

Potential Evolution of Host Range in Herbivorous Insects

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Abstract

Many clades of herbivorous insects are remarkably conservative in the plants that they attack, and in many groups, related insects tend to feed on related plants. However, rapid evolution of host range has been documented in several species. Managers who contemplate introducing a host-specific insect for biological control of a weed would like to predict whether or not the species to be introduced poses an appreciable risk that it might evolve rapidly in host range and adapt to non-target plants. Guidelines as to which plants might most readily be incorporated into the insect's diet may be provided, in some cases, by their phylogenetic relationship to the insect's normal host and by the diet of insects closely related to the proposed control agent. The likelihood of rapid evolution of a shift to a non-target plant may be judged to some extent by screening populations of the insect for genetic variation in behavioral responses to and performance on the plant, since genetic variation is the prerequisite for evolutionary change.

I describe a series of studies on species of *Ophraella* Wilcox (Coleoptera: Chrysomelidae) that were intended to assess the likelihood that constraints on genetic variation might make some imaginable host shifts less likely than others, and might, indeed, have influenced the history of evolution of host association in the genus. This history was inferred from mapping host associations onto a phylogeny based on DNA sequence data. Four species of *Ophraella*, host-specific on various Asteraceae, were assayed for evidence of genetic variation in consumption of and larval survival on several hosts of their congeners. Significant variance among full-sib or half-sib families was taken as evidence of probable genetic variation. In about half the beetle-plant combinations in which consumption was assayed, no evidence of genetic variation was found (in some such instances almost no feeding was recorded). Genetic variation for larval survival on non-host plants was discerned in a small minority of cases. Genetic variation was most often displayed in responses to plants that were relatively closely related to the insect's normal host (i.e., in the same tribe of Asteraceae), but genetically variable feeding on more distantly related plants within the Asteraceae was recorded in some cases.

The results indicate that: (1) all these species harbor genetic variation that might enable rapid adaptation to some plants other than their normal host; and (2) the plants most at risk of adaptation are especially but not exclusively those most closely

related to the insect's normal host. Although this study provides evidence that paucity of genetic variation in responses to novel plants could constrain or influence the direction of the evolution of insect diet, the methods used in this study are inadequate to reveal rare alleles that might enable rapid response to natural selection for expansion of diet. In order to judge the likelihood that a proposed weed control agent might adapt to a non-target plant, large samples of insects should be screened, ideally by selection experiments.

Keywords: adaptation, Chrysomelidae, genetic variation, host specificity, novel host plants, *Ophraella*

Introduction

Although management of pest species by integrated pest management or by biological control has clear advantages over chemical control alone, it is not risk-free. Precautions must be taken against introduction of organisms that may attack not only the target, but also crop plants or other economically important organisms. It is perhaps equally important to guard against introducing organisms that will attack elements of the native biota, and an argument has arisen about whether or not there exist adequate safeguards against this potential danger (e.g., Howarth, 1991; DeLoach, 1991; Simberloff and Stiling, 1996). For instance, the predatory snail *Euglandina rosea* (Ferrusac), widely introduced to control the African snail *Achatina fulica* Bowditch, has extinguished many endemic species of tree snails in the Hawaiian and Tahitian archipelagoes, and the weevil *Rhinocyllus conicus* (Frölich), released in the United States to control several Eurasian thistles, is severely reducing seed production of several native thistles as well (Louda et al., 1997). It is well understood that in order to avert such disasters, potential biological control agents must be tested for specificity. The ideal biocontrol agent will attack only the target pest species, and no others.

As an outsider to the field of biological control, I would not presume to prescribe testing procedures, and indeed am not familiar with standard procedures in any detail. I assume that in screening herbivorous arthropods for potential control of weeds, non-target plant species are presented to the arthropod in no-choice tests, since this would resemble the decision-making context for dispersing insects that encounter plants singly. (*Note: No-choice tests are not always used. In some instances, choice tests are used in which plant species are presented concurrently or in sequence; see Heard and van Klinken [1998] for a review of testing*

procedures used for screening weed biocontrol agents.)

Likewise, intraspecific variation in plants may affect the outcome of feeding or oviposition trials. Taking these and other considerations into account, it may be possible to achieve considerable confidence that a non-target species of plant is not acceptable to the arthropod population - at the present time.

