

EVIDENCE FOR NONADAPTIVE EVOLUTION IN PARASITOID VIRULENCE FOLLOWING A BIOLOGICAL CONTROL INTRODUCTION

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Abstract. Biological control systems can evolve; founder effects, drift, inbreeding, and adaptation to new environments can occur during the introduction and establishment of exotic pests and their natural enemies. It has been hypothesized that successful biological control agents are those that become locally adapted to their new environment or populations of hosts. However, there are no explicit comparisons of native and introduced populations of biological control agents testing for local adaptation. The pea aphid, *Acyrtosiphon pisum*, is an introduced pest of legumes. *Aphidius ervi*, a parasitoid wasp, was introduced from France to control pea aphids. Using reciprocal transplant experiments, I compared introduced populations of pea aphids and *A. ervi* from New York State with native populations from France. I documented patterns of aphid resistance to parasitism and *A. ervi* ability to overcome aphid resistance (virulence) in the two localities and explored whether the introduced parasitoids are locally adapted to the introduced pea aphids. I found that parasitoids from native French populations have high rates of parasitism on pea aphids specialized on clover and on pea aphids specialized on alfalfa, regardless of whether the aphids are collected from France or New York. Introduced parasitoids from New York have high rates of parasitism on pea aphids from clover, but low rates of parasitism on pea aphids from either New York or French alfalfa fields. Thus, there is no evidence that the introduced wasps have become adapted to their local populations of pea aphid hosts. On the contrary, the ability of the introduced parasitoids to develop successfully in pea aphids from alfalfa may have been compromised by the biological control introduction. This apparently nonadaptive evolution was cryptic until examined experimentally. A better understanding of the effects of biological control introductions on natural enemies will come through additional comparisons of native and introduced populations of biocontrol agents. Such comparisons may provide valuable insights into the role of microevolutionary change in biological control and suggest useful avenues to enhance the success of biological control introductions.

Key words: *Acyrtosiphon pisum*; *Aphidius ervi*; biological control; bottleneck; evolution; invasive species; local adaptation; parasitoid; pea aphid; reciprocal transplant; resistance; virulence.

INTRODUCTION

Classical biological control is the introduction of natural enemies of exotic pests into new areas in an attempt to reduce the population sizes of those pests. Success rates for biological control are relatively low. In the biological control of insects and arachnids, 25–34% of introductions have resulted in establishment of the control agents (Hall and Ehler 1979, van Lenteren 1983), and only 16% of introductions provided complete control of targeted pest species (Hall et al. 1980). In the biological control of weeds, from 60 to 63% of introductions succeeded in establishing phytophagous insects, but only 10–18% of all introductions have provided good to complete control (Crawley 1989, Lawton 1990). The underlying causes of these low success rates are not well understood, although it is clear that many

factors (e.g., climate-matching and Allee effects) are involved in the successful establishment of biological control agents and control of pests (Huffaker and Messenger 1976, Hopper and Roush 1993, Van Driesche and Bellows 1996). Understanding the circumstances that contribute to successful biological control is a particularly vital issue because of the potential dangers of biological control introductions to non-target organisms (Howarth 1983, Simberloff and Stiling 1996, Louda et al. 1997). Ideally, to minimize these dangers fewer introductions should be made, while to achieve control of pest species a greater proportion of introductions need to be successful. Thus, in order to improve both the safety and success of biological control, it is vital to move toward a better understanding of the fundamental ecological and evolutionary processes involved.

Biological control represents a basic application of ecological theory, and relies heavily upon ecological groundwork in demography and population regulation. While connections to population ecology are clear, the role of genetics and of adaptive evolution in the success of biological control efforts have long been under de-

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bate (Force 1967, Remington 1968, Lucas 1969, Mackauer 1972, Messenger et al. 1976, Myers and Sabath 1981, Roush 1990a, b, Hopper et al. 1993, Blossey and Notzold 1995, Blossey and Kamil 1996, Holt and Hochberg 1997, Jervis 1997). The main hypothesized role of genetics comes through the literature on founder effects and the consequences of bottlenecks in populations size (e.g., Kerr and Wright 1954, Mayr 1954, Crow 1964, Baker and Stebbins 1965, Barton and Charlesworth 1984). Bottlenecks are known to reduce levels of genetic variation in neutral loci (Nei et al. 1975), and this has been implicated in inbreeding depression (Woodworth et al. 1994, Frankham 1998) and may constrain further adaptive evolution. However, bottlenecks may also increase additive genetic variance in quantitative traits (Carson 1990, Goodnight 1987, 1988), and this may allow a faster response to selection. As yet there are no data that directly explore the effects of bottlenecks on the success of biological control.

The role of adaptive microevolution in the success of biological control is even less clear. It has been suggested that successful biological control agents are those that evolve to become locally adapted to their target pest population or new environment (DeBach 1964, Messenger and van den Bosch 1971, Peschken 1972). However, Murray (1985) and Lawton (1990) both point out that most evidence for local adaptation of natural enemies to their new environment is anecdotal; when biological control agents stay at low densities for several generations after their introduction, and then their populations grow rapidly, they are presumed to have become adapted to the new environment. While this pattern of population growth may indicate short-term adaptive evolution to local conditions (Bazzaz 1986), it also can arise from Allee effects (Lewis and Kareiva 1993, Grevstad 1999a, b), or from the practical difficulty of detecting a small, dispersed population at a release site before it grows to a sufficiently large size (Shigesada and Kawasaki 1997, Grevstad 1999a, b).

There is some experimental evidence that biological control agents can become adapted to hosts in their new range. *Bathyplectes curculionis* (Hymenoptera: Ichneumonidae) was introduced from Europe to southern California to control the alfalfa weevil (*Hypera postica*), and it was also observed to attack a related species in the new range, *H. brunneipennis*. Survival in *H. brunneipennis* initially was low due to encapsulation of the parasitoid eggs by host defensive cells. The same population was studied 15 yr after the release, and the parasitoid was encapsulated only 5% of the time by *H. brunneipennis*, suggesting that the parasitoid population had adapted to the physiological immune response of its new host (van den Bosch 1964, Salt and van den Bosch 1967). *Cotesia glomerata* (Hymenoptera: Braconidae) was introduced to the United States from Great Britain. Lemasurier and Waage (1993) compared an introduced U.S. population with

a native British population. The main host of the U.S. population is *Pieris rapae*, while the main host of the British population is *P. brassicae*. They found that the introduced population has higher rates of parasitism on *P. rapae* than the British population, suggesting that the introduced population has adapted to *P. rapae* since its introduction.

Both these examples provide strong evidence for adaptation of a parasitoid to its new range. However, to test explicitly for local adaptation to host in the new range, comparisons should be made of replicate populations of the biological control agent from the native and introduced range, on replicate populations of the native and introduced hosts.

