistent (e.g., Mopper et al. 1995, Dybdahl and Lively 1996, Stiling and Rossi 1998). Hufbauer and Via (1999) explored the mechanism of the difference in parasitism rates of aphids from alfalfa and clover, but they assayed only two fields of each crop. Particularly if the parasitoids are mobile, they are only likely to experience a consistent selective regime that could lead to wasp adaptation to the aphid populations in the two crops if the difference in resistance of the aphids is widespread.

In this paper, I address three main questions: (1) Do parasitoids experience consistent differences in levels of aphid resistance in alfalfa and clover fields that could select for different levels of virulence? (2) Are parasitoids adapted to the aphids from their home crop? (3) Are parasitoids locally adapted to aphids from their home field? The first question centers on aphid resistance, and I addressed it in two ways. I measured rates of parasitism in natural populations of pea aphids on alfalfa and clover to examine whether rates of parasitism in the field reflect the pattern of resistance documented in the laboratory. Also, I assayed resistance levels of additional populations of aphids from alfalfa and clover in two reciprocal transplant experiments performed in the laboratory. The second and third ques-

tions center on interactions between aphid resistance and the parasitoid's ability to overcome resistance. I addressed these questions using the same two reciprocal transplant experiments that elucidate levels of aphid resistance.

#### MATERIALS AND METHODS

##### *Study system*

Pea aphids are cyclically parthenogenetic: They reproduce asexually during the summer, passing through 8–10 clonal generations in temperate areas. Sexual forms develop in the fall, and produce eggs that overwinter on the host plant (Lamb and Pointing 1972). Aphid parthenogenesis is apomictic; meiosis and recombination are suppressed and development proceeds through mitosis (Blackman 1980, 1987, Hales et al. 1997). In pea aphids, asexual reproduction is maintained by long photoperiods, which makes it possible to replicate experiments with clonal individuals. Individuals from the same clone are likely to be genetically identical; however, mutations during replication may lead to some genetic variation within long-term clonal lines (e.g., Lushai et al. 1998).

*Aphidius ervi* is a solitary endoparasitoid of the pea aphid: The female wasp lays a single egg inside an aphid, the egg hatches, and the larva feeds on the aphid's internal tissues, eventually killing it. The parasitoid pupates inside the dried and hardened exoskeleton of the dead aphid, or mummy. Adults of *A. ervi* feed on nectar and aphid honeydew, but do not feed on their hosts. In temperate areas, *A. ervi* completes  $\approx 10$  generations per year. This parasitoid was introduced into the northeastern United States by the USDA in 1959 to control the pea aphid. It was collected from alfalfa fields in France for release (Halfhill et al. 1972, Mackauer and Campbell 1972, Angalet and Fuester 1977). Since *A. ervi* became established, it has spread rapidly to become the dominant parasitoid of pea aphids in North America (Angalet and Fuester 1977).

##### *Field sampling and analysis*

To estimate rates of parasitism by *A. ervi* in field populations, I reared aphids collected from the field. In 1996, I sampled three fields of each crop on active dairy farms in Tompkins County, New York,  $\approx$  every two weeks (eight times) from late May through early September. The six fields sampled include the four fields in the New York reciprocal transplant experiment described below. In 1997, I performed one-time samples of two fields of each crop in Maryland in mid-May, and in New York in mid-June. The fields sampled in Maryland were the same four fields sampled for the Maryland reciprocal transplant experiment described below, while the fields sampled in 1997 in New York were different from those sampled in 1996.

The sampling protocols were similar for all fields. Third and fourth instar aphid nymphs were collected

from 8 to 10 separate areas in each field using a beat tray. Sample locations were placed every 30–40 paces along three randomly chosen transects. When aphids were abundant, 20 or more nymphs were taken in each sample. When aphids were rare, a space of  $\approx 2$  m<sup>2</sup> was searched thoroughly for 10–12 min in each sample location before moving to the next location. On average, I collected 136 aphids per field per date with which to estimate parasitism rates. The aphids in these collections were brought back to the laboratory, and placed in cages on their appropriate host plant to mature. The proportion of the aphids sampled that became *A. ervi* mummies was recorded.

To test whether the proportion of aphids parasitized by *A. ervi* differed by crop, I used an ANOVA with crop and sample (New York 1996, New York 1997, or Maryland 1997) treated as a fixed effects (JMP Version 3.2, SAS Institute, 1997b). For fields sampled in New York in 1996, I calculated the total proportion of aphids parasitized by combining data for the entire season.

