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Variation in hostplant preference and performance by the milfoil weevil, *Euhrychiopsis lecontei* Dietz, exposed to native and exotic watermilfoils

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Abstract Exotic plants often displace native plants and thus alter the availability of native hostplants for specialist herbivorous insects. The submersed aquatic weevil *Euhrychiopsis lecontei* Dietz is endemic to North America, but there are now source populations on the exotic Eurasian watermilfoil (*Myriophyllum spicatum* L.) as well as on the weevil's ancestral host, northern watermilfoil (*Myriophyllum sibiricum* Komarov). This provides an opportunity to examine a host range expansion in progress. To further define the host range of the weevil and to determine how population source and rearing plant influence host plant preference and performance, we conducted a series of preference and rearing experiments with weevils from two source populations reared on northern milfoil, on Eurasian milfoil, switched late in larval development from northern to Eurasian milfoil, and vice versa. We also included two rearing treatments with milfoils on which the weevil has not been documented: the native *M. verticillatum* L. and the exotic *M. aquaticum* Verd. Preference by weevils in the switched rearing treatments was similar to preferences exhibited by weevils reared solely on the second (later) milfoil species and an increase in preference for Eurasian milfoil was induced by adult exposure to Eurasian milfoil for 2 weeks. In contrast, sizes and development times of weevils in the switched rearing treatments were similar to sizes and development times exhibited by weevils reared solely on the first (early) milfoil species. These results indicate that preference by the milfoil weevil is determined late in larval development or later and Hopkins' host selection principle is not supported. How-

ever, size and development time were most affected by hostplant quality during larval development when larvae must acquire the resources needed for pupation. Oviposition preference in the milfoil weevil was a population attribute, not a fixed individual attribute and there was no significant variation in preference among individuals reared on northern milfoil, but significant variation in preference was detected among weevils reared on Eurasian milfoil. Weevils oviposited on all four milfoil species and completed development on three of them, but did not develop beyond the larval stage on *M. aquaticum*. Weevils reared on Eurasian milfoil developed faster and reached larger adult sizes than weevils in any other rearing treatment. The smallest sizes and longest development times were for weevils reared on the natives, northern milfoil and *M. verticillatum*. The milfoil weevil oviposits on an array of milfoil species and is unable to distinguish an unsuitable host (*M. aquaticum*) within this genus. The influence of rearing plant and adult exposure to Eurasian milfoil on hostplant preference suggests that host range expansion to novel congeners may occur more rapidly than predicted by models which assume that genetic variation is required. Significant variation among individuals in hostplant preference suggests the potential for a host shift to a plant for which *E. lecontei* appears pre-adapted.

Keywords Oviposition preference · Host range · Freshwater macrophyte · *Myriophyllum* · Hopkins' host selection

Introduction

The invasion of exotic plant species is now considered a major component of global change (Vitousek et al. 1996). Exotic plant species can displace native plants (Vitousek et al. 1996) and thereby alter the availability of hostplants for herbivorous insects. The simultaneous decreasing availability of the ancestral host and increasing availability of the exotic host may result in an increased preference for the exotic plant species (Bowers

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et al. 1992). Therefore, the effects of exotic plant invasions may provide scientific opportunity (e.g., Potvin and Tousignant 1996) to study host range expansions by herbivorous insects as they occur, rather than post facto as is usually practiced (Karowe 1990). This approach may offer insight on host range expansion or host shifts by herbivorous insects exposed to novel hostplants, and may also provide information on how insects may be better used to control invasive plants.

Both terrestrial and aquatic systems are faced with invasive plant species. However, current theory on host range expansion was developed primarily through investigation in terrestrial systems, and may not be a realistic model of herbivore responses to habitat changes in aquatic systems. Little is known about freshwater host choice (Newman 1991) and our work with the weevil *Euhrychiopsis lecontei* Dietz (= *Eubrychiopsis lecontei*), which has expanded its host range to include Eurasian watermilfoil *Myriophyllum spicatum* L. (e.g., Sheldon and Creed 1995; Solarz and Newman 1996; Newman et al. 1997), suggests that freshwater specialist herbivores may respond to hostplants in ways similar to terrestrial insects. Determining the response to an exotic plant by this specialist herbivore will contribute to our understanding of herbivory in aquatic systems and to how specialist herbivores in both terrestrial and aquatic systems may respond to the introduction of exotic plant species.

To predict the response of herbivorous insects to changes in hostplant availability, such as invasive species, examining the mechanisms of change in hostplant preference is important (Singer et al. 1989; Mayhew 1997). For example: is oviposition preference concordant with location or attraction to hostplants? Do females oviposit on the plant to which they are attracted? Oviposition and other behavioral preferences do not always agree and most authors are careful to make this distinction (e.g., Fox 1993) since Singer (1986) pointed out that ecologists often mistakenly use an assay of one preference to draw inferences about another. Is there intrapopulation variation in hostplant preference? The extent to which there is potential for evolution in the short term may be limited by the amount of existing variation in preference (Singer et al. 1989) and several authors have found significant variation in hostplant preference by specialist insect herbivores (e.g., Singer et al. 1988, 1989; Thompson 1988). In addition, many authors have sought to discover the developmental stage at which hostplant preference is determined (e.g., Corbet 1985; Anderson et al. 1995). This crucial stage may affect the feeding or oviposition preferences of all other life stages of the organism. If preference is determined at the larval stage, then an 'accidental' switch by larvae may lead to a change in adult behavior. However, if preference is determined much later in development, adults may shift their hostplant preference from the species upon which they were reared to a novel species. This could lead to oviposition on novel hostplant species.

