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Intraspecific variation in feeding preference and performance of *Galerucella nymphaeae* (Chrysomelidae: Coleoptera) on aquatic macrophytes

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Abstract. The feeding preferences and performance of a freshwater macrophyte-feeding chrysomelid beetle (*Galerucella nymphaeae*) were assessed in laboratory experiments. Populations of *Galerucella* had a relatively narrow diet breadth, preferring species of *Nuphar*, *Polygonum*, and in 1 case *Brasenia*, while largely ignoring the remaining macrophytes offered in assays. However, because of interpopulation variation in host preferences, the species *G. nymphaeae* should be considered polyphagous. Distant populations from the Upper Peninsula of Michigan, Indiana, and North Carolina collected from *Nuphar* spp. all readily consumed *Nuphar* spp. and *Polygonum* spp., but beetles from a site in south Michigan collected from *P. amphibium* or from *Brasenia schreberi* treated *Nuphar* as a low-preference host. The performance of *Galerucella* in no-choice assays was clearly related to the behavioral preferences of the larvae: larvae performed well on 3 macrophyte species that they willingly consumed, but performed poorly when they refused to consume alternative host plants and presumably starved to death. It is unknown if the starved beetles could have performed well physiologically if they had eaten alternative hosts. Performance was also related to the quality of host plant, given that beetles collected from *Nuphar luteum* or *N. advena* reached a larger adult mass on *N. luteum* than on *N. advena*. The feeding preferences of *Galerucella* were largely non-plastic within a single generation, although there were sometimes behavioral differences among clutchmates raised on different host plants. Morphometric and preliminary allozyme data suggest the North American populations used in this paper are conspecific, but they probably represent a different species than European *G. nymphaeae*. The intraspecific variation of our North American *G. nymphaeae* in host preferences and performance suggests that at least 2 different ecotypes occur in North America.

Key words: behavior, chrysomelid, ecotypes, feeding specificity, freshwater macrophytes, herbivory, host race, host specificity, intraspecific variation, plant-herbivore interactions, water lily.

The study of plant-herbivore interactions has been overwhelmingly terrestrial (Rosenthal and Janzen 1979, Crawley 1983, Rosenthal and Berenbaum 1992). However, evidence is mounting that herbivory on freshwater macrophytes is often substantial, contrary to past conventional wisdom (Lodge 1991, Newman 1991, Lodge et al. 1998). Thus, opportunities abound to extend experimental and comparative analyses of plant-herbivore relationships into a new habitat. The freshwater habitat might offer new insights into the ecological and evolutionary questions central to plant-herbivore studies because fresh waters have many taxa of macrophytes and in-

vertebrate herbivores that are evolutionarily independent, or at least divergent, from their terrestrial counterparts.

Although freshwater vascular plants (macrophytes) are of terrestrial origin, many are highly specialized for their aquatic existence and have few close terrestrial relatives. For example, the water lily family (Nymphaeaceae), which is a focus of this paper, is entirely aquatic (Voss 1985). It includes *Nuphar* and several species known to have complex secondary compounds (Ostrofsky and Zettler 1986, Kerfoot 1988). Recent work demonstrated that *Nuphar* is defended by these chemicals against generalist herbivores (Cronin 1998). Despite possessing compounds that deter feeding, the leaves, seed pods, and rhizomes of water lilies are consumed by mammals (e.g., beavers, D. M. Lodge, personal observation), primary aquatic insect larvae (sensu Newman 1991, Cronin et al. 1998), and secondary and semiaquatic insects (Wallace and O'Hop 1985, Kerfoot 1988, Otto and Wallace 1989, Juliano

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1988, Setälä and Mäkelä 1991, Kouki 1991a, 1991b, 1991c, 1993a, 1993b, Cronin et al. 1998).