##### *Insect collections for experiments*

Aphids used in the two experiments described below were laboratory reared, clonally produced offspring of parthenogenetic females collected from Tompkins County, New York in June 1996, and from Montgomery and Prince Georges Counties, Maryland, in May 1997. Aphids were collected from two clover fields and two alfalfa fields in both states, with each alfalfa field paired with a nearby clover field. The fields comprising each pair in New York were  $<1$  km from each other, and within 16 km of the other pair. These fields were part of active dairy farms, and are different from fields in Hufbauer and Via (1999). The fields comprising each pair in Maryland were within 5 km of each other, and were  $\sim 80$  km from the other pair. The two fields in Montgomery County were from dairy farms, and the pair in Prince Georges County were at the National Agricultural Research Center in Beltsville, Maryland.

A single parthenogenetic female was collected  $\approx$  every 40 paces within each of these eight fields, for a total of  $\approx 45$  aphids per field. Sampling from widely spaced intervals early in the season decreased the chances that individuals of a single clonal genotype would be sampled more than once. Fifteen clonal lines of aphids were initiated from the individuals collected from each field for a total of 60 separately maintained clonal lines for each state. Pea aphids collected from clover were maintained as individual clones on potted red clover plants, and those from alfalfa were maintained on potted alfalfa plants. Rigorous protocols for maintenance of the clones were followed to ensure that no cross-contamination occurred. Cages were maintained at  $\approx 21^\circ\text{C}$  and 16:8 L:D.

*Aphidius ervi* were collected as larvae in parasitized immature aphids from the same eight fields from which aphids were collected, and used to found eight separate wasp colonies. I sampled from widely spaced intervals

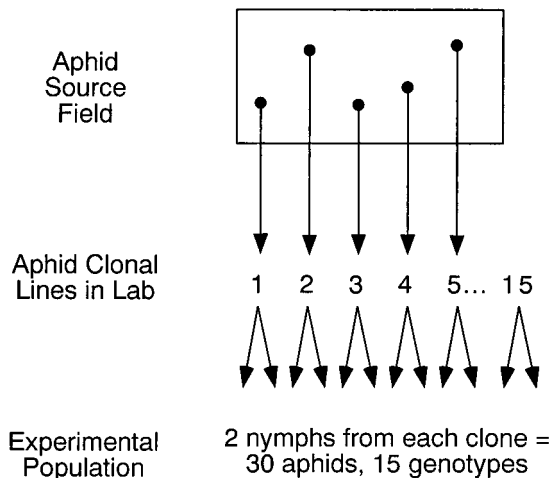


FIG. 1. Diagram illustrating the formation of experimental populations. Parthenogenetic female pea aphids were collected from the field and used to initiate clonal lineages. Experimental populations comprised two second instar nymphs from each of the 15 clones of a source field. Members of an experimental population were placed together on a potted alfalfa or potted clover plant.

in each field to maximize the genetic variation in the wasps collected. Approximately 50 individual wasps from each field founded the colonies, which were maintained at ~200 individuals. Wasps were reared on two aphid clones: one clover specialist and one alfalfa specialist. Neither of these clones was used in experiments. To minimize problems associated with wasps becoming adapted to laboratory conditions or becoming inbred, experiments were performed as quickly as possible: Experiments using wasps from the New York colonies took place within 2 mo of colony initiation ( $\approx$  four generations), and those using Maryland wasps took place within 1.5 mo of colony initiation.

#### *Experimental design and analysis*

Aphid resistance and the ability of parasitoids to overcome resistance were assayed in two reciprocal transplant experiments: One in which aphids from New York alfalfa and clover were subjected to wasps from New York alfalfa and clover, and one in which aphids from Maryland alfalfa and clover were subjected to wasps from Maryland alfalfa and clover. For these two experiments, aphids from different clonal lines were combined to form experimental populations that represented each aphid source field, and then were subjected to parasitism by wasps from each of those fields. The experimental populations consisted of 30 aphids: two second-instar pea aphids from each of the 15 clones of a source field (Fig. 1) placed together on either a potted clover or potted alfalfa plant. Pea aphid clones from parthenogenetic females collected from clover were tested on clover, and pea aphids from alfalfa were tested on alfalfa. This restriction of aphid clones to their normal host plant is necessary due to the extreme

host-plant specificity of the aphids. However, previous experiments (Hufbauer and Via 1999) demonstrate that resistance to parasitism is not induced by feeding on one or the other plant in these assays.

After the nymphs had settled on the test plant for 24 h, a single female parasitoid was introduced to each cage, allowed to search and parasitize for 24 h, and was then removed. All wasps used in these experiments had eclosed 2–4 d earlier, and were maintained with a supply of honey and water. After parasitism, the experimental aphids were raised on their host plant until mummies formed  $\approx$  10 d later. The aphids were scored as either living, successfully parasitized, or missing and presumed dead. Four replicates of each aphid-source-field/wasp-source-field combination were performed, two in each of two temporal blocks.