The acceptance by herbivorous insects of novel hostplants is of interest because it can be an important mech-

anism of diversification of insect-plant relationships (Berenbaum and Zangerl 1991; Bowers et al. 1992; Bush 1993) by leading to new feeding and oviposition preferences. Furthermore, understanding the mechanisms of novel host choice may improve our ability to predict unwanted host range expansions to natives when exotic herbivores are introduced for the control of exotic weeds (e.g., Louda et al. 1997). The mechanisms of change in hostplant preference that we seek to understand in this paper are concordance of host location and oviposition preference, intrapopulation variation in hostplant preference, the developmental stage at which hostplant preference is determined, development on and preference for novel hostplant species, and the effect that development on novel species has on oviposition preference.

The weevil *E. lecontei* is native to North America and appears to be a specialist on watermilfoil, *Myriophyllum* (Haloragaceae), species (Solarz and Newman 1996). The life history and basic ecology of the weevil have been described (Sheldon and Creed 1995; Newman et al. 1996; Sheldon and O'Bryan 1996). All life stages are associated with the submersed plant, indicating that the weevil is fully aquatic. Females oviposit one to several yellow eggs (ca 0.5 mm in diameter) on the meristem of the plant and a single female can oviposit several hundred eggs within her lifetime (Sheldon and O'Bryan 1996). The larvae feed on the meristem and then burrow down through the stem where they pupate. Adults (ca. 3 mm long) feed on the stem and leaves usually within the upper meter of the plant. Typical generation time ranges from 20 to 30 days (Newman et al. 1997).

Known hostplants for *E. lecontei* include *M. spicatum* and *M. sibiricum* Komarov (= *M. exalbescens* Fernald; Creed and Sheldon 1994). Another species, *M. verticillatum* L., is native to North America (Sculthorpe 1985) and exists within the range of *E. lecontei*. It is likely a natural hostplant for *E. lecontei* and here we document that *M. verticillatum* is a suitable hostplant for weevil development. We also test weevil oviposition preference for *M. aquaticum* Verd. (= *M. brasiliense* Cambess.), a native to South America which is not found in the northern United States, including Minnesota. The weevil oviposits and successfully develops on both its native host, northern watermilfoil (*M. sibiricum*), and the exotic, Eurasian watermilfoil (*M. spicatum*; Newman et al. 1997). However, hostplant preference by the weevil is affected by rearing-plant species. Weevils collected from or reared on the exotic Eurasian watermilfoil (hereafter Eurasian milfoil) preferred Eurasian milfoil in a multiple-plant genera oviposition experiment (Solarz and Newman 1996). However, weevils reared on the native northern watermilfoil (hereafter northern milfoil), exhibit an equal oviposition preference for both Eurasian and northern milfoil. Thus, weevils reared on the native northern milfoil show no oviposition preference between the native and exotic watermilfoils, whereas weevils reared on the exotic Eurasian milfoil greatly prefer it over their native milfoil.

We now ask (1) whether behavioral attraction to a hostplant is concordant with oviposition preference, (2)

whether the differences in oviposition preference exhibited by Eurasian and northern milfoil-reared weevils are indicative of fixed individual preferences or are a mean population response, (3) whether oviposition preference is determined early in development or whether adult weevils are capable of switching their preferences upon exposure to a novel watermilfoil, (4) whether differences in preference exhibited by weevils persist after the populations have been exposed to both Eurasian and northern milfoil, (5) whether weevils oviposit on and successfully develop on watermilfoil species other than Eurasian and northern milfoil, and (6) whether rearing on other watermilfoils influences oviposition preference.

Materials and methods

Y-tube oviposition series

To determine the concordance of attraction and oviposition preference, if preference is fixed within individuals or is a measure of the population response, and the effect of adult exposure to a novel hostplant on host range and hostplant preference by *E. lecontei*, we compared preference responses of individual females in a series of four preference experiments: a pair of Y-tube and oviposition preference experiments, followed by a 2-week exposure to the novel plant, followed by another pair of Y-tube and oviposition experiments (Y-O-Y-O). Throughout this series of laboratory experiments, preferences of individual female weevils from each of two populations were recorded. One population was collected from Eurasian milfoil in Lake Auburn, Carver County, Minn. (T116N;R24W;S10). The second population was from Christmas Lake, Hennepin County, Minn. (T117;R23W;S35,36), which lacks Eurasian but contains northern milfoil (Newman and Maher 1995), the weevil's native host (Creed and Sheldon 1994). Weevil populations from each source were maintained on their respective hostplant species in greenhouse cultures for several days (<10) until the experiments were performed. Greenhouse cultures of weevils were maintained on healthy plants rooted in lake sediment in 0.545-m³ steel tanks (61×183×76 cm). The greenhouse light cycle was 18 h light:6 h dark and the water temperature remained between 20 and 25°C.

The Y-tube bioassays were conducted to measure weevil preferences between meristem samples of Eurasian and northern milfoil. The glass Y-tube was 1.0 cm in diameter, with 10.0 cm-long branches and a 10-cm stem. Each end of the tube was stoppered with cork. Weevils are positively phototactic and tend to swim upward. Therefore, the branches of the tube were inclined 10° with respect to the horizontal and the introductory stem was fitted with an opaque sleeve to encourage weevils to move up the stem and into one of the branches. Samples to be tested were placed in the ends of the Y-tube branches and an individual weevil was introduced through the stem. Weevils swam up the stem to the end of one branch, and a preference was recorded. Weevils were considered to exhibit a preference when they landed on the plant sample. Not every trial resulted in the exhibition of preference. A no choice was recorded when the weevil did not leave the introductory stem for 10 min. However, the weevils usually exhibited a preference in less than 5 min.

Meristem samples were all 3–4 cm long and undamaged. The Y-tube branches were rotated randomly after each trial so that the branches were not necessarily in the same position for each trial. Samples were replaced every five trials at which time the Y-tube was rinsed with water, then absolute ethanol, then distilled water. A χ^2 analysis indicated that there was no significant bias in choice among individual plant samples ($\chi^2 P>0.1$) so each trial was used as one observation. Data from the Y-tube experiment were analyzed with a sign test (Devore and Peck 1993). Trials in which no preference was exhibited were excluded from the analysis but more than 90% of tests resulted in a choice.