There exists, however, the possibility that the control agent, having been released, will adapt to the non-target plant and become, itself, a pest in the future. That is, evolutionary change in the introduced species may alter its specificity, or host range (Roderick, 1992; Simberloff and Stiling, 1996).

Evolutionary changes that have transpired within the last century have been documented in hundreds of species of organisms (Bishop and Cooke, 1981; Travis and Futuyma, 1993; Thompson, 1998; Futuyma, 1998). Most such changes have occurred in response to human alterations of a species' environment, or in populations that have been transplanted into new environments. The most conspicuous and familiar examples are the evolution of antibiotic resistance in many bacteria and other disease-causing organisms and of insecticide resistance in more than 500 species of insects and other arthropods (Metcalf and Luckmann, 1994). Other examples of rapid evolutionary change include changes in migration patterns of birds, life history features of fishes, and the ability of insects and other crop pests to attack previously resistant crop varieties (Travis and Futuyma, 1993). Of particular relevance to biological management of weeds are the several well-documented cases in which insects have altered their host range within the last century by adapting to introduced plants (Thompson, 1998). For instance, populations of the checkerspot butterfly *Euphydryas editha* Boisduval have added the plantain *Plantago lanceolata* L. (Plantaginaceae) to their diet (of

several Scrophulariaceae) (Singer et al., 1993); the rhopalid bug *Jadera haematoloma* (Herrich-Schaeffer) has adapted morphologically and physiologically to several introduced Sapindaceae (Carroll et al., 1997); the clouded sulfur *Colias philodice* Godart has added alfalfa (*Medicago sativa* L.) to its diet of native legumes (Tabashnik 1983); and the apple maggot fly (*Rhagoletis pomonella* [Walsh]) adapted to and became a serious pest of apple, on which it has formed a distinct “host race” from the native hawthorn-feeding population, and is evidently becoming a distinct species (Feder et al., 1990; Filchak et al., 1999). It has been clear for some time to evolutionary biologists that populations of most organisms have the potential ability to evolve rapidly in many of their characteristics - so rapidly that their ecological interactions with other species, including humans, may change appreciably on the scale of decades (Thompson, 1998).

Evolution consists of change in the genetic composition of populations: changes in the proportions of different genotypes. In some cases, a prevailing genotype may be completely replaced by another; in other instances, proportions are less fully altered, and no one genotype is fixed (i.e., reaches a frequency of 100%). The change in proportions may result in some instances from genetic drift (i.e., random changes due to accidents of sampling, but adaptive changes result from natural selection, a nonrandom difference in reproductive success between genotypes, due often to their interaction with environmental factors). Because evolution consists of genetic change, it cannot occur unless there exists genetic variation, consisting of two or more alleles at a gene locus, or at some of the several or many loci, that affect a characteristic. Each such allele arises *de novo*, by mutation, at a low rate, so if a population is initially genetically homogeneous, the “waiting time” for genetic variation to arise and enable evolution to occur may be quite long. In most populations, however, mutations at many loci that have arisen in the past persist for a considerable time, so that many characters are genetically variable and can change to at least some degree almost immediately if changes in the environment alter the regime of natural selection, and favor a different character state. Indeed, over the last 50 years, population geneticists have so consistently found genetic variation in the features of diverse organisms that the majority of workers are inclined to think that evolution is seldom constrained by lack of genetic variation (Lewontin, 1974; Barker and Thomas, 1987). Most of the time, characters do not evolve noticeably

simply because the environment selects for a stable, quasi-optimal trait, or because the selection regime fluctuates without favoring change consistently in any one direction (Endler, 1986). But a consistent change in the selection regime, as when a population is introduced into a new region that differs in climate and possible food sources, is likely to evoke rapid, often substantial, genetic responses.

Managers who contemplate releasing a species into a new region, such as an insect that promises to control a weed, should assume that the population will undergo some evolutionary changes. (Indeed, if it is so genetically homogeneous that the capacity for evolutionary adaptation is unlikely, the population probably has a dim future.) The question is whether these changes are likely to include expansion of diet to include native plants or crops. Since it is impossible to test the insect against all the plant species it will encounter in its new home – or in places it might disperse to from the site of introduction – it would be useful to judge which plants might be most at risk of becoming included in the insect’s diet.