Here, I examine whether populations of an introduced biological control agent, *Aphidius ervi* (Hymenoptera: Braconidae), have become adapted to local populations of their host, the pea aphid, *Acyrtosiphon pisum* (Hemiptera: Aphididae). *Aphidius ervi* is considered to be a successful biological control agent (Hagen et al. 1976, Gonzalez et al. 1995) and therefore seems a suitable system with which to explore the hypothesis that successful control is associated with local adaptation. I employ a reciprocal transplant design that allows me to test experimentally for local adaptation of the introduced parasitoids. This design also enables me to examine more generally how this aphid-parasitoid system may have evolved since the introductions of the aphid and parasitoid to North America from Europe with respect to two quantitative traits: physiological resistance to parasitism in the aphids, and the ability of wasps to overcome resistance, or virulence.

MATERIALS AND METHODS

Study system

The pea aphid is a pest of herbaceous legumes. Red clover (*Trifolium praetense*) and alfalfa (*Medicago sativa*) grown by dairy farmers for hay are two of its main host plants. Pea aphids are cyclically parthenogenetic: they reproduce asexually during the summer, going through 8–10 clonal generations in temperate areas. This clonal reproduction can be maintained in the laboratory by long photoperiods making it possible to replicate experiments with clonal individuals. In the field, sexual forms develop in the fall and produce eggs that overwinter on the host plant (Lamb and Pointing 1972). Pea aphids are native to Europe, and they were first noted as pests in North America in the 1870s (Angalet and Fuester 1977). Despite this recent introduction, large amounts of genetic variation (quantitative genetic and at several allozyme loci) exist in North American populations of pea aphids (Via 1991a, 1994, 1999, Henter and Via 1995, Via and Shaw 1996).

Aphidius ervi is a solitary endoparasitoid: 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. Thus, death of the aphid is a necessary

consequence of development of the parasitoid. The parasitoid pupates inside the dried exoskeleton of the dead aphid, or mummy. *Aphidius ervi* was introduced into the United States in 1959 to control the pea aphid. Since its release, *A. ervi* has become the dominant parasitoid of pea aphids in North America (Angalet and Fuester 1977).

The importation of *A. ervi* to North America consisted of about 1,000 individuals that were shipped from France to the United States Department of Agriculture (USDA) in Maryland. Of these, 800 were collected as pupae in mummies from alfalfa fields in France, and 200 were from a French lab culture (USDA Beneficial Insects Research Laboratory second quarter report 1959, USDA European Parasite Laboratory second quarter report 1959, Halfhill et al. 1972, Mackauer and Campbell 1972, Angalet and Fuester 1977). In addition, in 1965 scientists at the University of California, Berkeley imported a small colony of *A. ervi* from Lebanon and subsequently released it in Glenn County, California (Mackauer and Finlayson 1967). Theoretically, with this many individuals few detrimental effects due to drift and inbreeding are expected, and very little of the genetic variation present in the French populations should have been lost during the introduction. However, it is not known from how many fields the parasitoids were collected in France, how many of the field-collected mummies were hyperparasitized, how many of the unparasitized individuals survived shipment to found the laboratory population, whether the laboratory culture fluctuated in size before release, how many individuals were released into the field, or what proportion of those released successfully reproduced. Thus, even with a collection of 1000 individuals, it is not safe to assume there to be no detrimental effects of the introduction process.

Pea aphids collected from clover and alfalfa are specialized on their host plants and are locally adapted (Via 1991a, b, 1994, Sandstrom 1994a, b, 1996). Pea aphids from both crops are also differentiated at allozyme loci and there is little gene flow across crops, even when fields of clover and alfalfa are adjacent to each other (Via 1999), thus it seems warranted to call these host-plant specialists "host-races" (sensu Bush 1969). The most recent key (Eastop 1973) classifies the alfalfa host-race and the red clover host-race within the subspecies *A. pisum pisum*.

Aphids specialized on alfalfa are known to be 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). The exact mechanism of resistance is unknown, but it appears to be humoral (Henter 1995, Henter and Via 1995), rather than a cellular encapsulation response (see Vinson 1990 and Strand and Pech 1995 for reviews of insect defenses against parasitism).

In New York and Maryland, the alfalfa host-race of

pea aphids is about two times more resistant to parasitism by local *A. ervi* than is the clover host-race (Hufbauer and Via 1999, Hufbauer 2001a). This striking difference in susceptibility to parasitism between alfalfa and clover specialists is genetically based, and is due to differences in aphid physiology rather than to indirect effects of the plants or to differences in parasitoid behavior on the two host plants (Hufbauer and Via 1999).

Introduced *A. ervi* are genetically variable in their ability to overcome pea aphid resistance (Henter 1995). The ability of a parasitoid to overcome physiological host defenses is called virulence in the literature on insect-parasitoid interactions (Godfray 1993, Kraaijeveld et al. 1998). This usage is somewhat problematic because a parasitoid can not 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 an unusual exception). Nonetheless, the term virulence is a useful way to discuss the ability of parasitoids to overcome host immune responses. This terminology has been used in experimental systems, particularly *Drosophila* parasitoids and *A. ervi* (e.g., Carton and Nappi 1991, Henter 1995, Hufbauer and Via 1999, Kraaijeveld and Godfray 1999, Green et al. 2000), and in verbal and mathematical models of host-parasitoid interactions (e.g., Holt and Hochberg 1997, Kawecki 1998, Sasaki and Godfray 1999, Tuda and Bonsall 1999, Fellowes and Travis 2000). Typically, relative rates of parasitism in laboratory assays are used to gauge both host resistance, and parasitoid virulence, but in the *Drosophila* system, researchers are coming closer to characterizing traits that can be measured independent of the rate of parasitism (e.g., Russo et al. 2001).

The exact mechanism by which *A. ervi* overcomes host resistance is unknown, however it appears to involve teratocytes, cells that dissociate from the wasp embryo, enlarge, and float free in the host haemolymph (Falabella et al. 2000). The heritable variation in *A. ervi* virulence found by Henter (1995) suggests that the evolution of local adaptation in the introduced wasps to the physiology of their host aphids is possible.

Experimental overview

In this paper, I explore how pea aphid resistance to parasitism and *A. ervi* virulence may have evolved since the introductions of the aphids and wasps to North America, by comparing introduced and native populations. I ask two main questions: (1) Are introduced wasps in New York locally adapted to the introduced pea aphids with respect to their ability to overcome aphid immune responses and develop successfully? (2) More generally, what are the patterns of aphid resistance and parasitoid virulence in introduced New York and native French populations? Comparing the introduced and native aphid populations may provide in-

sights into the intriguing difference in susceptibility to parasitism between the alfalfa and clover host races. For example, if the greater resistance of pea aphids specialized on alfalfa relative to those on clover that is found in the northeastern U.S. is also present in native French populations, then the host-races of aphids may have colonized North America separately, already differing in resistance.

I performed two experiments that jointly address both questions. In the first experiment, I set up two reciprocal transplants simultaneously. I subjected pea aphids from French alfalfa and clover to wasps from French alfalfa and clover to examine the pattern of aphid resistance and wasp virulence in the native French populations. To directly relate my findings to results from New York, I performed a simultaneous reciprocal transplant, using aphids and wasps from New York alfalfa and clover. This experiment allowed me to compare the patterns of resistance and virulence *within* France with those *within* New York. This experimental design also permitted me to assess whether wasps were adapted to aphids from their home crop within either France or New York.