Following the first Y-tube experiment, each weevil entered the multi-genera oviposition experiment. This experiment, which follows the general methods of Solarz and Newman (1996), was designed to determine first and second oviposition preference of individual female weevils offered an array of plant species common to weevil habitat. Individual females were placed in 9.5-l glass aquaria filled with well water and containing undamaged, 10 cm-long plants of each species tested. Light was provided by fluorescent bulbs on a 16 h light:8 h dark cycle and all aquaria were aerated. The experiment was divided into two rounds. Round one (R1) weevils were allowed access to all seven plants used in the experiment: *M. spicatum*, *M. sibiricum*, *Elodea canadensis* Michx., *Potamogeton zosteriformis* Fern., *P. pectinatus* L., *Ceratophyllum demersum* L., and *Ranunculus aquatilis* L. or *Megalodonta beckii* Greene. All plant species used in the oviposition experiments were collected from either Lake Auburn or Christmas Lake. Once the female oviposited in R1, she was moved to a round two (R2) tank that contained the same array of plant species as in the R1 aquaria, except for her first oviposition choice. This allowed us to investigate second oviposition choice in the absence of the preferred plant. Plants were inspected twice daily for eggs. Oviposition plant preference was determined with a χ^2 test and binomial probabilities (Solarz and Newman 1996) with females that oviposited.

After the first Y-tube and oviposition preferences had been determined for each weevil, they were exposed for 2 weeks to the watermilfoil species on which they had not been reared (i.e., weevils reared on Eurasian were exposed to northern milfoil for 2 weeks, and vice versa) in individual aerated 0.95-l mason jars. Each weevil then repeated the Y-tube and oviposition preference experiments described above. For each population of weevils, we compared preferences between Y-tube and oviposition experiments and determined consecutive preferences for each individual weevil. We also compared preferences by weevils of the two populations before and after exposure to the new host.

Four-watermilfoil oviposition experiment

To compare the watermilfoil hostplant preferences of Eurasian versus northern milfoil-reared weevils exposed sequentially to both Eurasian and northern milfoil, we tested the oviposition preference of females, from weevil populations that had been exposed to both Eurasian and northern milfoil, in a four-watermilfoil oviposition experiment. As described above, these weevils had been exposed either by being reared upon the plant or by being exposed to it for 2 weeks (a subset of these populations had been tested in the Y-O-Y-O series). This experiment was conducted exactly as the oviposition experiment described above, except that four watermilfoil species were offered in R1 rather than seven macrophytes of various genera: two natives (northern milfoil and *M. verticillatum*) and two exotics (Eurasian milfoil and *M. aquaticum*). All plant species used in this experiment were collected from local lakes except *M. aquaticum*, which was purchased from a commercial greenhouse in Minneapolis, Minn.. Again, both first (R1) and second (R2) oviposition preferences were recorded. We tested for significant preferences for each population for both rounds using a χ^2 test.

Rearing experiment

To compare weevil development on an array of watermilfoil species, to determine whether weevils would successfully develop on watermilfoil species outside of their known host range, and to determine the subsequent oviposition preferences of weevils successfully reared on each watermilfoil species, we attempted to rear adult weevils from eggs on all four watermilfoil species tested in the four-watermilfoil oviposition experiment. To determine if early larval experience is important to adult hostplant preference we included two rearing treatments in which larvae were switched late in development either from Eurasian to northern milfoil or vice versa. We wanted to compare development between weevil popu-

lations with a long history on their respective hostplants and, therefore, a new Eurasian milfoil source population was needed. Weevils collected from Eurasian milfoil were from Fish Lake near Sauk City, Wis. (T9N;R7E;S3), where weevils have been reported on Eurasian milfoil for 30 years, while those collected from northern milfoil were from Christmas Lake. Because northern milfoil is their native host, we may assume a long association between weevil and milfoil in Christmas Lake.

The eggs reared in this experiment were from two different generations. The first generation was from weevils collected from the Eurasian and northern milfoil source populations and assigned to the Eurasian, northern, and switched watermilfoil treatments. Each female parent was randomly assigned either a Eurasian or a northern milfoil plant and allowed to oviposit several eggs per plant to ensure that at least one adult female would eventually emerge. Only female progeny were used, because oviposition preference was of particular interest. Progeny were reared individually in 0.95-l mason jars extended with 20 cm-long, clear plastic tubing. Each jar was randomly assigned a position in 1 of 12, 0.545-m³ steel tanks (61×183×76 cm). The tanks were positioned in a greenhouse courtyard with no shade. Water temperature (measured mid-watercolumn) was maintained between 20 and 30°C by changing or adding water daily. Rooted Eurasian or northern milfoil plants were added to the jars assigned to weevils selected for the switched treatments. Just prior to pupation, weevil larvae emerge from the stem and move downward along the outside of the stem to find a suitable undamaged location for pupation. We had previously observed that larvae will preferentially move onto an undamaged stem that is touching the damaged plant just below the extent of larval mining. Weevils switched plants when the larvae emerged from the stem (immediately before pupation, about 2 weeks after oviposition). Most weevils moved from the original damaged plant onto the touching new hostplant in 1–2 days at which point the old plant was discarded. Upon their emergence as adults, we recorded development time, females were mated, and their hostplant preference was recorded in the four-watermilfoil oviposition experiment. For this experiment, preference was tested for R1 (all four species) only. All weevils in this experiment were collected after eclosion and thus fed on their rearing plants (as adults) prior to being tested in the four-watermilfoil experiment. Finally, we estimated the size of each newly eclosed weevil in all treatments using a Nikon stereoscope by multiplying the length (base of the head to the posterior end of the elytra) by the width (widest point across the dorsal side of the abdomen) for each individual.