These experiments allow both aphid avoidance and wasp searching behaviors; however, Hufbauer and Via (1999) documented that rates of parasitism in such assays are not significantly affected by either aphid or wasp behavior. Thus, high rates of parasitism in the laboratory assays indicate that aphids are relatively susceptible, and that wasps are relatively successful at overcoming aphid defenses (i.e., they are virulent). Low rates of parasitism indicate that aphids are relatively resistant, and wasps do not have effective counter-defenses.

Aphids might be scored as missing and presumed dead in these experiments for two reasons. First, parasitism itself can sometimes kill hosts without resulting in successful parasitoid reproduction (e.g., Rahman 1970, Tamaki et al. 1970, Cate et al. 1973). Second, transferring the aphid nymphs several times to set up experiments can cause some mortality. To help account for these sources of aphid death, and to increase the accuracy of my measure of the rate of successful parasitism, the proportion of aphids successfully parasitized was calculated as (number of mummies)/(number of mummies + number living), rather than as (number of mummies)/30, with 30 being the initial size of the cohort of experimental aphids. However, the first temporal block of the New York reciprocal transplant was not scored early enough to distinguish between aphids in the original test cohort and their adult offspring. Thus, in this block of the New York reciprocal transplant, the proportion of aphids successfully parasitized was calculated as (number of mummies)/30. The increased variability in parasitism rate due to this difference in calculation is accounted for in the block effect of the statistical model described below. For both experiments, the proportion of aphids successfully parasitized was arcsine square-root transformed to improve the normality of the residuals.

The data from the two reciprocal transplant experiments were analyzed using mixed linear models (The Mixed Procedure, SAS Institute 1997a). The Mixed Procedure is designed explicitly for the analysis of models including both fixed and random effects. The

TABLE 1. ANOVA analyzing the rate of parasitism in alfalfa and clover fields estimated from three sets of samples: 1996 and 1997 in New York, and 1997 in Maryland.

Source	<i>F</i>	df	<i>P</i>
Crop	55.09	1, 1	<0.0001
Sample	19.74	1, 2	<0.001
Crop × sample	1.55	1, 2	0.270

Satterthwaite approximation was used to obtain appropriate denominators for *F* tests of fixed effects (Littell et al. 1996). Individual random effects are tested with likelihood ratio tests. These likelihood ratios are distributed approximately as  $\chi^2$  with one degree of freedom. As variances cannot be negative, tests of single random effects are one sided, and  $\chi^2$  probabilities are divided by two to obtain *P* values (Littell et al. 1996). The crop from which the aphids were collected (aphid crop), and the crop from which the wasps were collected (wasp crop) were treated as fixed effects. The field from which the aphids and wasps were collected (aphid field and wasp field) were considered to be random effects, and were nested within aphid crop and wasp crop, respectively. The date of the trial (block) was treated as a random effect, and all interactions were tested. This design tests for adaptation at two different scales: The aphid crop × wasp crop interaction term tests for adaptation of wasps to aphids from the same crop from which they had been collected (or vice versa), and the aphid field(aphid crop) × wasp field (wasp crop) interaction term tests for local adaptation of wasps to aphids from the same field (or vice versa).

### RESULTS

The rate of parasitism by *A. ervi* in field samples of pea aphids was significantly affected by crop and sample (Table 1), and followed the pattern expected from laboratory assays of aphid resistance (Hufbauer and Via 1999). Parasitism rates were significantly lower in alfalfa, where aphids are more resistant, than in clover, where they are more susceptible (Fig. 2).

In the New York reciprocal transplant, the crop from which the aphids were collected had a marginally non-significant effect on parasitism rates (Table 2a). Mean parasitism levels of aphids from alfalfa were less than those of aphids from clover (mean for aphids from alfalfa = 0.42, mean for clover = 0.77, Fig. 3a). Thus, the aphids from alfalfa in New York appear to be somewhat more resistant to parasitism than the aphids from clover. There was also a significant difference in parasitism levels between the aphids from the different fields (aphid field term in Table 2a). A subsequent *F* test revealed a significant difference between the two New York alfalfa fields (means of 0.34 and 0.52,  $F_{1,66.4} = 18.27$ ,  $P = 0.0001$ , Fig. 3a). In the Maryland reciprocal transplant, the crop from which the aphids were collected had a significant effect on parasitism rates (Table 2b). Mean parasitism levels of aphids from al-

alfa were one-third those of aphids from clover (mean for aphids from alfalfa = 0.29, mean for clover = 0.88, [Fig. 3b]). I combined the results from the New York and Maryland reciprocal transplants in a simple meta-analysis to examine the effect of aphid crop in more detail (Sokal and Rohlf 1995). The test statistic,  $-2 \sum \ln P$ , is distributed as  $\chi^2$  with  $2k$  degrees of freedom, where *P* is the *P* value from each experiment, and *k* is the number of experiments performed. This analysis shows that the crop from which the aphids are collected significantly affects the parasitism rate, with aphids from alfalfa being more resistant than aphids from clover (Table 2c).