In the second experiment I performed a reciprocal transplant in which I subjected aphids collected from alfalfa and clover from both France and New York to wasps from alfalfa and clover from both France and New York. Thus, in this experiment, aphids from both countries and crops were subjected to parasitoids from both countries and crops. This allowed me to test for adaptation of the introduced wasps to the introduced aphids, and to assess whether any differences in patterns of resistance and virulence revealed in the first experiment were due to differences between the native and introduced aphid populations, to differences between the native and introduced wasp populations, or both.

Insect collections for experiments

Pea aphids and *A. ervi* were collected from Tompkins County, New York in late June 1997, and from the Loire River Valley between the towns of Poitiers and Thouars in France in early July 1997. In both regions, aphids were collected from two red clover fields and two alfalfa fields within 30 km of each other. From each field a single parthenogenetic female was sampled every forty paces, for a total of ~45 aphids per field. This sampling design decreases the chances that individuals of a single clonal genotype would be sampled more than once. With the parthenogenetic females collected from the field, I initiated from 10 to 15 clonal lineages of aphids from each field and maintained them in laboratory facilities at Cornell University in Ithaca, New York (Fig. 1). I had a total of 59 distinct clonal lineages of aphids from New York (30 from alfalfa and 29 from clover), and 49 clonal lineages from France (24 from alfalfa and 25 from clover). Pea aphid clones originating from clover were individually maintained on

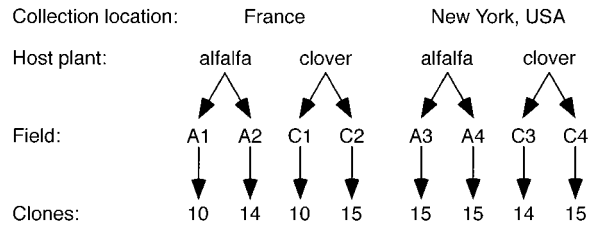


FIG. 1. Diagram of aphid and wasp collection locations, and the number of aphid clonal lineages initiated from each source field.

potted red clover plants (*Trifolium praetense* var. Medium Red), and those from alfalfa were individually maintained on potted alfalfa plants (*Medicago sativa* var. Oneida VR). These varieties are typically grown in the upstate New York area, and the French aphids fed and grew successfully on them.

Aphidius ervi were collected both as pupae (in mummies) and as larvae (in parasitized immature aphids) to initiate wasp colonies from each of the four New York and four French source fields. Wasp colonies from New York populations were initiated with at least 50 individuals, while colonies from French populations were initiated with 10–20 individuals. All eight wasp colonies were maintained on two pea aphid clones from New York, one alfalfa specialist and one clover specialist. Neither of these aphid clones was used in any experiments. After the first experiment, wasp colonies from the same country and crop that did not differ in virulence (see *Results*) were merged. All experiments with these wasps took place within three months of collection, or ~6 generations.

Experimental protocols and analysis

Aphids used in the reciprocal transplant experiments were clonally produced offspring of the parthenogenetic females collected from the field. In both experiments, I formed experimental composite populations of aphids in the lab to represent field populations, and then I subjected them to parasitism. To create the experimental populations, I combined 30 second-instar aphid nymphs from different clones to obtain a genetically variable group of aphids that represented a field population. The exact composition of these experimental populations differed in the two experiments as described below, but in other respects the experimental protocols were the same. The nymphs comprising an experimental population were placed on their appropriate host plant in a test cage and allowed to settle for 24 h, and were then subjected to parasitism by a single parasitoid wasp. The wasp was held in the test cage with the aphids for 24 h, and then removed. All wasps used in these experiments had eclosed 2–4 d prior to the trial and were maintained with a supply of honey and water, to minimize variance in parasitism rates due to wasp age and nutritional status. When mummies formed, ~10 d after parasitism, the aphids from the

original experimental population were scored as either living, dead, or successfully parasitized. The aphids that survived parasitism gave birth to offspring within the experimental cages, however trials were scored before the offspring reached adulthood, making it easy to distinguish the offspring from the experimental subjects.

In these bioassays, both aphids and wasps were free to move around the cages. Thus, behavioral factors could affect the rate of successful parasitism in addition to resistance of the aphids and virulence of the wasps. However, previous experiments document that neither the behavior of aphids or wasps on the two host plants, nor effects on the aphid physiology of feeding on different host plants (i.e., induced resistance or altered nutritional quality) affect parasitism rate significantly in these trial arenas (Hufbauer and Via 1999). Thus, parasitism rate in these standardized assays reflects the resistance of the aphids, and the virulence of the parasitoids (Hufbauer and Via 1999, Hufbauer 2001a).

Aphid resistance and parasitoid virulence are intimately related. Because there is not yet an absolute measure of pea aphid resistance or *A. ervi* virulence, these traits can only be measured relative to each other in the assays I performed. Low rates of parasitism indicate that the aphids are relatively resistant to the particular parasitoids attacking them, and that the parasitoids are not successful at overcoming aphid immune responses (i.e., low virulence). High rates of parasitism indicate that the aphids are relatively susceptible to the particular parasitoids attacking them, and that the parasitoids are relatively virulent.

The proportion of aphids successfully parasitized within each test enclosure was calculated as (number of mummies)/(number of mummies + living aphids). This is preferable to calculating parasitism rates as (number of mummies)/(initial number of aphids) because parasitism itself can sometimes kill an aphid outright without resulting in successful reproduction of the wasp. Analyses using (number of mummies)/(initial number of aphids), and thus including death due to parasitism without parasitoid reproduction, gave the same qualitative results and are not presented here. Adult wasps emerged from almost all mummies, and no differences in emergence rates from aphids collected from the two host plant species were noted. Parasitism data were natural log-transformed [$\ln(\text{proportion parasitized} + 1)$] for analysis to improve the normality of the residuals.

I analyzed the data using a mixed linear model (PROC MIXED, SAS Institute, Incorporated 1997) that is designed explicitly for use with models containing both fixed and random effects. In the Mixed Procedure, the significance of each random effect is tested using likelihood ratio tests (Littell et al. 1996). The significance of fixed effects is tested with *F* tests that account for both the variance from the random effects and the error variance. I used the Satterthwaite approximation

to calculate appropriate degrees of freedom for the *F* tests (Neter et al. 1990, Littell et al. 1996), and thus degrees of freedom may be fractional. A consequence of using PROC MIXED is that the complex mean square denominators traditionally associated with mixed models are not employed or reported (Littell et al. 1996, SAS Institute Incorporated 1997).

Experiment 1: Levels of resistance and virulence within countries.—Aphids from both fields of the two crops within France were subjected to wasps from those same four fields in a fully reciprocal design. The experimental populations of aphids were formed to represent each of the four fields from which I collected in France. For the field from which I had 15 clonal representatives, the experimental populations of 30 aphids were composed of two nymphs from each clone. For the field with 14 clonal representatives, three nymphs rather than two were taken from two randomly chosen clones, to obtain 30 individuals. For the two fields with 10 clonal representatives, three aphid nymphs from each clone were used, for a total of 30 individuals. Using the same protocols, I set up assays for New York aphids and wasps simultaneously, so that rates of successful parasitism in the French and New York assays could be compared directly.