Eggs that were oviposited onto the two new watermilfoil species (*M. verticillatum* and *M. aquaticum*) were reared on these plants and the resulting development times and hostplant preferences of these second-generation adults were recorded. For these rearing treatments, we haphazardly selected from *M. verticillatum* and *M. aquaticum* plants that were chosen for oviposition in the four-watermilfoil oviposition experiment. Therefore, we know how many females from each source oviposited on these two novel species, but there is no record of source population for each weevil reared in the *M. verticillatum* and *M. aquaticum* rearing treatments.

We estimated the effect of treatment on hostplant preference using logistic regression and on size and development time using ANOVA. We determined significant preferences within treatments and significant differences in preference among treatments using a χ^2 -test and significant differences in development time and size among treatments using Tukey's HSD. All tests were conducted with SYSTAT (Wilkinson 1989) except logistic regression, which was conducted with Statistix (Analytical Software).

Results

Y-O-Y-O series

Weevils reared on and exposed to only Eurasian milfoil exhibited a significant preference for Eurasian over

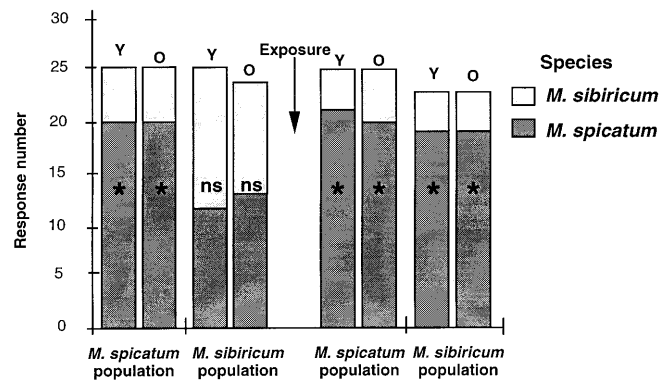


Fig. 1 Weevil preferences in the Y-tube-oviposition (Y-O-Y-O) series ($n=25$). When the total for a particular bar is less than 25, the difference is composed of no choices. The arrow indicates exposure to the watermilfoil opposite from the one on which the weevils had been reared. The left half of the histogram represents preferences by weevils before exposure and the right half, preferences by these same weevils after exposure to the watermilfoil species opposite to the one on which were reared. Asterisks indicate significant differences ($P < 0.05$) in preference between the two watermilfoil species, while ns indicates no significant difference in preference. Y-tube data were analyzed using a sign test and the oviposition data were analyzed using a χ^2 -test. In addition to *Myriophyllum spicatum* and *M. sibiricum*, five other genera of aquatic macrophytes were offered in this preference experiment. However, there was no oviposition on any of them

northern milfoil, whereas weevils reared on and exposed only to northern milfoil exhibited an equal preference for both watermilfoil species in both Y-tube and multiple-plant genera oviposition assays (Fig. 1). Throughout the Y-O-Y-O series, there were no ovipositions on non-watermilfoil species (out of 97 trials). After exposure to the watermilfoil species opposite to the one on which they had been reared, Eurasian milfoil-reared weevils maintained their significant preference for Eurasian milfoil (Y-tube preference for Eurasian milfoil was 80% pre- and 86% post-exposure to northern milfoil, $n=25$), but northern milfoil-reared weevils now also exhibited a significant preference for Eurasian milfoil (Y-tube preference for Eurasian milfoil was 48% pre- and 83% post-exposure to Eurasian milfoil, $n=23$; Fig. 1). There was no significant difference between oviposition and Y-tube preferences throughout the series (χ^2 , $P > 0.5$).

Although northern milfoil-reared weevils shifted their preference upon exposure to Eurasian milfoil, indicating that preference is not fixed in this population, the preference of the Eurasian milfoil-reared population remained unchanged after exposure to northern milfoil. Therefore, we wanted to determine whether for Eurasian milfoil-reared weevils, preference is fixed in individual females or whether individuals change their preference despite the population tendency to choose Eurasian milfoil consistently. The Eurasian milfoil-reared weevil population exhibited an 80–84% preference for Eurasian milfoil throughout the Y-O-Y-O series; however, consecutive preferences by individuals decreased over the series of experiments so that only 36% of Eurasian milfoil-reared individuals exhibited a preference for Eurasian milfoil in

all four trials. If hostplant preference is a fixed behavior, we expected the same individuals to exhibit preference for Eurasian milfoil in each of the four tests ($p=1$). In contrast, if preference is variable within individuals, we expected all weevils within a population to exhibit a preference for Eurasian milfoil with a similar probability. If this is the case, when we observe that 80% of weevils in one test prefer Eurasian milfoil, then each weevil tested should exhibit a probability of $p=0.8$ for choosing Eurasian milfoil throughout the Y-O-Y-O series. We used the partial score test (Dean 1992) to test the null hypothesis that all weevils within a population exhibit the same probability of preferring Eurasian or northern milfoil, versus the alternative hypothesis that the probability of preferring Eurasian or northern milfoil varies significantly among individuals within a population. The partial score test is calculated as:

$$t = \frac{\{\sum [y_i - n_i * p]^2\} / \{p(1-p)\} - \{\sum n_i\}}{\sqrt{\{2 * [\sum n_i * (n_i - 1)]\}}}$$

where y_i is the number of times weevil i prefers Eurasian milfoil, n_i is the number of trials, and p is the average probability of choosing Eurasian milfoil based on the population-level response (e.g., because on average 80% of the population preferred Eurasian milfoil, $p=0.8$). The null hypothesis is rejected if t exceeds a critical value, from a t -distribution, based on $n-1$ degrees of freedom. We determined that preference was not fixed within individuals (only 36% preferred Eurasian milfoil in all four trials) but there was significant variation in preference among individual Eurasian milfoil-reared weevils (partial-score test, $P=0.04$) with an average individual probability of 0.72 for choosing Eurasian milfoil. In contrast, there was no significant difference (partial-score test, $P=0.32$) in hostplant preference among individual northern milfoil-reared weevils. The average probability for choosing Eurasian milfoil was 0.58. Thus, individual northern milfoil-reared weevils all appear to exhibit an equal preference for both Eurasian and northern milfoil, whereas Eurasian milfoil-reared weevils prefer Eurasian milfoil, but some prefer it more than others.