Phylogenetic Patterns

A conspicuous pattern in the diet of many, although not by any means all, groups of herbivorous insects is that related species tend to feed on related plants. That is, species in a higher taxon of insects, such as a genus or subfamily, commonly feed on taxonomically related plants, often in the same family. This pattern, long known to insect systematists, was the basis of an influential theory of coevolution by Ehrlich and Raven (1964), who proposed that chemical compounds shared by related plants (due to common descent) elicit feeding and egg-laying by specialized insects. Similar responses to chemical stimuli are shared by insect species derived from recent common ancestors. Subsequent research has provided some confirmation of this hypothesis. For instance, iridoid glycosides are feeding and oviposition stimulants for species of *Euphydryas* butterflies (Bowers 1991); these compounds characterize the butterflies’ host plants in the Scrophulariaceae, as well as the Plantaginaceae, a lineage of Scrophulariaceae that has become adapted for wind-pollination. That *E. editha* has recently expanded its diet to include *Plantago* is thus readily understandable.

Many classes of insects are remarkably conservative in diet. For example, all species in the butterfly tribe Heliconiini feed on Passifloraceae as larvae; all

tetraopine cerambycid beetles feed on Asclepiadaceae or the closely related Apocynaceae; among the true fruit flies (Tephritidae), the huge subfamily Tephritinae is almost exclusively associated with Asteraceae. Based on the fossil record, biogeography, and levels of DNA sequence divergence, it is clear that many such monophyletic groups are 40 to 60 million years old, or even older (Mitter et al., 1991; Mitter and Farrell, 1991; Farrell and Mitter, 1993). The most parsimonious interpretation is that these lineages have retained much the same host association throughout their long history of diversification while continents have moved, climates have changed drastically, and whole orders of mammals have originated and become extinct. Evolution may be rapid in some respects, yet slow in others: for example, a molecular phylogeny of the aphid clade consisting of the genera *Uroleucon* and *Macrosiphoniella* implies that the 319 described species have evolved in only 5-10 million years, yet they have retained similar host associations, all feeding on Asteraceae or the closely related family Campanulaceae (Moran et al., 1998).

Such examples of very conservative feeding habits strongly suggest that there exist constraints on the ability of these insects to adapt to plants distantly related from their normal hosts. On the other hand, these constraints are not universal among insects. In some clades, related species are host-specific, but on distantly related plants; for example, species of the leaf beetle genus *Tricholochmaea* specialize on willow (Salicaceae), blueberry (Ericaceae), or meadowsweet (Rosaceae), which tend to grow in similar habitats but do not otherwise have obvious traits in common. In some taxa, one or a few species depart far from an otherwise conservative pattern; in the large leaf beetle genus *Trirhabda*, for example, all the species feed on Asteraceae except for two that have Hydrophyllaceae species as hosts. In other cases, many species have broad diets, and their more specialized relatives may occupy a great variety of plant taxa (e.g., the aphid genus *Aphis*).

Nevertheless, a pattern of strong phylogenetic conservatism of diet in a higher taxon that includes a potential biocontrol agent does suggest that the non-target plants at greatest risk of unintended attack are those closely related to the insect's known normal hosts. Even if the insect does not show an immediate ability to feed, survive, and reproduce on a related plant, we may hypothesize that plants closely related to its normal hosts would be more likely to elicit adaptation than distantly related plants. More generally, we might be concerned about adaptation not only to plants that are closely

related to the insect's normal host, but also plants that are hosts to the near relatives of the insect. For example, if a species of *Trirhabda* were a candidate for release, one might be concerned about the possibility of its evolving the ability to feed not only on various non-target Asteraceae, but also Hydrophyllaceae.