In contrast to the aphids, wasps from the different crops and fields in New York did not differ in parasitism rates in the reciprocal transplant experiment, indicating that they do not differ in their ability to overcome aphid resistance (wasp field and wasp crop terms in Table 2c). Furthermore, there was no significant interaction between the source crop of the aphids and the wasps; thus there is no detectable adaptation of the wasps from alfalfa to aphids from alfalfa, or the wasps from clover to the aphids from clover in New York. There is also no evidence of local adaptation of New York wasps to aphids from their source field (aphid field[aphid crop] × wasp field[wasp crop] term in Table 2a).

In the Maryland reciprocal transplant experiment, as in the New York experiment, wasps from the different crops and fields did not differ in their ability to overcome aphid resistance, and there is no evidence of either adaptation of the wasps to the aphids from their home crop, or local adaptation to the aphids from their home field (aphid crop × wasp crop and aphid field [aphid crop] × wasp field[wasp crop] terms in Table 2b). However, unlike the New York experiment, there was a significant interaction between aphid crop and wasp field(wasp crop) (Table 2b, Fig. 4). This inter-

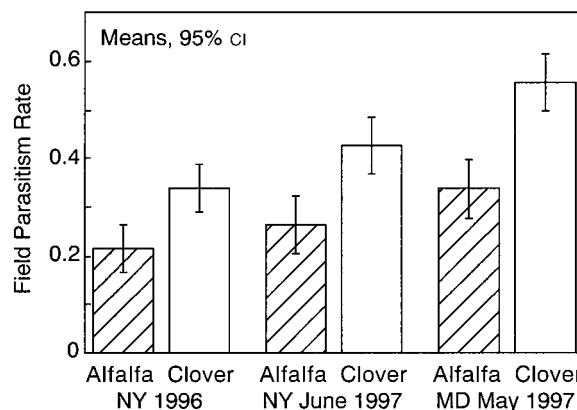


FIG. 2. Rate of parasitism by *A. ervi* in field samples of pea aphids from New York (NY) in 1996, and from New York and Maryland (MD) in 1997. Pea aphids from alfalfa fields are represented by hatched bars; pea aphids from clover fields are represented by open bars. Bars are least square means with 95% confidence intervals.

TABLE 2. Analysis of the effects of aphid and wasp sources on parasitism rates in fully reciprocal transplant experiments.

Fixed effects				Random effects			
Source	<i>F</i>	df†	<i>P</i>	Source	Likelihood ratio	df	<i>P</i>
a) 1996 New York collections‡							
Aphid crop	11.88	1, 1.99	0.075	Block	2.47	1	0.058
Wasp crop	0.28	1, 56	0.599	Aphid field(aphid crop)	5.48	1	0.010
Aphid crop × wasp crop	0.14	1, 56	0.706	Wasp field(wasp crop)	0	1	>0.50
				Wasp field(wasp crop) × aphid field(aphid crop)	0	1	>0.50
b) 1997 Maryland collections§							
Aphid crop	9.08	1, 3.64	0.045	Block	0	1	>0.50
Wasp crop	0.13	1, 4	0.736	Aphid field(aphid crop)	1.53	1	0.108
Aphid crop × wasp crop	2.18	1, 4	0.214	Wasp field(wasp crop)	0	1	>0.50
				Aphid crop × wasp field (wasp crop)	4.24	1	0.020
				Wasp field(wasp crop) × aphid field(aphid crop)	0	1	>0.50
c) Meta-analysis							
	Test statistic¶	df	<i>P</i>				
Aphid crop	11.32	4	0.023				

Note: Random effects are tested with likelihood ratio tests distributed as  $\chi^2$  with one degree of freedom, and fixed effects are evaluated with approximate *F* tests (Littell et al. 1996).

† Degrees of freedom are estimated using the Satterthwaite approximation which can produce fractional values.

‡ Mixed model ANOVA of the experiment with aphids and wasps from 1996 New York collections.

§ Mixed model ANOVA of the experiment with aphids and wasps from 1997 Maryland collections.

|| Meta-analysis of the overall effect of aphid crop on proportion parasitized (Sokal and Rohlf 1995). See Results for details.

¶ Fisher's test ( $-2\sum \ln P$ ).

action term indicates that there are differences in the proportion of aphids from clover and alfalfa successfully parasitized by wasps from particular fields. However, these differences in parasitism rate were very small (about 0.10, Fig. 4) relative to the differences in parasitism between aphids from alfalfa and clover, and they did not show any pattern that might relate to the crop or the location from which the wasps were collected.