Four-watermilfoil oviposition experiment

The populations tested in the Y-O-Y-O series, now exposed to both Eurasian and northern milfoil, were next tested in the oviposition experiment with four watermilfoil species. Analysis of this experiment revealed first that both Eurasian and northern milfoil-reared weevils oviposited on all four watermilfoil species (Fig. 2). Second, only the Eurasian milfoil-reared population exhibited a significant preference for Eurasian over all watermilfoil species offered, although significantly more than 25% of the northern milfoil-reared population preferred Eurasian milfoil (χ^2 , $P<0.05$). Eurasian milfoil-reared weevils exhibited a 23% higher preference than northern milfoil-reared weevils for Eurasian milfoil, while northern milfoil-reared weevils distributed their preferences

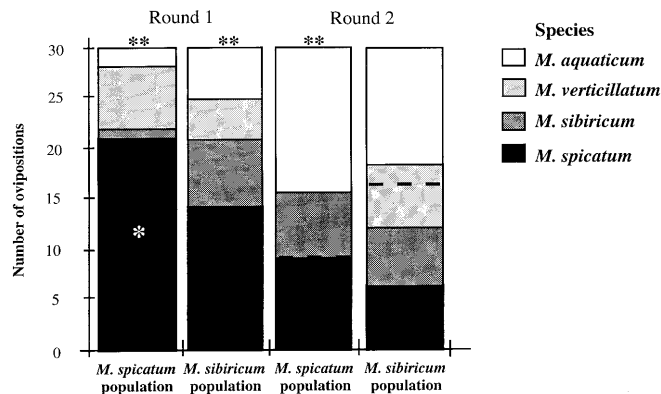


Fig. 2 Oviposition preference by weevils in the four-watermilfoil oviposition experiment. Weevils tested ($n=30$) had been exposed for 2 weeks to the watermilfoil species on which they had not been reared. *Round 1* displays preferences when all four watermilfoils were offered. *Round 2* displays preferences when the first species on which they oviposited was removed. In round 2, the area of each histogram bar from 0 to the heavy dashed line indicates the number of weevils that could have oviposited on Eurasian milfoil (e.g., all *M. spicatum*-reared weevils offered Eurasian milfoil in round 2 oviposited on it; thus, the *M. spicatum* segment of the histogram extends up to the dashed line). Asterisks indicate significant preference (χ^2 , $P<0.05$) for Eurasian milfoil over all watermilfoil species offered. Double asterisks indicate a preference significantly different from 25% (χ^2 , $P<0.05$) for at least one of the milfoil species offered

more evenly across the four watermilfoil species offered (Fig. 2). For Eurasian milfoil-reared weevils, preferences for *M. spicatum*, *M. verticillatum*, *M. aquaticum*, and *M. sibiricum* were 70, 20, 7, and 3%, respectively (Fig. 2). For northern milfoil-reared weevils preferences were 47, 13, 17, and 23%, respectively. For their second oviposition preference (R2), 100% ($n=9$) of Eurasian milfoil-reared weevils offered Eurasian milfoil oviposited on it, whereas only 38% of northern milfoil-reared weevils offered Eurasian milfoil ($n=16$), oviposited on it (Fig. 2). Indeed, for all four of the watermilfoil species offered (*M. spicatum*, *M. sibiricum*, *M. verticillatum*, and *M. aquaticum*) to northern milfoil-reared weevils for their second oviposition, there was no significant difference in preference (χ^2 , $P=0.38$). Thus, Eurasian and northern milfoil-reared weevils exhibited distinct host rank orders, indicating that the difference in watermilfoil hostplant preference between Eurasian and northern milfoil-reared weevils appears to persist after the populations have been exposed to both Eurasian and northern milfoil.

Rearing experiment

About equal numbers of the ovipositions on *M. aquaticum* and *M. verticillatum* were by female progeny of parents from the Eurasian and northern milfoil sources. Oviposition on *M. aquaticum* was by 46.5% Fish Lake progeny and 53.5% Christmas Lake progeny, and oviposition on *M. verticillatum* was by 38% Fish Lake progeny and 62% Christmas Lake progeny.