Plant taxonomy is already used as a guide to screening biocontrol agents for specificity. For instance, the reliably reported host plants of the American leaf beetle *Ophraella communa* LeSage include *Ambrosia* (ragweeds) and several other genera of Ambrosiinae, a subtribe of the tribe Heliantheae, family Asteraceae. Palmer and Goeden (1991) tested this insect's responses to several species of Heliantheae and found that it reproduced and survived successfully on cultivated sunflower, *Helianthus annuus* L. Hence the species was considered unsuitable for introduction into Australia as a control agent for *Ambrosia artemisiifolia* L. and two other adventive ambrosiine weeds. It must be noted that the use of plant taxonomy in this way depends on accurate assessment of evolutionary, i.e., phylogenetic, relationships among plant species. If *Ambrosia* had been wrongly classified with sagebrush (*Artemisia*) in the tribe Anthemideae, on the basis of their convergently similar wind-pollinated flowers, Palmer and Goeden might well not have been led to test this beetle's response to sunflower. By the same token, the feeding habits of insect species closely related to a proposed biocontrol agent might legitimately alert us to possibly susceptible non-target plant species only insofar as the phylogenetic relationships among insects are accurately known.

Chemical and Phenetic Similarity of Plants

Dethier (1954), and later Ehrlich and Raven (1964), suggested that insects adapt most readily to plants that share key features with their ancestral hosts, and that this accounts for the association of related insect species with related plant species. The key features, they suggested, are often the "secondary compounds" that characterize higher taxa of plants, such as the glucosinolates of Brassicaceae (mustards) and the cardiac glycosides of Asclepiadaceae (milkweeds). Thus one might propose to ignore phylogenetic relationships among plants, and test the responses of candidate insect species against native or cultivated plants that are chemically similar to the insect's normal hosts.

Although reasonable in principle, this may not always be a practical approach. First, the plant characters that may have acted as “bridges” for the evolution of new host associations seem not to be chemical in all cases, as illustrated by insect taxa whose various host plants have in common their habitat rather than their chemistry (Mitter and Farrell, 1991). Second, plants generally have not one secondary compound but many, often representing several very different chemical families. These compounds affect both insect behavior and post-ingestion physiology by acting as toxins or interfering with digestion. Behavioral and physiological responses of insects to such compounds are often very complex (Rosenthal and Berenbaum, 1992; Bernays and Chapman, 1994). Although in some insects a single compound may elicit feeding or oviposition, and so account for host specificity, it is far more common for these behaviors to be based on a multifactorial response to several or many compounds. Some compounds act as stimulants, others as deterrents, often with synergistic effects. Determination of these effects may require assaying responses to compounds not only singly, but also in combinations. The Colorado potato beetle (*Leptinotarsa decemlineata* [Say]), for instance, is attracted to a complex blend of volatile compounds produced by its *Solanum* hosts, not to any single compound. Its feeding behavior is stimulated not by any identifiable *Solanum*-specific compounds (much less by the steroidal glycoalkaloids that are most characteristic of these plants), but instead by a wide array of compounds that are not host-specific (Mitchell, 1988; Hare, 1990). Thus individual compounds shared by plants (such as the steroidal glycoalkaloids of *Solanum*) may not play any role in the adaptation of insects to new hosts, and the similarity of plant species with respect to their overall chemical profile likewise need not predict host associations, especially in those cases in which a few critical compounds do indeed play a leading role.

Genetic Variation

Another approach may be to screen populations of an insect species (being considered as a biological control agent) for genetic variation in its capacity to oviposit, feed, and develop on non-target plant species. Note that we are not concerned with the simple question of whether or not a small sample of insects will readily attack a plant, which might indicate that the species might immediately include the plant in its diet if released. Rather, we are asking if a plant that is rejected

by the majority of the population, or which is unsuitable for development of the majority, may nevertheless become a suitable host plant due to rapid evolution of the introduced insect population. Showing that the population harbors genetic variation in features required to develop on the plant may indicate that the population could readily adapt to the non-target species. The prudent course of action would be to assume that such evolution could occur. However, it is impossible ever to say that an insect species absolutely lacks now and must forever lack the genetic variation that is the prerequisite for such adaptation to occur. I return to this point below.