## DISCUSSION

### *Differential aphid resistance and rates of parasitism in the field*

Pea aphids specialized on alfalfa are more resistant to parasitism than pea aphids specialized on clover in laboratory assays. This difference in the aphids' resistance is reflected in the data on rates of parasitism in the field. That is, parasitism rates are significantly low-

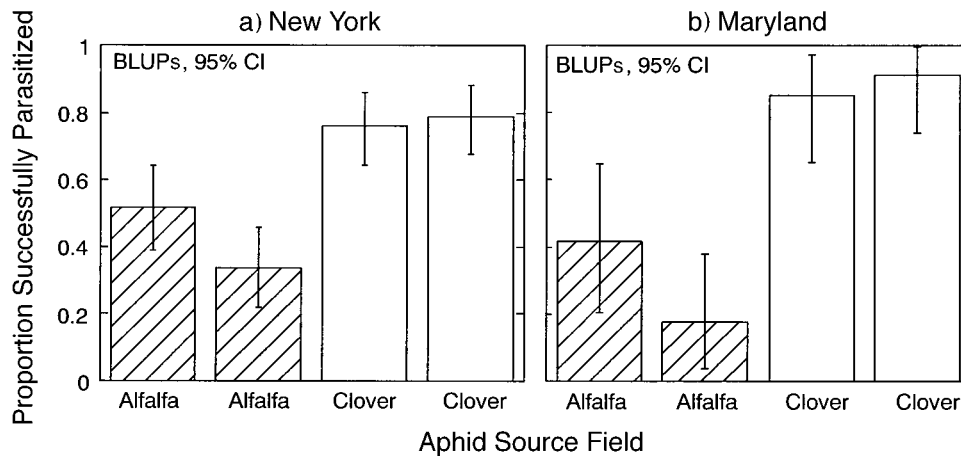


FIG. 3. The rate of successful parasitism by *A. ervi* on experimental pea aphid populations in the two reciprocal transplant experiments. Bars are best linear unbiased predictors (BLUPs) with 95% confidence intervals. Hatched bars represent aphids from alfalfa fields; open bars represent aphids from clover fields. In panel (a), aphids and parasitoids are from 1996 New York collections; panel (b) depicts aphids and parasitoids from 1997 Maryland collections.

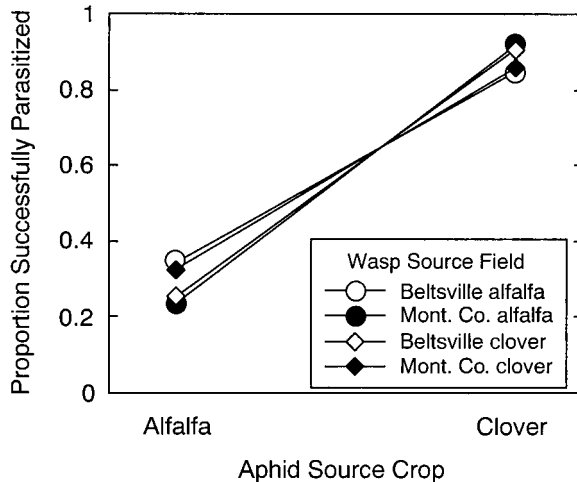


FIG. 4. Norm of reaction plot showing the rate of successful parasitism in the Maryland reciprocal transplant for aphids from alfalfa and clover attacked by parasitoids from the four source fields. The diamonds represent wasps from the clover fields; circles represent wasps from the alfalfa fields. Filled symbols represent the clover field-alfalfa field pair in Montgomery County, Maryland, while open symbols represent the pair of fields at Beltsville.

er in alfalfa fields where the aphids are resistant than in clover fields where the aphids are susceptible. This result suggests that the genetically based difference in resistance documented in laboratory experiments influences rates of parasitism in the field. The pattern of differential resistance appears to be general and robust: Including the earlier work (Hufbauer and Via 1999), it has been found across three years, six fields of each crop, and in Maryland as well as in New York.

Most research on plant-insect natural-enemy interactions has focused on behavioral and physiological effects of the host plant on rates of successful attack by natural enemies. However, Hufbauer and Via (1999) demonstrated that in this system, differences in the rates of parasitism in laboratory assays are due to genetic differences in physiological resistance to parasitism among pea aphid populations, not to insect behavior or host-plant-induced resistance. Here, I have shown that the differences in resistance of pea aphids specialized on clover and alfalfa are widespread, fairly stable through time, and that they are paralleled by differences in parasitism rates in the field. Thus, when differences in rates of parasitism are observed on different host plants in other tritrophic study systems, they should not automatically be attributed to effects of the host-plant environment. Particularly when populations of phytophagous insects are structured by their specialization on different host plants, they may diverge in traits other than host-plant use that affect their interactions with their natural enemies.