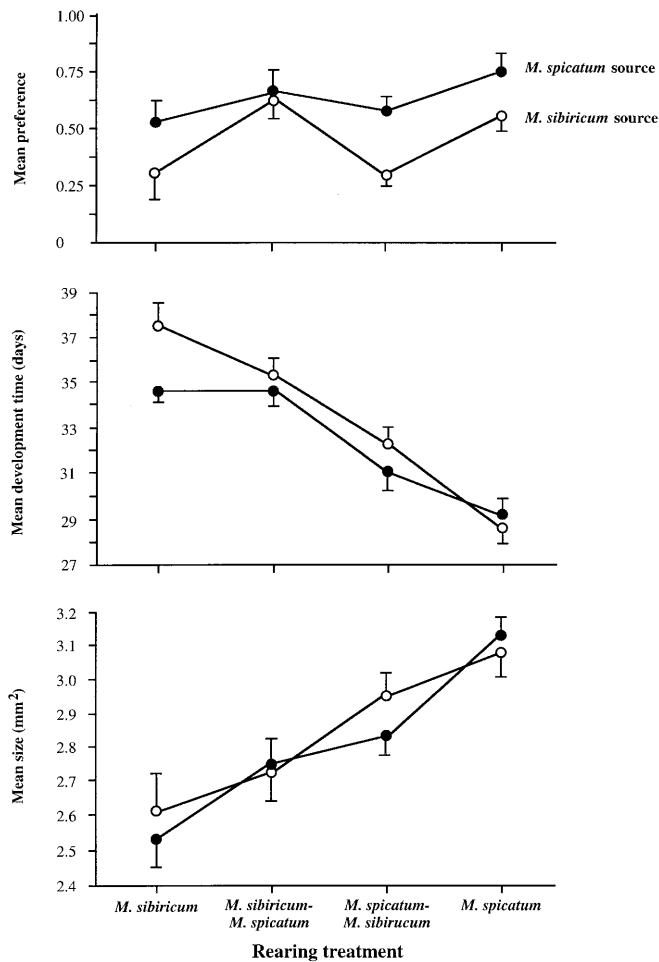


Fig. 3 Reaction norms of oviposition preference (A), size (B), and development time (C) across the four rearing environments for both sources of the milfoil weevil (*M. spicatum* source=Fish Lake; *M. sibiricum* source=Christmas Lake). Means \pm or -1 SE are plotted. Mean preference refers to preference for Eurasian milfoil (i.e., the proportion of progeny that preferred the exotic watermilfoil in the four-watermilfoil oviposition experiment). Mean development time on *M. verticillatum* (not shown) was 37.6 ± 1.24 days ($n=22$) which was not significantly longer than on *M. sibiricum* for weevils from the *M. sibiricum* source ($n=63$, Tukey's HSD, $P=0.96$). Weevils reared on *M. verticillatum* (not shown) were significantly smaller (2.90 ± 0.06 mm) than *M. spicatum*-reared weevils (Tukey's HSD, $P=0.02$), but significantly larger than *M. sibiricum*-reared weevils (Tukey's HSD, $P<0.01$)

Female progeny of the milfoil weevils examined in the rearing experiment were largest and reached the adult stage with the shortest development times when reared completely on Eurasian milfoil, regardless of source population (Tukey's HSD, $P<0.01$; Fig. 3). Preference for Eurasian milfoil was also highest for weevils in this rearing treatment. The lowest preference for Eurasian milfoil was exhibited by weevils from Christmas Lake reared either completely on northern milfoil or switched from Eurasian to northern milfoil late in larval development. Weevils from Fish Lake reared on Eurasian milfoil ($n=66$) were significantly larger than any other weevils reared (Tukey's HSD, all $P<0.05$). The smallest mean

adult size and second-longest mean development time were for weevils reared completely on northern milfoil (Fig. 3). Development time on *M. verticillatum*, northern milfoil, or the series of northern and Eurasian milfoil was significantly longer than on Eurasian milfoil for both source populations ($n=59-68$ for all except the *M. verticillatum*-rearing treatment, Tukey's HSD, all $P<0.001$; Fig. 3). Development time for weevils switched from Eurasian to northern milfoil ($n=64$ for Fish Lake and $n=62$ for Christmas Lake weevils) was significantly shorter than for weevils reared on *M. verticillatum* or northern milfoil (Tukey's HSD, all $P=0.03$) but not significantly different from development time on Eurasian milfoil (Tukey's HSD, $P=0.41$). We attempted to rear 21 progeny oviposited upon *M. aquaticum* and 30 progeny upon *M. verticillatum* in this rearing experiment. Of these attempts, no progeny survived past the larval stage on *M. aquaticum* (100% mortality). However, 22 female adults emerged from the *M. verticillatum* ovipositions with a mean development time of 37.6 ± 1.24 days and mean size of 2.9 ± 0.06 mm. Of all the rearing treatments, this is the longest development time reported. However, mean size for *M. verticillatum*-reared weevils was approximately midway between Eurasian and northern milfoil-reared weevils.

For each source, weevils in the Eurasian and in the switched northern-Eurasian milfoil rearing treatments exhibited a significant preference for Eurasian over all other watermilfoil species offered (χ^2 , $P<0.05$), whereas weevils in the northern and in the switched Eurasian-northern rearing treatments did not exhibit a significant preference for one milfoil species over all others (χ^2 , $P>0.2$). Next, we determined if preferences, sizes, and development times by weevils reared on a series of two watermilfoil species were consistent with preferences, sizes, and development times by weevils reared entirely on either the early or the late plant species. For the preference data, we used a logistic regression model and for the performance data, ANOVA. For each model, the response was the trait measured and the factor was rearing treatment. After determining that treatment was significant for all three traits (all $P<0.03$), we tested for significant differences between those treatments that were most similar (Fig. 3) and found no significant difference for any of the traits tested ($P>0.3$). Progeny reared in the Eurasian and northern-Eurasian milfoil-rearing treatments exhibited similar oviposition preferences and progeny reared in the northern milfoil and Eurasian-northern rearing treatments exhibited similar oviposition preferences. For development time and size, the Eurasian and Eurasian-northern milfoil treatments were similar and the northern and northern-Eurasian milfoil treatments were similar. Therefore, we simplified the models by pooling the data from the four rearing treatments into two groups (factors) based on the two treatments that were the most similar. For preference, we combined the Eurasian-reared with the northern-Eurasian milfoil switched treatment (late development on Eurasian) and the northern-reared with the Eurasian-northern switched