An Example: *Ophraella* Leaf Beetles and Their Hosts

The approach of screening for genetic variation in an insect species' responses to potential future host plants is illustrated by work in my laboratory on genetic variation in the leaf beetle genus *Ophraella* (Futuyma et al., 1993, 1994, 1995). This research was undertaken in order to determine if the course of historical evolution of host shifts in this genus may have been influenced by genetic constraints on variation. We tested the hypothesis that populations harbor genetic variation in responses to only certain plants, so that adaptation to new hosts has been restricted to a limited number of possibilities. Thus according to this hypothesis, the plant species actually adopted as hosts during the course of evolution of *Ophraella* were more likely to have been adopted, due to genetic constraints, than many other plants that were available. It should surprise no one if species in a genus that feeds on Asteraceae (sunflower family) were to display absolutely no ability at all to feed on, say, ferns, club mosses, or pines. Thus, in order to restrict our analysis to plants that could be regarded as plausible potential hosts, we screened species of *Ophraella* for genetic variation in responses to plants that are hosts of other species of *Ophraella*, species that are either very close or relatively distant relatives of the particular species tested. Thus, if limits on genetic variation had closely guided the evolution of host shifts, we might expect a species to display genetic variation in response to the host of its nearest relative, but with lesser likelihood to the host of a phylogenetically more remote species of *Ophraella*. It might be noted that many or most population geneticists would expect genetic variation to be revealed in a species' responses to any of the plants on which they were tested, since genetic variation has been found in most characters of organisms, when sought.

Ophraella (Coleoptera: Chrysomelidae, Galerucinae) is a North American genus with at least 13 species (LeSage, 1986; Futuyma, 1990, 1991). Both larvae and adults are externally feeding folivores. Oviposition and usually pupation occur on the host plant. Adults overwinter; some species are univoltine, but most appear to be multivoltine, with egg-to-egg generation time (in the laboratory) of a month or slightly more (Futuyma, 1990). The hosts fall into four tribes of the Asteraceae (Compositae). Some species of *Ophraella* have been recorded from only a single host species, but most are known from several congeneric hosts, and *Ophraella communis* LeSage, which is geographically variable in host association, has been found breeding on several genera in two subtribes of the tribe Heliantheae.

Because this work addressed the relation of patterns of genetic variation to the actual history of evolution of host associations of these insects (an issue that would not necessarily arise in screening biocontrol agents), part of the research program consisted of inferring phylogenetic relationships among the species of *Ophraella*, to provide a framework for inferring a most likely history of host associations. First, using morphological and allozyme data, and later DNA sequences (866 base pairs of the mitochondrial cytochrome oxidase I and 16s ribosomal RNA genes), we obtained an estimate of phylogeny, in which most clades are well supported statistically (Funk et al., 1995). The beetle phylogeny does not closely match that of the host plants, and the levels of DNA sequence divergence among species of *Ophraella* strongly suggest that they have originated much more recently than the divergence of the several tribes of Asteraceae that include their host plants. Thus most of the diversity of host plant use has arisen as populations or species have shifted from one host plant to another, rather than by cospeciation and contemporaneous divergence of insect-plant associates. These host shifts, however, have been rather conservative: the phylogeny implies that about eight of the approximately twelve host shifts in the history of the group have been between genera in the same tribe of plants, and only four shifts between tribes have occurred.

We screened four species of *Ophraella* for genetic variation in responses to their normal hosts, as well as to five or six plant species that are hosts of species of *Ophraella* other than the focal species. For example, *O. communis* was tested for responses not only to one of its normal hosts, the common ragweed *Ambrosia*

artemisiifolia, but also to *Solidago bicolor* L., *Solidago altissima* L., *Chrysopsis villosa* (Pursh) Nutt. Ex DC., *Eupatorium perfoliatum* L., *Artemisia vulgaris* L. and *Iva frutescens* L., which are the respective hosts of *Ophraella pilosa* LeSage, *O. conferta* (LeConte), *O. bilineata* (Kirby), *O. notata* (Fabricius), *O. artemisiae* Futuyma, and *O. notulata* (Fabricius). (*Artemisia vulgaris* was actually a surrogate for the species of *Artemisia* that *O. artemisiae* normally feeds on.) These species of *Ophraella* span the range from the closest to the most distant relative of *O. communis*, and their host plants are included both in the same tribe as *O. communis*'s normal hosts and in the other tribes of Asteraceae. We assayed behavioral (feeding) responses to these plants, and in most cases we assayed larval survival as well. Feeding responses were tested for neonate larvae in 22 *Ophraella*-plant combinations (other than on the species' normal host plant), and for newly eclosed adults in 18 such combinations, by placing insects individually in small petri dishes with discs of leaf tissue of a single plant species (no-choice tests) on moist filter paper. Consumption was measured 24 h later (and in a few cases repeatedly thereafter) by counting squares in an ocular grid in a dissecting microscope. The adults used in consumption tests had been reared on their normal host plant. In tests of larval survival, we maintained individual larvae in dishes with leaf fragments of the test plant, which were replaced every 2 days until death or pupation. Further details are described in the original papers (Futuyma et al., 1993, 1994, 1995).