#### *Have parasitoids adapted to differential resistance?*

The differential resistance of the aphids, and the differences in parasitism rate in the field, suggest that

selection for wasps to overcome pea aphid resistance may be consistently stronger in alfalfa than in clover. An extremely virulent wasp in an alfalfa field may perform several times better than the average wasp in the field, while an extremely virulent wasp in clover will only perform a little better than the average wasp. However, despite this apparent difference in selective regime, wasps collected from clover and alfalfa fields do not differ in their abilities to overcome aphid resistance, and are not more successful on the aphids from their home crop. For example, wasps from alfalfa fields are as ineffective at parasitizing the resistant pea aphids specialized on alfalfa as are wasps from clover fields. Thus, the wasps from the two crops are not adapted to the aphids specialized on those crops. The wasps are also not locally adapted to aphids from their individual source fields. This lack of adaptation to the different levels of aphid resistance is surprising given that there is substantial genetic variation within populations in the ability of *A. ervi* to overcome aphid resistance (Henter 1995).

Most examples of local adaptation come from systems in which gene flow is restricted (e.g., Parker 1985, Karban 1989, reviewed in Mopper and Strauss 1998; however, see Mopper et al. 1995, Dybdahl and Lively 1996, and Stiling and Rossi 1998 for exceptions). Thus, one factor that may play a role in the lack of adaptation of the wasps to the aphids is that they are very mobile, as indicated by their rapid spread across North America after their release in 1959 (Angalet and Fuester 1977). High levels of gene flow among wasp populations could constrain the evolution of adaptation to populations of pea aphids from a particular crop or field (e.g., Strauss 1997, Storfer and Sih 1998). In addition, there may have been insufficient time since the wasps' introduction to North America for them to adapt to the host races of pea aphids.

Wasps from the fields in Maryland vary in their success on aphids from alfalfa and clover, but there are no differences in virulence of the wasps from the different fields in New York. The variable success rates of wasps from Maryland fields is not related to either the crop, or the location of the field from which they were collected. Possibly, the Maryland wasps have gone through more generations since their introduction than wasps in New York, and this additional time for evolution to occur might explain the greater variability among the Maryland populations of wasps in their ability to parasitize pea aphids. When the wasps were originally introduced for biological control, they were released in Delaware and New Jersey, thus they probably have been present in Maryland longer than in New York. Furthermore, the longer season and warmer temperatures in Maryland could increase the number of generations per year there relative to New York. It is possible, however, that variation between the conditions of the two experiments could account for the differences that were observed among parasitoid popu-

lations in Maryland, but were not observed among New York populations.

#### CONCLUSIONS

Has *Aphidius ervi* adapted to the different resistance regimes of pea aphids from alfalfa and clover? The evidence presented here does not support either reciprocal adaptation of the parasitoids at the level of the two crops, or local adaptation of the parasitoids to aphids from their home field. Thus, this system does not appear to have been strongly shaped by coevolution, at least since the introduction of pea aphids and *A. ervi* to North America. It would be interesting to examine whether coevolutionary dynamics occur over a similar geographic scale where the system is native. The interaction between *Drosophila* and its parasitoids has been examined closely in its native range. Kraaijeveld and Godfray (1999) review the *Drosophila*-parasitoid literature, and find evidence that virulence in the parasitoid *Asobara tabida* may have evolved in response to differential resistance of *Drosophila* populations. However, there does not appear to be reciprocal adaptation of the flies, and Kraaijeveld and Godfray (1999) conclude that there is little evidence for coevolutionary dynamics. Host-parasitoid systems may indeed be good systems for studying the evolution of species interactions as suggested by Price et al. (1980), Thompson (1994), and Kraaijeveld et al. (1998); however, thus far there is little evidence of truly reciprocal coevolutionary dynamics. Nonetheless, taking an explicitly geographical approach enhances our understanding of the complex nature of the evolution of species interactions.

#### ACKNOWLEDGMENTS

I thank Chris George for help in the field and laboratory in New York, and Sara Via and Stasia Skillman for help with Maryland field collections. Thanks to the many dairy farmers in New York and Maryland, and to the USDA Beltsville facility, for access to fields. I had many fruitful discussions about statistics with Charles F. McCulloch and David W. Winkler, and the members of Biostats Lunch Bunch at Cornell. Todd E. Dawson, Richard B. Root, Sara Via, Timothy G. Carr, two anonymous reviewers, and particularly William E. Bradshaw, Andrew P. Norton, and Susanna K. Remold gave comments and insights that improved the manuscript. This study was supported by the Mellon Foundation, the Cornell Chapter of Sigma Xi, and a dissertation improvement grant from the National Science Foundation. I was supported by a predoctoral fellowship from the National Science Foundation for part of the project.