One replicate of each of the 32 distinct aphid source–wasp source combinations (16 French aphid–French wasp source combinations, and 16 New York aphid–New York wasp combinations) was performed in each of four temporal blocks, for a total of four replicates. The data for France and New York were first analyzed together. The country being tested and the crop from which the aphids and wasps were collected (country, aphid crop, and wasp crop) were treated as fixed effects. The specific fields 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. Each field was labeled with a unique name, making additional nesting within country unnecessary. Block was treated as a random effect. Interactions with block were tested and none were significant, so they were dropped from the model.

Experiment 2: Test for local adaptation between countries.—Pea aphids from France and New York were subjected to wasps from France and New York in a reciprocal lab transplant. The design was dependent upon some of the results from the first experiment; within each country, aphids from the two fields of each crop did not differ significantly in resistance (see *Results*). Thus, aphids from the same crop and country were pooled together in the second experiment to create four distinct groups: aphids from alfalfa in New York (30 clones), aphids from clover in New York (29 clones), aphids from alfalfa in France (24 clones), aphids from clover in France (25 clones). The experimental populations representing these four sources of aphids consisted of a single aphid nymph from each clone, with a second nymph chosen randomly from the

TABLE 1. Mixed-model ANOVA for proportion of pea aphids from alfalfa and from clover in France successfully parasitized by *Aphidius ervi* from France, and for proportion of pea aphids from alfalfa and clover in New York successfully parasitized by *A. ervi* from New York.

Source	Type III <i>F</i>	df	<i>P</i>
Fixed effects			
Aphid crop	47.69	1, 39.4	0.002
Wasp crop	0.44	1, 3.96	NS
Country	0.15	1, 4.06	NS
Aphid crop × wasp crop	0.03	1, 4.07	NS
Aphid crop × country	59.80	1, 3.94	0.002
Wasp crop × country	0.01	1, 3.96	NS
Aphid crop × country	0.21	1, 4.07	NS
Source	Likelihood ratio	df	<i>P</i>
Random effects			
Block	7.13	1	0.004
Aphid field(aphid crop)	0.03	1	NS
Wasp field(wasp crop)	15.69	1	<0.0001
Aphid crop × wasp field(wasp crop)	0	1	NS
Wasp crop × aphid field(aphid crop)	0.01	1	NS
Country × aphid field(aphid crop)	0	1	NS
Country × wasp field(wasp crop)	0	1	NS
Aphid field(crop) × wasp field(crop)	0	1	NS
Country × aphid field(crop) × wasp field(crop)	0	1	NS

Note: Replicate experimental populations of aphids were tested in two simultaneous reciprocal transplants as described in *Materials: Experimental overview*.

clones of a group so that all experimental populations had a total of 30 nymphs.

The first experiment also showed that the wasps from the two fields of each crop within each country did not differ significantly in virulence with one exception; wasps from one French clover field were more virulent than wasps from the other French clover field (see *Results*). Thus, the wasps were pooled where appropriate to create five groups: wasps from alfalfa in New York, wasps from clover in New York, wasps from alfalfa in France, wasps from clover field 1 in France, and wasps from clover field 2 in France. All four test groups of pea aphids were subjected to parasitism by all five groups of wasps in a fully reciprocal lab transplant.

Two replicates of each of the 20 aphid source–wasp source combinations were performed in each of two temporal blocks for a total of four replicates. In the analysis, wasp country, wasp crop, aphid country, and aphid crop were treated as fixed effects. Block, and a dummy variable that distinguished between wasp colonies from the two French clover fields were treated as random effects. All interactions of fixed and random effects were treated as random; none were significant and so they were dropped from the final model.

RESULTS

Experiment 1: Levels of resistance and virulence within countries.—The initial analysis of the data from the within-country reciprocal transplants showed a strong interaction between the crop and the country from which the aphids were collected (aphid crop ×

country effect, Table 1). This indicates that rates of successful parasitism on the aphids from the two crops differed between New York and France (Fig. 2). A linear contrast written to examine the differences in resistance associated with the host races in the two countries indicated that the aphids from New York alfalfa were significantly more resistant to New York wasps (mean rate of parasitism = 0.33) than were the aphids from French alfalfa to French wasps (mean rate of parasitism = 0.60, $F_{1,632} = 13.79$, $P = 0.009$). The rates of parasitism on aphids from clover also differed between the countries, with aphids from New York clover being significantly more susceptible to New York wasps than were aphids from French clover to the average French wasp (mean rate of parasitism of aphids from New York clover = 0.79, and from French clover = 0.58, $F_{1,6.8} = 6.23$, $P = 0.044$). Because of this interaction between aphid crop and country, further analyses were performed separately by country to more easily discern the patterns of resistance and virulence within each country.

The analysis of the reciprocal transplant with New York aphids and parasitoids revealed that, within New York, the aphids from alfalfa were parasitized significantly less than the aphids from clover (Table 2, Fig. 2a), indicating that the aphids from alfalfa are more resistant to parasitism than the aphids from clover, and that the parasitoids are more virulent on aphids from clover than aphids from alfalfa. This confirms the pattern of pea aphid resistance to wasps observed in previous experiments, with populations from New York

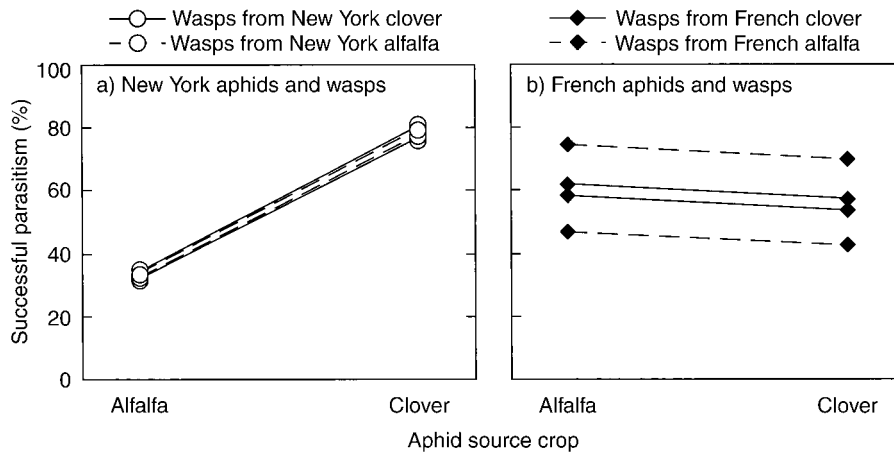


FIG. 2. Norm-of-reaction plots for the within-country reciprocal transplants showing the percentage of aphids from clover and alfalfa successfully parasitized. The lines are for illustrative purposes and are not regression lines. (a) Aphids from New York attacked by wasps from New York. (b) Aphids from France attacked by wasps from France.

and Maryland (Hufbauer and Via 1999, Hufbauer 2001a). The source of the parasitoids (alfalfa or clover) had no effect on the parasitism rate in New York (Table 2, Fig. 2a) indicating that on average the New York wasps from alfalfa and clover do not differ in their ability to overcome aphid resistance. Furthermore, wasps were not adapted to aphids from their home crop (aphid crop \times wasp crop interaction, Table 2, Fig. 2a) or locally adapted to aphids from their home field [aphid field(aphid crop) \times wasp field(wasp crop) interaction, Table 2, Fig. 2a].