We assumed that the methods of quantitative genetics (Falconer and Mackay, 1996) are appropriate for assaying genetic variation in phenotypic characters of this kind. Our main interest was in determining whether or not genetic variance could be detected, on the principle that heritable characters display greater variation among than within families (i.e., significant among-family variance). We succeeded in most cases in using a half-sib design, in which each male was mated to two or more virgin females, and a trait (such as consumption of *Ambrosia*) was scored on several offspring of each female. Significant variance among sires (i.e., among families of half-sibs) is generally taken to indicate additive genetic variance, the kind of genetic variation that enables ready response to natural selection. Significant variance among dams (females) within sires may be attributed not only to additive genetic variance, but also to nonadditive genetic variance (owing to dominance and epistasis), to maternal effects (including both nongenetic effects and

common environment that full sibs often share (e.g., neonate feeding on a leaf on which the eggs were laid, before dispersal or removal). My concern in these experiments was to search for evidence of possible constraints on the genetic variation needed for host shifts to evolve, so any data interpretable as evidence of genetic variation would count against my hypothesis that genetic constraints exist, and in favor of the widespread belief that paucity of genetic variation does not generally affect the direction of evolution. Therefore, instances of significant variance among dams were counted as evidence for genetic variation (even though maternal effects or common environment cannot be entirely ruled out) because doing so provided a bias against my hypothesis. (If I were screening a proposed biological control agent, I might take the same position, on the grounds that it would be prudent to assume that the insect population harbors genetic variation enabling it to adapt to a non-target plant, unless rigorous screening strongly suggested otherwise.)

In most of the assays, we tested progeny of 20 to 40 sires, each mated to 2 virgin dams (cf. Table 1). The parents were taken from one locality or a few sites near each other. All populations displayed genetic variation in feeding responses to at least one plant species, so none of them was lacking in genetic variation due to inbreeding. (Electrophoretic studies and DNA sequence variation have shown that local populations of all *Ophraella* species are typically as highly heterozygous as insect species generally [Futuyma and McCafferty, 1990; Knowles et al., 1999].)

Table 1 presents a sample of the data on variation in larval feeding responses. The original papers (Futuyma et al., 1993, 1994, 1995) may be consulted for the full data, a summary of which is provided by Futuyma et al. (1995). From the point of view of possible genetic constraints, perhaps the most important result of these studies was that in 14 of the 16 tests of larval survival, and in 18 of 39 tests of larval or adult feeding on plants other than the insect's normal host, no genetic variation was demonstrable. In 10 combinations of beetle and plants, no larvae survived to pupation, and in only 2 of the other 6 cases was there evidence of genetic variation, manifested as significant variance among full-sib families (from different mothers), among half-sib families (progenies of different males), or both. These 2 cases involved 2 species that feed on different members of the subtribe Ambrosiinae (*Ambrosia artemisiifolia* and *Iva frutescens*): each displayed genetic variation in survival on the other's host.

Certainly a major cause of larval mortality was failure to feed, although in some instances death occurred after several days of feeding, suggesting that toxic or other post-ingestion effects also played a role. For larval consumption, we found evidence of genetic variation (Table 1) in 15 of the 22 combinations; of the 7 combinations in which no genetic variation was discerned, virtually no feeding occurred in 6; at most, trace feeding was exhibited by a small minority. Of 17 tests of adult consumption, evidence of genetic variation was found in 6; among the other 11 combinations, at least modest feeding occurred in 7, and virtually no feeding in 4. Thus failure to discern evidence of genetic variation in a feeding response was sometimes but not always a result of non-acceptance of a novel plant by the experimental animals.