#### LITERATURE CITED

- Althoff, D. M., and J. N. Thompson. 1999. Comparative geographic structures of two parasitoid-host interactions. *Evolution* **53**:818–825.
- Angalet, G. W., and R. Fuester. 1977. The *Aphidius* parasites of the pea aphid *Acyrtosiphon pisum* in the eastern half of the United States. *Annals of the Entomological Society of America* **70**:87–96.
- Blackman, R. L. 1980. Chromosomes and parthenogenesis in aphids. Pages 133–148 in R. L. Blackman, G. M. Hewitt, and M. Ashburner, editors. *Insect cytogenetics*. Blackwell Scientific, Oxford, UK.
- Blackman, R. L. 1987. Reproduction, cytogenetics and development. Pages 209–220 in A. K. Minks, and P. Harrewijn, editors. *Aphids: their biology, natural enemies and control*. Volume 2. Elsevier, Amsterdam, The Netherlands.
- Carton, Y., and A. J. Nappi. 1991. The *Drosophila* immune reaction and the parasitoid capacity to evade it: genetic and coevolutionary aspects. *Acta Oecologica* **12**:89–104.
- Cate, R. H., T. L. Archer, R. D. Eikenbary, K. J. Starks, and R. D. Morrison. 1973. Parasitization of the greenbug by *Aphelinus asychis* and the effect of feeding by the parasitoid on aphid mortality. *Environmental Entomology* **2**:549–553.
- Chau, A., and M. Mackauer. 1997. Dropping of pea aphids from feeding site: a consequence of parasitism by the wasps, *Monoclonus paulensis*. *Entomologia Experimentalis et Applicata* **83**:247–252.
- Dybdahl, M. F., and C. M. Lively. 1996. The geography of coevolution: comparative population structure for a snail and its trematode parasite. *Evolution* **50**:2264–2275.
- Godfray, H. C. J. 1993. *Parasitoids*. Princeton University Press, Princeton, New Jersey, USA.
- Hales, D. F., J. Tomiuk, K. Wohrmann, and P. Sunnucks. 1997. Evolutionary and genetic aspects of aphid biology: a review. *European Journal of Entomology* **94**:1–55.
- Halfhill, J. E., P. E. Featherson, and A. G. Dicke. 1972. History of *Praon* and *Aphidius* parasites of the pea aphid in the Pacific Northwest. *Environmental Entomology* **1**:402–405.
- Henter, H. J. 1995. The potential for coevolution in a host-parasitoid system. II. Genetic variation within a population of wasps in the ability to parasitize an aphid host. *Evolution* **49**:439–445.
- Henter, H. J., and S. Via. 1995. The potential for coevolution in a host-parasitoid system. I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. *Evolution* **49**:427–438.
- Holt, R. D., and M. E. Hochberg. 1997. When is biological control evolutionarily stable? *Ecology* **78**:1673–1683.
- Hufbauer, R. A., and S. Via. 1999. Evolution of an aphid-parasitoid interaction: variation in resistance to parasitism among aphid populations specialized on different plants. *Evolution* **53**:1435–1445.
- Ives, A. R., S. S. Schooler, V. J. Jagar, S. E. Knuteson, M. Grbic, and W. H. Settle. 1999. Variability and parasitoid foraging efficiency: a case study of pea aphids and *Aphidius ervi*. *American Naturalist* **154**:652–673.
- Janzen, D. H. 1980. When is it coevolution? *Evolution* **34**:611–612.
- Jervis, M. A. 1997. Parasitoids as limiting and selective factors: can biological control be evolutionarily stable? *Trends in Ecology and Evolution* **12**:378–379.
- Karban, R. 1989. Fine-scale adaptation of herbivorous thrips to individual host plants. *Nature* **340**:60–61.
- Karban, R., and G. English-Loeb. 1997. Tachinid parasitoids affect host plant choice by caterpillars to increase caterpillar survival. *Ecology* **78**:603–611.
- Kawecki, T. J. 1998. Red Queen meets Santa Rosalia: arms races and the evolution of host specialization in organisms with parasitic lifestyles. *American Naturalist* **152**:635–651.
- Kraaijeveld, A. R., and H. C. J. Godfray. 1999. Geographic patterns in the evolution of resistance and virulence in *Drosophila* and its parasitoids. *American Naturalist* **153**:S61–S74.
- Kraaijeveld, A. R., J. J. M. van Alphen, and H. C. J. Godfray. 1998. The coevolution of host resistance and parasitoid virulence. *Parasitology* **116**:S29–S45.
- Lamb, R. J., and P. J. Pointing. 1972. Sexual morph determination in the aphid *Acyrtosiphon pisum*. *Journal of Insect Physiology* **18**:2029–2042.
- Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wol-