The data from the French insects show a very different pattern: aphid crop did not affect rates of parasitism, but the field from which the wasps were collected from did, indicating significant variation in the virulence of wasps from the French fields (Table 3, Fig. 2b). A subsequent contrast revealed that wasps from one of the French clover fields had particularly high mean rates of parasitism (mean = 0.70), while wasps from the other French clover field performed poorly (mean = 0.43, $F_{1,6.06} = 93.14$, $P = 0.0001$, Fig. 2b).

However, as in New York, the French wasps were not adapted to the French aphids from their home crop or field [aphid crop \times wasp crop and aphid field(aphid crop) \times wasp field(wasp crop) interactions, Table 3, Fig. 2b].

Experiment 2: Test for local adaptation between countries.—The reciprocal transplant of aphids from France and New York attacked by wasps from both France and New York provided no evidence of local adaptation of the wasps to the aphids from their own geographic region (wasp country \times aphid country interaction, Table 4). However, there was a very strong wasp country \times aphid crop interaction (Table 4, Fig. 3). This interaction was due to wasps from France (both crops) performing much better than wasps from New York on aphids from alfalfa, ($F_{1,2.91} = 73.53$, $P = 0.004$, Fig. 3), and performing slightly worse, though not significantly so, on aphids from clover than the wasps from New York ($F_{1,2.94} = 4.30$, $P = 0.132$, Fig. 3). This was true whether the aphids were from New York (Fig. 3a) or from France (Fig. 3b), and whether the

TABLE 2. Mixed-model ANOVA for proportion of pea aphids from alfalfa and from clover successfully parasitized by *A. ervi* from alfalfa and clover in a fully reciprocal transplant, using aphids and wasps from New York collections.

Source	Type III <i>F</i>	df	<i>P</i>
Fixed effects			
Aphid crop	75.38	1, 2	0.013
Wasp crop	1.41	1, 2	NS
Aphid crop \times wasp crop	0.22	1, 53	NS
Source	Likelihood ratio	df	<i>P</i>
Random effects			
Block	0.08	1	NS
Aphid field(aphid crop)	0.46	1	NS
Wasp field(wasp crop)	0.22	1	NS
Aphid field(crop) \times wasp field(crop)	0	1	NS

TABLE 3. Mixed-model ANOVA for proportion of pea aphids from alfalfa and from clover successfully parasitized by *A. ervi* from alfalfa and clover in a fully reciprocal transplant, using aphids and wasps from French collections.

Source	Type III <i>F</i>	df	<i>P</i>
Fixed effects			
Aphid crop	0.23	1, 10.2	NS
Wasp crop	0.16	1, 2.01	NS
Aphid crop × wasp crop	0.05	1, 10.2	NS
Source	Likelihood ratio	df	<i>P</i>
Random effects			
Block	7.57	1	0.003
Aphid field(aphid crop)	0	1	NS
Wasp field(wasp crop)	15.03	1	<0.0001
Aphid field(crop) × wasp field(crop)	0.45	1	NS

wasps were from alfalfa or clover (solid and dashed lines, respectively, Fig. 3ab). Thus, not only was there no evidence for local adaptation of the introduced wasps, but the introduced wasps performed significantly worse than the French wasps on pea aphids from alfalfa.

There was also a significant four-way interaction between wasp country, wasp crop, aphid country, and aphid crop, however the magnitude of this effect was much smaller than the two-way interaction, as indicated by the size of the *F* statistics (Table 4). The significance of the four-way interaction was due to several smaller differences in parasitism rate and can be visualized in the crossing of the norm of reaction lines connecting wasps from each country in Fig. 3.

DISCUSSION

Patterns of aphid resistance and wasp virulence within introduced New York populations and native French populations.—The pattern of parasitism of pea aphids by *Aphidius ervi* within New York clearly differs from the pattern within France. As found previously, pea aphids from clover in New York are very susceptible to New York wasps, while pea aphids from alfalfa in New York are very resistant (Hufbauer and Via 1999, Hufbauer 2001a). However, French aphids from both clover and alfalfa are relatively susceptible to French parasitoids. Thus, the overall patterns of aphid resistance and wasp virulence observed in New York do not appear to be imported unchanged from European populations. One difference is that the French wasp

TABLE 4. Mixed-model ANOVA for proportion of pea aphids from alfalfa and from clover in both New York and France that were successfully parasitized by *A. ervi* from alfalfa and clover in both New York and France (W stands for wasp and A stands for aphid).

Source	Type III <i>F</i>	df	<i>P</i>
Fixed effects			
Wasp country	14.37	1, 1.58	0.091
Wasp crop	0	1, 1.58	NS
Aphid country	3.65	1, 61	0.061
Aphid crop	38.36	1, 61	0.0001
Wcountry × W crop	0.24	1, 1.58	NS
A country × A crop	0.07	1, 61	NS
W country × A country	0.65	1, 61	NS
W country × A crop	105.35	1, 61	0.0001
W crop × A country	0.30	1, 61	NS
W crop × A crop	0	1, 61	NS
W country × A country × A crop	0.11	1, 61	NS
W country × W crop × A country	0.17	1, 61	NS
W country × W crop × A crop	1.13	1, 61	NS
W crop × A country × A crop	0.07	1, 61	NS
W country × W crop × A country × A crop	6.26	1, 61	0.015
Source	Likelihood ratio	df	<i>P</i>
Random effects			
Block	4.61	1	0.016
French wasp field code	2.20	1	0.069

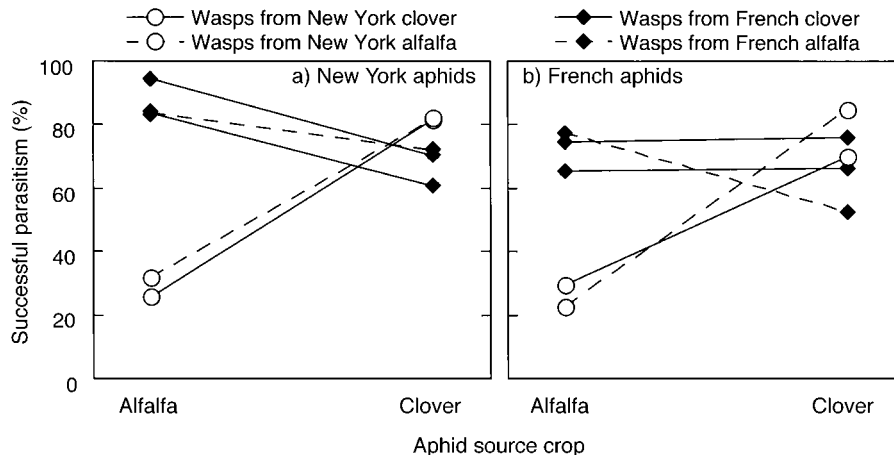


FIG. 3. Norm-of-reaction plots for the France–New York reciprocal transplant showing the percentage of aphids from alfalfa and clover successfully parasitized. (a) New York aphids from clover and alfalfa attacked by wasps from both France and New York. (b) French aphids from clover and alfalfa attacked by wasps from both France and New York.

populations are more variable in virulence than the New York wasp populations. Because the French and New York wasps had been raised in the same environment for a minimum of one generation before the experiment ran, this difference is unlikely to be environmental, and may have a genetic basis. This suggests that the process of introducing the wasps as a biological control agent has reduced genetic variation in an important quantitative trait: virulence. Alternatively, the selective regime might differ between France and New York in a way that has led to a reduction of standing genetic variation.