As already noted, the two cases in which we discerned genetic variation in survival entailed growth on a plant closely related to the insect's normal host. A similar, though less dramatic pattern, was found for feeding response. In 7 of the 21 cases in which genetic variation in feeding response was detected, the test plant was in the same tribe as the insect's normal host, whereas in only 1 case out of 18 was a plant that elicited no genetically variable feeding a member of the same tribe as the normal host. The association is significant by likelihood-ratio test ($P = 0.0373$). Phylogenetic relations among the beetle species, on the other hand, provided no additional prediction of which plants would elicit genetically variable feeding behavior. Hosts of *Ophraella* species in the same major clade as the test species were significantly more likely to elicit genetically variable feeding than hosts of more distantly related species of *Ophraella*, but species in the same major clade of *Ophraella* generally feed on plants in the same tribe.

Interpretations and Implications

These beetles are more likely to display genetically variable feeding responses to plants that are closely related to their normal hosts than to more distantly related plants. However, out of the 31 different combinations in which larval or adult feeding was scored on plants in a different tribe of Asteraceae than the beetle's normal host, 14 showed evidence of genetically variable feeding. Thus, one cannot assume a priori that the feeding response to a relatively distantly related plant could not evolve. Bear in mind, however, that the test plants in this study are all in the same family, and moreover are a highly biased sample: they are all hosts of one or another species of *Ophraella*,

Table 1. Examples of analyses of variance of consumption of test plants by neonate larvae of *Ophraella artemisiae* Futuyama and *O. notulata* (Fabricius)

Plant Species	Source ¹	df	MS	F
<i>Ophraella artemisiae</i> (natural hosts: <i>Artemisia ludovicana</i> Nutt., <i>A. carruthii</i> Wood ex Carruth., [Anthemideae])				
<i>Artemisia vulgaris</i> L.	S	37	0.1869	1.27
(Anthemideae)	D(S)	22	0.1859	1.31
	E	175	0.1418	
<i>Ambrosia artemisiifolia</i> L.	S	37	0.3740	0.66
(Ambrosiinae)	D(S)	22	0.5628	2.42***
	E	177	0.2330	
<i>Eupatorium perfoliatum</i> L.	S	35	0.0881	1.01
(Eupatorieae)	D(S)	21	0.0877	1.55
	E	156	0.0566	
<i>Chrysopsis villosa</i> (Pursh) Nutt. Ex DC.	S	32	0.0637	0.44
(Astereae)	D(S)	19	0.1436	1.97*
	E	149	0.0727	
<i>Ophraella notulata</i> (natural hosts: <i>Iva frutescens</i> L., <i>I. annua</i> L. [Ambrosiinae])				
<i>Iva frutescens</i> L.	S	28	0.2526	0.88
(Ambrosiinae)	D(S)	29	0.2885	1.43
	E	406	0.2016	
<i>Artemisia vulgaris</i>	S	28	0.2595	0.84
(Anthemideae)	D(S)	28	0.3089	2.61***
	E	395	0.1186	
<i>Eupatorium perfoliatum</i>	S	28	0.4027	2.81**
(Eupatorieae)	D(S)	26	0.1432	1.54*
	E	381	0.0931	
<i>Chrysopsis villosa</i>	S	28	0.2526	0.88
(Astereae)	D(E)	27	0.5810	2.53***
	E	388	0.2293	

¹The terms in the ANOVAs are sire (S), dam within sire (D[S]), and error (E). Significant S or D(S) terms were taken as evidence of genetic variance in the character scored. (Data from Futuyama et al. 1994, 1995).

* $p < 0.05$

** $p < 0.01$

*** $p < 0.0001$

chosen specifically because such plants might be expected to elicit genetically variable reactions, given that at least one species of *Ophraella* has adapted to each of them. This choice was made in order to see if evidence of constraints on genetic variation might come to light even in characters that an orthodox population geneticist might least expect to be genetically invariant. It is certainly likely that many other plants would elicit no feeding response at all, and thus no genetic variation, as was the case in some instances studied. Likewise, even given some feeding, complete larval mortality was observed on some test plants, and this surely would be even more conspicuously true if a wider variety of plants were presented.