- finger. 1996. SAS system for mixed models. SAS Institute, Cary, North Carolina, USA.
- Lushai, G., P. J. De Barro, O. David, T. N. Sherratt, and N. Maclean. 1998. Genetic variation within a parthenogenetic lineage. *Insect Molecular Biology* **7**:337–344.
- Mackauer, M., and A. Campbell. 1972. The establishment of three exotic parasites (Hymenoptera: Aphidiidae) in British Columbia. *Journal of the Entomological Society of British Columbia* **69**:54–58.
- Midori, T., and M. B. Bonsall. 1999. Evolutionary and population dynamics of host-parasitoid interactions. *Researches on Population Ecology* **41**:81–91.
- Mopper, S., M. Beck, D. Simberloff, and P. Stiling. 1995. Local adaptation and agents of selection in a mobile insect. *Evolution* **49**:810–815.
- Mopper, S., and S. Y. Strauss, editors. 1998. Genetic structure and local adaptation in natural insect populations. Chapman & Hall, New York, New York, USA.
- Parker, M. A. 1985. Local population differentiation for compatibility in an annual legume and its host specific fungal pathogen. *Evolution* **39**:713–723.
- Price, P. W., C. E. Bouton, P. Gross, B. A. McPherson, J. N. Thompson, and A. E. Weis. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics* **11**:41–65.
- Rahman, M. 1970. Mutilation of the imported cabbageworm by the parasite *Apanteles rubecula*. *Journal of Economic Entomology* **63**:1150–1153.
- Sandstrom, J. 1994. High variation in host adaptation among clones of the pea aphid, *Acyrtosiphon pisum*, on peas, *Pisum sativum*. *Entomologia Experimentalis et Applicata* **71**:245–256.
- SAS Institute. 1997a. SAS/STAT software: changes and enhancements through release 6.12. SAS Institute, Cary, North Carolina, USA.
- SAS Institute. 1997b. JMP Version 3.2. SAS Institute, Cary, North Carolina, USA.
- Sasaki, A., and H. C. J. Godfray. 1999. A model for the coevolution of resistance and virulence in coupled host-parasitoid interactions. *Proceedings of the Royal Society of London Series B* **266**:455–463.
- Sokal, R. R., and F. J. Rohlf. 1995. *Biometry: the principles and practice of statistics in biological research*. Third edition. W. H. Freeman, New York, New York, USA.
- Stiling, P., and A. M. Rossi. 1998. Deme formation in a dispersive gall-forming midge. Pages 22–36 in S. Mopper, and S. Y. Strauss, editors. Genetic structure and local adaptation in natural insect populations. Chapman & Hall, New York, New York, USA.
- Storfer, A., and A. Sih. 1998. Gene flow and ineffective antipredator behavior in a stream-breeding salamander. *Evolution* **52**:558–565.
- Strauss, S. Y. 1997. Lack of evidence for local adaptation to individual plant clones or site by a mobile specialist herbivore. *Oecologia* **84**:111–116.
- Tamaki, G., J. E. Halfhill, and D. O. Hathaway. 1970. Dispersal and reproduction of colonies of pea aphids by *Aphidius smithi* (Hymenoptera: Aphidiidae). *Annals of the Entomological Society of America* **63**:973–980.
- Thompson, J. N. 1988. Variation in interspecific interactions. *Annual Review of Ecology and Systematics* **19**:65–87.
- Thompson, J. N. 1994. The coevolutionary process. University of Chicago Press, Chicago, Illinois, USA.
- Thompson, J. N. 1999. The evolution of species interactions. *Science* **284**:2116–2118.
- Travis, J. 1996. The significance of geographic variation in species interactions. *American Naturalist* **148**:S1–S8.
- Via, S. 1991a. The genetic structure of host plant adaptations in a spatial patchwork: demographic variability among reciprocally transplanted pea aphid clones. *Evolution* **45**:827–852.
- Via, S. 1991b. Specialized host plant performance of pea aphids clones is not altered by experience. *Ecology* **72**:1420–1427.
- Via, S. 1994. Population structure and local adaptation in a clonal herbivore. Pages 58–85 in L. Real, editor. *Ecological genetics*. Princeton University Press, Princeton, New Jersey, USA.
- Via, S. 1999. Reproductive isolation between sympatric races of pea aphids: gene flow restriction and habitat choice. *Evolution* **53**:1446–1457.
- Vinson, S. B. 1990. How parasitoids deal with the immune system of their hosts: an overview. *Archives of Insect Biochemistry and Physiology* **13**:3–27.