The reciprocal transplant design employed in the first experiment allows me to test for adaptation of wasps *within* each country to the aphids from their home crop. However, as in previous experiments (Hufbauer 2001a), there is no evidence that wasps from clover and alfalfa are adapted to the aphids from their home crop, within either France or New York.

Patterns of aphid resistance between New York and France.—The difference in successful parasitism rates of aphids from alfalfa when attacked by wasps from France and New York are striking. Aphids from New York and French alfalfa are very resistant to New York wasps, and very susceptible to French wasps. This suggests that the aphids from alfalfa in New York did not evolve greater resistance to parasitism since their introduction, because the French populations in France are also resistant.

Previous work (Hufbauer and Via 1999) has demonstrated that differences in pea aphid resistance to parasitism in New York are due to a genetically based physiological response as found in Henter and Via (1995), and are not a function of aphid or wasp behavior, or of differences in host-plant chemistry. Here, I find that the differences in resistance between aphids from the two crops are consistent between the two countries. This suggests that the pea aphids may have

colonized North America from Europe as two distinct groups, one specialized on clover and one on alfalfa, that were already differentiated with respect to the physiological factors that confer resistance to the introduced parasitoid. The possibility of separate aphid colonization events makes sense in light of what is known about the extreme host-plant specificity of pea aphids in North America (Via 1991a, b, 1994) and Europe (Sandstrom 1994a, b, 1996), and data on high rates of gene flow within both crops and low rates between alfalfa and clover (Via 1999). However, mtDNA sequence data do not support the hypothesis that pea aphids specialized on the two crops represent phylogenetically distinct host races (Boulding 1998). These different conclusions may be reconciled if the differentiation of the aphids into host-races is a recent phenomenon. In this case, the mtDNA tree may not yet reflect that lineage sorting (Hudson 1990).

Patterns of parasitoid virulence between New York and France: a test for local adaptation of the introduced wasps.—There is no evidence of local adaptation of the introduced New York wasps to aphids from New York, despite predictions that successful biological control agents are locally adapted. On the contrary, the introduced wasps are less virulent than the French wasps when attacking the aphids from alfalfa. The reduction in virulence of the introduced wasps is surprising given the large number of wasps imported from France.

Aphidius ervi in North America are thought to be descendants of individuals collected from French alfalfa. The difference in the ability of wasps from France and New York to successfully parasitize aphids from alfalfa might be explained if the wasps I collected are not representative of those that were introduced for biological control. For example, in 1959 the researchers could have collected from a population that was for some reason less virulent on aphids from alfalfa than

from clover. This seems unlikely since the collection was made from an alfalfa field. Furthermore, there is no evidence from the French reciprocal transplant that sampling from one particular population would give rise to the low virulence on alfalfa seen in the introduced wasps. However, there may be greater variation within France than my samples from a relatively small region indicate.

Another explanation for the difference in the performance of the French and New York wasps is that the French populations have evolved to overcome resistance in the alfalfa specialists in a coevolutionary arms race. French wasps may have evolved greater virulence on the resistant aphids from alfalfa subsequent to the 1959 collections for introduction to North America. If this is the case, then the populations from which I sampled would not be representative of those collected for biological control. This explanation suggests that even the large sample of wasps taken from France for the introduction was not sufficient to ensure the adaptability of the introduced populations. Native populations in France may have been able to adapt to the aphids from alfalfa faster than the introduced populations, because the populations were presumably large from 1959 on, and genetically more variable than the introduced population.

If the wasps I collected are representative of the populations imported for biological control, then the data from the reciprocal transplant experiments suggest that either at the time of introduction or since the wasps were introduced in 1959, the North American populations of wasps have evolved to become less virulent on aphids from alfalfa than the native French wasps. There are four non-mutually exclusive ways that this may have occurred.

1) The introduced wasps may have become adapted to laboratory conditions (e.g., Frankham et al. 1986, Frankham and Loebel 1992) in ways that made them less fit in the field environment. For example, if prior to their introduction the wasps were raised in the lab on pea aphid genotypes that were particularly susceptible to parasitism, then the ability of the wasps to develop successfully in alfalfa specialists may have been lost. A rapid loss of virulence upon relaxation of selection requires the additional assumption of a cost to virulence, either a quantitative cost, or a trade-off with some other trait important to fitness in the laboratory environment. Such adaptation to lab conditions would be potentially maladaptive in the field, and also would further reduce genetic variation. However, if this were the explanation then we should expect to see the evolution of increased virulence on aphids from alfalfa, which is not yet apparent. This is surprising, given the substantial genetic variation in virulence found by Henter (1995).

2) Similarly, a negative genetic correlation between virulence on aphids from alfalfa and other traits that are important to fitness in the novel New York field

environment could lead to a correlated reduction in virulence.

3) One of the mechanisms by which the parasitoids overcome the physiological defenses of pea aphids specialized on alfalfa may have been compromised during the introduction process. For example, *Aphidius ervi* eggs release cells called teratocytes into the host hemolymph that contribute to overcoming host defenses (Falabella et al. 2000). If a mutation arose in the genes coding for these cells when the introduced population was small, then it could have become fixed through genetic drift even if it was detrimental. Crosses of French and New York wasps and comparisons of their teratocyte production could be used evaluate this hypothesis.

4) The bottleneck imposed during the introduction process itself may have detrimentally affected the wasps. For example, the North American wasps may have lost genetic variation during the introduction that was vital in their ability to successfully parasitize aphids from alfalfa (Unruh et al. 1983), or epistatic interactions between loci may have been altered in a way that reduced virulence. The significant variation in virulence among wasp populations from France and corresponding lack of variation in virulence among populations of wasps from New York support this possibility.

Conclusions

Introduced *A. ervi* in New York perform very poorly on pea aphids specialized on alfalfa relative to native populations of *A. ervi* in France. Whatever the explanation for the differences in virulence of wasps from France and New York, the practical implication of this work for the pea aphid-*A. ervi* system is that a re-introduction of wasps from France should be considered. Field survey data (Hufbauer 2001b) suggest that the poor performance of the introduced wasps on pea aphids specialized on alfalfa inhibits their ability to control pea aphid populations in alfalfa. Thus, the more virulent wasps from France may be able to suppress pea aphid populations in alfalfa more effectively.