For evaluating candidate species for biological control, an important implication of the studies I have described is that even a small sample of an insect population may display genetic variation in some of the features that would enable it to colonize other species of plants than its normal host. For example, *Ophraella notulata* feeds only on two species of *Iva* (tribe Heliantheae, subtribe Ambrosiinae), as far as is known. It displayed genetically variable larval survival (as well as a capacity to oviposit) on *Ambrosia*, as well as genetically variable feeding responses not only to *Ambrosia*, but also to *Solidago bicolor*, *Chrysopsis villosa* (both in tribe Astereae), *Artemisia vulgaris* (tribe Anthemideae), and *Eupatorium perfoliatum* (tribe Eupatorieae). The second implication is more encouraging: survival requires more than feeding, and this measure of performance showed far less evidence of genetic variation. Moreover, feeding response of animals confined in a small space is only one of the several or many traits that may have to change in order for an insect population to adapt to a novel plant. Even the low levels of consumption often observed in these experiments might not occur in the field, where animals may disperse in search of more acceptable hosts, and establishment of viable populations may require changes not only in feeding, but also in postingestive physiological characters and oviposition behavior, to say nothing of factors such as phenology and avoidance of host-associated predators or parasitoids.

It is impossible, however, to prove a negative statement, such as a claim that a species has no genetic variation in a character (e.g., feeding response to a particular plant). Moreover, variation among geographic populations was not included in this study, and the sample sizes were relatively small, because the broad comparative nature of the study required assaying variation in numerous

combinations of species, and thus was labor-intensive. Thus genetic variation within the sample would not be discerned if the heritability of a character were very low, and rare alleles would have a high probability of not having been included in the sample. Screening for genetic variation by assaying variance among families may be particularly insensitive if the variation that enables a character to evolve is due to rare mutations at one or a few loci. The limited evidence to date suggests that behavioral responses of herbivorous insects and their performance on different hosts are generally multifactorial, although the effective number of loci may not be great (Jaenike, 1986; Hagen, 1990; Thompson et al., 1990; Sheck and Gould, 1996; Jones, 1998). Nevertheless, a polygenic character may respond to selection even if rare alleles at contributing loci are not readily detectable in small samples.

For these reasons, if an arthropod proposed for introduction as a weed-control agent is to be evaluated for its likelihood of adapting to a non-target plant species, the screen for genetic variation should entail much larger samples than those I have employed in my work, and should probably include assays of several geographic populations. If any evidence of growth, survival, or oviposition is found, it may be advisable to investigate further the role that variation in the plant (with respect to age, phenology, growth conditions, or provenance) may play in revealing a latent potential for the insect to adapt to particularly susceptible variants. Perhaps the best method of assay would be to impose mass selection on large experimental populations of the arthropod for adaptation to the plant, in order to determine if survival and other components of fitness increase over the course of 5-10 generations (e.g., Gould, 1979; Wasserman and Futuyma, 1981; Fry, 1990). This has the advantages that all characters that contribute to fitness are exposed to selection and that a response to selection may be obtained even if alleles contributing to the response are too rare to detect easily. Selection experiments may have various practical disadvantages, however, that depend on the species, such as the time required for a multigeneration experiment with univoltine insects, or the sometimes arduous logistics of rearing plant material in large quantity. Arduous as such experiments may be, the more difficult task is in extending such assays to all the species of plants that might plausibly be at risk of some day falling within the insect's range of diet.

The Role of Evolutionary Biology in Pest Management

Evolution is often cited as the single most important unifying principle of biology, but a broad recognition of its pervasive implications and applications is only slowly developing. Evolutionary biologists themselves are only now becoming fully aware of the range of applications of their science, as described in a recent report on "Evolution, Science, and Society" (Futuyma et al., 1999). These applications are particularly conspicuous in agriculture and pest management. The evolution of resistance to chemical pesticides in both arthropods and plants is only one reason for turning to alternative management methods or to integrated pest management. Breeding and genetically engineering plants for disease resistance and other useful traits relies in part on principles of evolutionary genetics. The possibility that beneficent agents of weed control might evolve into noxious plagues of crops or natural ecosystems provides yet another context in which the methods, principles, and data of evolutionary genetics are important. And, as noted earlier, any judgment of which plants might be most at risk depends on an accurate taxonomy and phylogeny of plants and often of the biological agents proposed to control them - and to infer phylogeny is to infer evolutionary history. It is imperative that at least the elementary principles of evolutionary biology be part of the training and awareness of pest managers and other applied ecologists.

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