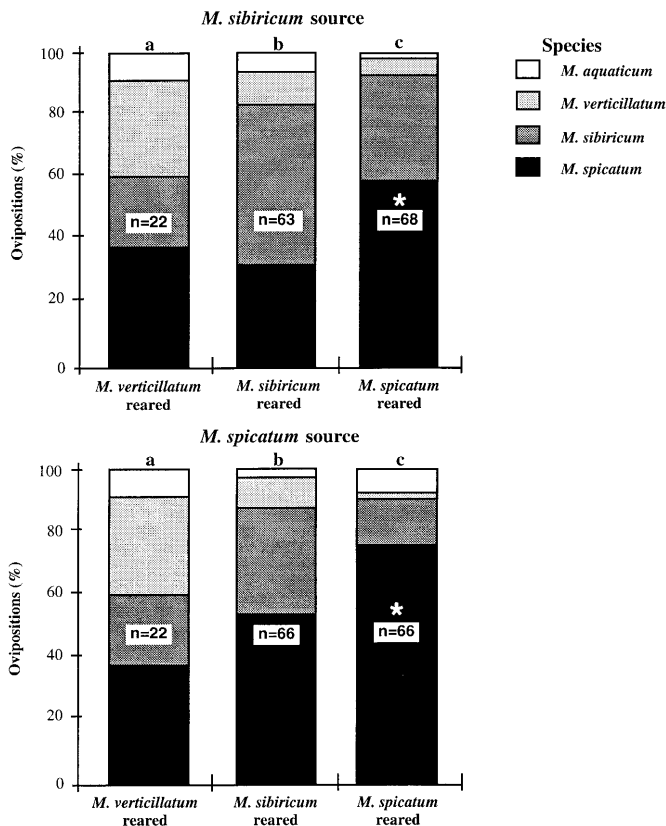


Fig. 4 Oviposition preference in the four-watermilfoil experiment by weevils from *M. sibiricum* (A) and *M. spicatum* (B) source populations reared on *M. spicatum*, *M. sibiricum*, or *M. verticillatum*. Asterisks indicate a significant preference (χ^2 test, $P < 0.05$) for Eurasian milfoil over all watermilfoil species offered. The distribution of preference for the four milfoil species offered differed significantly among rearing treatments (χ^2 , $P < 0.05$) as indicated by different lowercase letters. Letters apply to within-population comparisons only. The same results for *M. verticillatum*-reared weevils (from both sources) are presented in both source plant histograms for easy comparison

treatment (late development on northern). For size and development time, we combined the Eurasian-reared with the Eurasian-northern switched treatment (early development on Eurasian) and the northern-reared with the northern-Eurasian switched treatment (early development on northern). With these simplified models, we found that treatment was still significant (all three $P < 0.05$) suggesting that late-rearing plant affected preference and early-rearing plant affected performance. This indicates that weevils reared on a series of two watermilfoil species exhibit oviposition preferences which reflect their late hostplant species, whereas sizes and development times reflect their early hostplant species.

The distribution of preference for the four watermilfoil species offered differed significantly among weevils reared on *M. verticillatum*, *M. sibiricum*, and *M. spicatum* (Fig. 4). Weevils reared on Eurasian preferred Eurasian milfoil (χ^2 , $P < 0.05$), northern-reared weevils equally preferred northern and Eurasian milfoil (χ^2 , $P > 0.33$), and *M. verticillatum*-reared weevils exhibited

nearly equal preferences for *M. sibiricum*, *M. spicatum*, and *M. verticillatum* (36, 23, and 32%, respectively; $n = 22$, $P > 0.5$; Fig. 4). However, weevils from all five rearing treatments oviposited on all four watermilfoil species offered although they oviposited with fairly low frequencies on *M. aquaticum* and *M. verticillatum* (1–31% for Christmas Lake weevils and 1.5–17% for Fish Lake weevils).

Discussion

We have found no evidence that hostplant preference in the milfoil weevil is determined by early larval experience and thus no evidence for Hopkins' (1917) host selection principle, as has been suggested by others (e.g., Corbet 1985; Jaenike 1990; Futuyma et al. 1993). Oviposition preference by weevils which were switched prior to pupation, from Eurasian to northern milfoil or vice versa, was similar to weevils which were reared entirely on the second hostplant species (e.g., weevils switched from Eurasian to northern milfoil exhibited a hostplant preference similar to northern milfoil-reared weevils; Fig. 3). This suggests that hostplant preference in the milfoil weevil is determined quite late in larval development or later. Furthermore, in the Y-O-Y-O series, preference for Eurasian milfoil by northern milfoil-reared adult weevils was significantly increased upon exposure to Eurasian milfoil (Fig. 1). This indicates that hostplant preference can change in the adult stage.

Whereas oviposition preference by weevils in the switched rearing treatments reflected the second rearing-plant species, development time and size were similar to those of weevils reared entirely on the first rearing-plant species (e.g., weevils switched from Eurasian to northern milfoil exhibited a development time similar to weevils reared entirely on Eurasian milfoil; Fig. 3). This suggests that although hostplant preference is determined late in development, development time and size are most affected by hostplant quality during larval development when larvae must acquire the resources needed for pupation.

These experiments confirmed that the weevil is a watermilfoil specialist. The weevils did not oviposit on any non-watermilfoil species in the Y-O-Y-O series, yet oviposited on all four watermilfoil species offered, including *M. verticillatum*, a native species upon which *E. lecontei* has not been previously reported. Development time was longer and size smaller for weevils reared on the natives, *M. verticillatum* and northern milfoil, or switched from northern to Eurasian milfoil, compared to those reared on Eurasian milfoil or switched from Eurasian to northern milfoil (Fig. 3). This is consistent with previous results comparing development on Eurasian and northern milfoil (Newman et al. 1997) and further suggests that Eurasian milfoil is a superior hostplant compared to native watermilfoil species. Although small stem diameter (Sheldon and O'Bryan 1996) and decreased oxygen transport may explain the differential performance, the native watermilfoil stems were general-

ly as thick or thicker than the *M. spicatum* stems and thus stem diameter does not appear to explain the differences in performance among the species.

Weevils successfully developed on *M. verticillatum* and development time on *M. verticillatum* was not significantly longer than on northern milfoil. Weevils reared on *M. verticillatum* were even significantly larger than weevils reared on northern milfoil. This suggests that either *M. verticillatum* is a natural hostplant for *E. lecontei* (R. Newman, personal observation, found one occurrence of *E. lecontei* on *M. verticillatum* in Eagle Lake, Minn.), or that weevils are physiologically capable of development on watermilfoil species not currently within their host range. However, weevils also oviposited upon *M. aquaticum*, a species *E. lecontei* has not likely ever encountered. Most of these eggs developed to the larval stage (personal observation), but the larvae eventually suffered 100% mortality, indicating a physiological rather than a behavioral (weevils are willing to feed and oviposit on it) barrier to complete development. *M. aquaticum* develops emergent shoots and keeping these submersed for the rearing experiment (to avoid egg desiccation) may have stressed the plant, making it chemically or nutritionally unsuitable for weevil development. Emergent *M. aquaticum* shoots are denser and harder than submersed shoots and physical resistance may have been a factor. Conversely, because *M. aquaticum* is found in tropical South America and has not been found in lakes with *E. lecontei*, it may be an unsuitable hostplant. However, females are clearly not able to discern this and will readily oviposit on it.