This comparison of native and introduced populations of a biological control agent reveals surprising differences between the populations. Similar comparisons are needed in other biological control systems to determine whether differences of this magnitude are common. With enough comparative data, patterns may emerge indicating which methods of introduction or taxa are associated with detrimental changes vs. local adaptation in biological control agents.

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LITERATURE CITED

- Angalet G. W., and R. Fuerster. 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.
- Baker H. G., and G. L. Stebbins, editors. 1965. *The genetics of colonizing species*. Academic Press, New York, New York, USA.
- Barton, N. H., and B. Charlesworth. 1984. Genetic revolutions, founder effects and speciation. *Annual Review of Ecology and Systematics* **15**:133–164.
- Bazzaz, F. A. 1986. Life history of colonizing plants: some demographic, genetic, and physiological features. Pages 96–110 in H. A. Mooney and J. A. Drake, editors. *Ecology of Biological Invasions of North America and Hawaii*. Springer-Verlag, Berlin, Germany.
- Blossey, B., and J. Kamil. 1996. What determines the increased competitive ability of invasive nonindigenous plants? Pages 3–9 in V. C. Moran and J. H. Hoffmann, editors. *Proceedings of the IX International Symposium on Biological Control of Weeds*. University of Cape Town, Cape Town, South Africa.
- Blossey, B., and R. Notzold. 1995. Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *Journal of Ecology* **83**:887–889.
- Boulding, E. G. 1998. Molecular evidence against phylogenetically distinct host races of the pea aphid (*Acyrtosiphon pisum*). *Genome* **41**:769–775.
- Bush, G. L. 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). *Evolution* **23**:237–251.
- Carson, H. L. 1990. Increased genetic variance after a population bottleneck. *Trends in Ecology and Evolution* **5**:228–230.
- Carton, Y., and A. Nappi. 1991. The *Drosophila* immune reaction and the parasitoid capacity to evade it: genetic and coevolutionary aspects. *Acta Oecologia* **12**:89–104.
- Crawley, M. J. 1989. The successes and failures of weed biocontrol using insects. *Biocontrol News and Information* **10**:213–223.
- Crow, J. F. 1964. Breeding structure of populations. II. Effective population size. Pages 543–557 in O. Kempthorne, T. A. Bancroft, J. W. Gowen, and J. L. Lush, editors. *Statistics and mathematics in biology*. Hafner, New York, New York, USA.
- Debach, P., editor. 1964. *Biological control of insect pests and weeds*. Chapman and Hall, London, UK.
- Eastop, V. F. 1973. Keys for the identification of *Acyrtosiphon* (Hemiptera: Aphididae). *Bulletin of the British Museum (Natural History) Entomology* **26**:1–115.
- Falabella, P., E. Tremblay, and F. Pennacchio. 2000. Host regulation by the aphid parasitoid *Aphidius ervi*: the role of teratocytes. *Entomologia Experimentalis et Applicata* **97**:1–9.
- Fellowes, M. D. E., and J. M. J. Travis. 2000. Linking the coevolutionary and population dynamics of host–parasitoid interactions. *Population Ecology* **42**:195–203.
- Force, D. C. 1967. Genetics in the colonization of natural enemies for biological control. *Annals of the Entomological Society of America* **60**:722–729.
- Frankham, R. 1998. Inbreeding and extinction: island populations. *Conservation Biology* **12**:665–675.
- Frankham, R., H. Hemmer, O. A. Ryder, E. G. Cothran, M. E. Soulé, N. D. Murray, and M. Snyder. 1986. Selection in captive populations. *Zoo Biology* **5**:127–138.
- Frankham, R., and D. A. Loebel. 1992. Modeling problems in conservation genetics using captive *Drosophila* populations: rapid genetic adaptation to captivity. *Zoo Biology* **11**:333–342.
- Godfray, H. C. J. 1993. *Parasitoids*. Princeton University Press, Princeton, New Jersey, USA.
- Gonzalez, D., K. S. Hagen, P. Stary, G. W. Bishop, D. W. Davis, and K. S. Pike. 1995. Pea aphid and blue alfalfa aphid. Pages 129–135 in J. R. Nechols, L. A. Andrew, J. W. Beardsley, R. D. Goeden, and D. G. Jackson, editors. *Biological control in the Western United States*. University of California Division of Agriculture and Natural Resources Publication 3361.
- Goodnight, C. J. 1987. On the effect of founder events on epistatic genetic variance. *Evolution* **41**:80–91.
- Goodnight, C. J. 1988. Epistasis and the effect of founder events on the additive genetic variance. *Evolution* **42**:441–454.
- Green, D. M., A. R. Kraaijeveld, and H. C. J. Godfray. 2000. Evolutionary interactions between *Drosophila melanogaster* and its parasitoid *Asobara tabida*. *Heredity* **85**:450–458.
- Grevstad, F. S. 1999a. Experimental invasions using biological control introductions: the influence of release size on the chance of population establishment. *Biological Invasions* **1**:313–323.
- Grevstad, F. S. 1999b. Factors influencing the chance of population establishment: implications for release strategies in biocontrol. *Ecological Applications* **9**:1439–1447.
- Hagen, K. S., G. A. Viktorow, K. Yasumatsu, and M. F. Schuster. 1976. Range, forage, and grain crops. Pages 410–442 in C. B. Huffaker and P. S. Messenger, editors. *Theory and practice of biological control*. Academic Press, New York, New York, USA.
- 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.
- Hall, R. W., and L. E. Ehler. 1979. Rate of establishment of natural enemies in classical biological control. *Bulletin of the Entomological Society of America* **25**:280–282.
- Hall, R. W., L. E. Ehler, and B. Bisarbi-Ershadi. 1980. Rate of success in classical biological control of arthropods. *Bulletin of the Entomological Society of America* **26**:111–114.
- 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.
- Hopper, K. R., and R. T. Roush. 1993. Mate finding, dispersal, number released and the success of biological control introductions. *Ecological Entomology* **18**:321–331.
- Hopper, K. R., R. T. Roush, and W. Powell. 1993. Management of genetics of biological-control introductions. *Annual Review of Entomology* **38**:27–51.
- Howarth, F. G. 1983. Classical biocontrol: panacea or Pandora's box? *Proceedings of the Hawaiian Entomological Society* **24**:239–244.

- Hudson, R. R. 1990. Gene genealogies and the coalescent process. Pages 1–44 in D. Futuyma and J. Antonovics, editors. Oxford surveys in evolutionary biology. Volume 7. Oxford University Press, Oxford, UK.
- Hufbauer, R. A. 2001a. Pea aphid–parasitoid interactions: have parasitoids adapted to differential resistance? *Ecology* **82**:717–725.
- Hufbauer, R. A. 2001b. Aphid population dynamics: does resistance to parasitism influence population size? *Ecological Entomology*, in press.
- 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.
- Huffaker, C. B., and P. S. Messenger, editors. 1976. Theory and practice of biological control. Academic Press, New York, New York, USA.
- 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., 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.
- Kerr, W. E., and S. Wright. 1954. Experimental studies of the distribution of gene frequencies in very small populations of *Drosophila melanogaster*. *Evolution* **8**:172–177.
- 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.
- Lawton, J. H. 1990. Biological control of plants: a review of generalisations, rules, and principles using insects as agents. Pages 3–17 in C. Bassett, L. J. Whitehouse, J. A. Zabkiewicz, editors. Alternative to the chemical control of weeds. Proceedings of an International Conference, Rotorua, New Zealand, July 1989. Ministry of Forestry, FRI Bulletin 155.
- Lemasurier, A. D., and J. K. Waage. 1993. A comparison of attack rates in a native and an introduced population of the parasitoid *Cotesia glomerata*. *Biocontrol Science and Technology* **3**:467–474.
- Lewis, M. A., and P. Kareiva. 1993. Allee dynamics and the spread of invading organisms. *Theoretical Population Biology* **43**:141–158.
- Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS system for mixed models. SAS Institute, Cary, North Carolina, USA.
- Louda, S. M., D. Kendall, J. Connor, and D. Simberloff. 1997. Ecological effects of an insect introduced for the biological control of weeds. *Science* **277**:1088–1090.
- Lucas, A. M. 1969. The effect of population structure on the success of insect introductions. *Heredity* **24**:151–157.
- Mackauer, M. 1972. Genetic aspects of insect production. *Entomophaga* **17**:27–48.
- 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.
- Mackauer, M., and T. Finlayson. 1967. The hymenopterous parasites (Hymenoptera: Aphidiidae et Aphelinidae) of the pea aphid in Eastern North America. *Canadian Entomologist* **99**:1051–1082.
- Mayr, E. 1954. Change of genetic environment and evolution. Pages 157–180 in J. Huxley, A. C. Hardy, and E. B. Ford, editors. *Evolution as a process*. Allen and Unwin, London, UK.
- Messenger, P. S., and R. van den Bosch. 1971. The adaptability of introduced biological control agents. Pages 68–92 in C. B. Huffaker, editors. *Biological control*. Plenum, New York, New York, USA.
- Messenger, P. S., F. Wilson, and M. J. Whitten. 1976. Variation, fitness and adaptability of natural enemies. Pages 68–92 in C. B. Huffaker, editor. *Biological control*. Plenum, New York, New York, USA.
- Murray, N. D. 1985. Rates of change in introduced organisms. Pages 191–199 in E. S. Delfosse, editor. *Proceedings of the VI International Symposium on Biological Control of Weeds*, Vancouver, Canada, 1984. Agriculture Canada, Ottawa, Canada.
- Myers, J. H., and M. D. Sabath. 1981. Genetic and phenotypic variability, genetic variance, and the success of establishment of insect introductions for the biological control of weeds. Pages 91–102 in E. S. Del Fosse, editor. *Proceedings of the V International Symposium on Biological Control of Weeds*. Commonwealth Scientific and Industrial Research Organization, Brisbane, Australia 1980. Canberra, Australia.
- Nei, M., T. Maruyama, and R. Chakraborty. 1975. The bottleneck effect and genetic variability in populations. *Evolution* **29**:1–10.
- Neter, J., W. Wasserman, and M. H. Kutner. 1990. Applied linear statistical models. Richard D. Irwin, Boston, Massachusetts, USA.
- Peschken, D. P. 1972. *Chrysolina quadrigemina* (Coleoptera: Chrysomelidae) introduced from California to British Columbia against the weed *Hypericum perforatum*: comparison of behavior, physiology and color in association with post-colonization adaptation. *Canadian Entomologist* **104**:1689–1698.
- Remington, C. L. 1968. The population genetics of insect introduction. *Annual Review of Entomology* **13**:425–426.
- Roush, R. T. 1990a. Genetic variation in natural enemies: critical issues for colonization in biological control. Pages 563–588 in L. Ehler and M. Mackauer, editors. *Critical issues in biological control*. Intercept, Andover, UK.
- Roush, R. T. 1990b. Genetic considerations in the propagation of entomophagous species. Pages 373–387 in R. R. Baker and P. E. Dunn, editors. *New directions in biological control*. Liss, New York, New York, USA.
- Russo, J., M. Brehelin, and Y. Carton. 2001. Haemocyte changes in resistant and susceptible strains of *D. melanogaster* caused by virulent and avirulent strains of the parasitic wasp *Leptopilina boulardi*. *Journal of Insect Physiology* **47**:167–172.
- Salt, G., and R. van den Bosch. 1967. The defense reactions of three species of *Hypera* (Coleoptera: Curculionidae) to an ichneumon wasp. *Journal of Invertebrate Pathology* **9**:164–177.
- Sandstrom, J. 1994a. High variation in host adaptation among clones of the pea aphid, *Acyrtosiphon pisum*, on peas, *Pisum sativum*. *Entomologia Experimentalis et Applicata* **71**:245–256.
- Sandstrom, J. 1994b. Performance of pea aphid (*Acyrtosiphon pisum*) clones on host plants and synthetic diets mimicking the same plants' phloem amino acid composition. *Journal of Insect Physiology* **40**:1051–1057.
- Sandstrom, J. 1996. Temporal changes in host adaptation in the pea aphids, *Acyrtosiphon pisum*. *Ecological Entomology* **21**:56–62.
- 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.
- SAS Institute. 1997. SAS/STAT software: changes and enhancements through release 6.12. SAS Institute, Cary, North Carolina, USA.
- Shigesada, N., and K. Kawasaki. 1997. *Biological invasions: theory and practice*. Oxford University Press, Oxford, UK.
- Simberloff, D., and P. Stiling. 1996. How risky is biological control? *Ecology* **77**:1965–1974.
- Strand, M. R., and L. L. Pech. 1995. Immunological basis for compatibility in parasitoid–host relationships. *Annual Review of Entomology* **40**:31–56.
- Tuda, M., and M. B. Bonsall. 1999. Evolutionary and population dynamics of host–parasitoid interactions. *Researches on Population Ecology* **41**:81–91.
- Unruh, T. R., W. White, D. Gonzalez, G. Gordh, and R. F. Luck. 1983. Heterozygosity and effective population size in laboratory populations of *Aphidius ervi* (Hym.: Aphididae). *Entomophaga* **28**:245–258.
- van den Bosch, R. 1964. Encapsulation of the eggs of *Bathyplectes curculionis* (Thompson) (Hymenoptera: Ichneumonidae) in larvae of *Hypera brunneipennis* (Boheman) and *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae). *Journal of Invertebrate Pathology* **6**:343–367.
- Van Driesche, R. G., and T. S. Bellows Jr. 1996. *Biological control*. Chapman and Hall, New York, New York, USA.
- van Lenteren, J. C. 1983. The potential of entomophagous parasites for pest control. *Agriculture, Ecosystems and Environment* **10**:143–158.
- 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.
- Via, S., and A. J. Shaw. 1996. Short-term evolution in the size and shape of pea aphids. *Evolution* **50**:163–173.
- 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.
- Woodworth, L. M., M. E. Montgomery, R. K. Nurthen, D. A. Briscoe, and R. Frankham. 1994. Modeling problems in conservation genetics using *Drosophila*: consequences of fluctuating population sizes. *Molecular Ecology* **3**:393–399.