It should be noted that *M. spicatum*, *M. sibiricum*, and *M. verticillatum* are closely related congeners (see Galatowitsch et al. 1999 for a recent review). *M. sibiricum* was considered a variety or subspecies of *M. spicatum* by several taxonomists (Aiken 1981) and all three species were considered to be part of a circumboreal *M. verticillatum* complex (Aiken 1981; Ceska and Ceska 1986). Although these are now agreed as three separate species (Ceska and Ceska 1986; Galatowitsch et al. 1999), they are quite similar and have similar morphologies and likely chemistries (although they have different flavonoid profiles; Ceska and Ceska 1986). Thus, host expansion to the exotic *M. spicatum* is not as surprising as expansions to other genera or families (e.g., Berenbaum and Zangerl 1991; Bowers et al. 1992; Louda et al. 1997).

Our results also suggest that behavioral attraction to a hostplant may be a good indicator of oviposition preference because in the Y-O-Y-O series, weevil hostplant preference in the Y-tube bioassay was concordant with the preferences observed in the oviposition experiment (Fig. 1). This suggests that location and oviposition cues are either similar or covary similarly between Eurasian and northern milfoil. Therefore, for the milfoil weevil, one may be able to test the simpler behavioral attraction to draw inferences about oviposition preference.

Northern and Eurasian milfoil-reared weevils exhibit distinct host rank orders even after both populations

were exposed to both Eurasian and northern milfoil (Fig. 2). This indicates that the two populations differ in hostplant preference behavior and suggests that they represent two biotypes. Biotypes are populations which consistently differ in one or more biological traits, but do not necessarily exhibit reduced gene flow (Diehl and Bush 1984). The term is usually reserved for instances when investigators do not yet know whether differences in the biological traits are due to genetic or environmental differences (ecomorphs; Diehl and Bush 1984). These weevil biotypes differ in hostplant preference behavior which seems to be the result of rearing-plant species. Thus, the effect of rearing-plant species on hostplant preference may contribute to the establishment of biotypes.

Very few studies have detailed both the behavioral responses by herbivorous insects and their sources of variation (Berenbaum and Zangerl 1991; Bernays and Chapman 1994). Understanding the source of variation in hostplant preference is important for determining the mechanism for host range expansion in weevils. Physiological tradeoffs may be important in determining hostplant preference by the milfoil weevil because there are performance differences in development on Eurasian versus northern milfoil (e.g., development time; Fig. 3; see also Newman et al. 1997) and weevils readily oviposit on at least one hostplant (*M. aquaticum*) outside their physiological host range.

The observed variation in hostplant preference by weevils may be due to rearing-plant effects, genetic effects, or both. We have demonstrated that the hostplant species upon which weevils are reared and feed is important in determining weevil hostplant preference. Regardless of collection source, weevils reared on Eurasian milfoil (or reared on Eurasian milfoil from the last larval instar on) preferred it over all other species offered whereas weevils reared on northern milfoil (or reared on northern milfoil from the last larval instar on) usually exhibited an equal preference for both Eurasian and northern milfoil. In addition, the distribution of preference for the four watermilfoil species offered differed significantly among weevils reared on *M. verticillatum*, northern, and Eurasian milfoil. Furthermore, in the Y-O-Y-O series, weevil preference was affected by short-term exposure to a novel host (Eurasian milfoil) which indicates that hostplant preference is not fixed in adult weevils. These results suggest that hostplant preference may be largely determined by environmental (prior experience or rearing plant) effects rather than genetic effects.

There may also be a significant genetic contribution to variation in hostplant preference and in the ability of weevils to accept novel hosts. Results of individual consecutive preference trials revealed that northern milfoil-reared weevils demonstrated preferences at the population rather than individual level. However, for Eurasian milfoil-reared weevils, there was significant variation in preference among individuals. Northern milfoil is probably the weevil's original hostplant (Creed and Sheldon 1994) and individual weevils reared on northern milfoil

each have the same probability of choosing Eurasian over northern milfoil. Once Eurasian milfoil is introduced into the system, particular individuals are apparently more likely to consistently prefer it over northern milfoil. This indicates that for the lake populations studied here, some individuals have a stronger preference for Eurasian milfoil than others and suggests that there is individual variation in weevil ability to accept or to be induced to novel hosts. Induction is the phenomenon by which herbivorous insects prefer to feed or oviposit on the plant species upon which they were reared (Bernays and Chapman 1994). This means that some individuals may have greater potential for rapid host range expansion or host shifts than other individuals within a population, especially if preference for the novel host is favored evolutionarily. Population variation in the potential to accept novel hosts should be considered along with host-plant preference in studies of host choice or host range expansion.

The results reported here suggest that specialist herbivores may more readily expand their host range to include invasive species than current models would predict because most authors assume herbivorous insects must undergo a genetic change in either physiology or behavior in order to shift hosts or expand their host range (Futuyma 1983; Jaenike 1989, 1990; Prokopy et al. 1988; Futuyma et al. 1993; but see Leclaire and Brandl 1994). We have demonstrated that for the milfoil weevil, hostplant preference is affected by rearing plant and even adult exposure to a novel hostplant species. However, we have also demonstrated individual variation in adults' ability to accept novel hosts, suggesting that when invasive species successfully displace native hostplants, selection may favor those individuals that tend to prefer the novel hostplant species.

Host range expansions to at least novel congeners may be more common than predicted (e.g., Leclaire and Brandl 1994; Louda et al. 1997). Host range testing for biocontrol introductions should include preference tests in which the herbivores are reared on novel hosts, even if these hosts are uncommon, because prior experience or rearing-plant effects may contribute significantly to further host range expansion.

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