Feeding specialization is less common among aquatic than terrestrial herbivorous insects, although there are several semiaquatic insects that specialize in feeding on aquatic macrophytes (Newman 1991, Cronin et al. 1998). Behavioral responses of insects to their hosts are believed to be central in maintaining specialized associations (Futuyma and Moreno 1988). Indeed, a behavioral shift in host preferences is thought to be an initial step that can lead to the formation of sympatric host races, local reproductive isolation, and speciation (Bush 1975, Diehl and Bush 1989). Sympatric speciation has reportedly occurred in northern Europe within the species complex of a chrysomelid beetle (*Galerucella* [= *Pyrrhalta*] *nymphaeae* L.) that feeds on aquatic plants (Hippan and Koponen 1986, Nokkala and Nokkala 1994). The differences between *G. nymphaeae* that eats species of the water lily *Nuphar* (Nymphaeaceae) and *Galerucella* that eats *Rubus* or *Comarum* (both Rosaceae) were apparently great enough to warrant the latter beetles being designated a separate species, *G. sagittariae* (Hippan and Koponen 1986, Nokkala and Nokkala 1994).

We focus exclusively on North American populations of *G. nymphaeae* (hereafter referred to by genus only), which is usually referred to as a specialist on *Nuphar* (e.g., Kouki 1991a, Cronin et al. 1998). The *Galerucella*-*Nuphar* system is probably the best-studied case of herbivory on a freshwater plant. Herbivory by *Galerucella* causes considerable damage to the leaves of *Nuphar* (e.g., Kouki 1991a, Cronin et al. 1998), reduces the longevity of floating leaves (Otto and Wallace 1989, Kouki 1991a, 1991c, Setälä and Mäkelä 1991), alters the production of submersed vs floating leaves (Kouki 1993b), and possibly alters nutrient cycling in aquatic habitats (Wallace and O'Hop 1985, Setälä and Mäkelä 1991). Predators can reduce the impact of *Galerucella* on host plants by reducing their population size and by decreasing their feeding rates (T. Schlacher and G. Cronin, unpublished data). Intraspecific competition among *Galerucella* occurs primarily through habitat degradation (i.e., sinking of leaves resulting from heavy herbivory; Juliano 1988, Kouki 1991c, 1993a) and cannibalism (larvae eat eggs; Scott 1924, G. Cronin, personal observation). Despite this body of work, no experiments on diet choice or its plas-

ticity have been conducted on North American *Galerucella* populations.

We address the following questions: 1) What is the diet breadth of North American *Galerucella*? 2) Does food choice differ among geographically separated populations? 3) Are food preferences altered by prior experience with different plants? 4) Are food choice and performance (survival and growth) related? The answers to these questions should illuminate ongoing work on host-race formation and sympatric speciation of similar European species (Nokkala and Nokkala 1994, Nokkala et al. 1998). We evaluated these questions using 4 or 5 populations of *Galerucella* from different host plants: 1) North Carolina *Galerucella* from *Nuphar luteum* (L.); 2) Upper Peninsula of Michigan *Galerucella* from *N. variegata* Durand; 3) Indiana *Galerucella* from *N. advena* (Ait.); and 4) or 5) south Michigan *Galerucella* from *Polygonum amphibium* L. or *Brasenia schreberi* Gmel.

Methods

Feeding preferences of field-collected Galerucella

On 25–26 June 1995, *Galerucella* was collected from Morris Lake (lat 46.26°N, long 89.52°W) at the University of Notre Dame Environmental Research Center in Michigan's Upper Peninsula (UP Michigan population); from Lake Papakeechee (lat 41.37°N, long 85.68°W) near Syracuse, Indiana (Indiana population); and from *Galerucella* Pond (lat 34.73°N, long 76.80°W) in Morehead City, North Carolina (North Carolina population) (Fig. 1). *Galerucella* from these lakes used species of *Nuphar* as host plants even though several other species of floating, emergent, or terrestrial plants were available at each of these sites. Larvae and adults were collected for assays from each site with the exception of Lake Papakeechee, which did not have larvae at this time. Leaves of macrophytes were collected on the same dates from the same sites from where *Galerucella* were collected, or from locations within 50 km of *Galerucella* source sites.

On 23 August 1995, larval *Galerucella* from Gray Lake (lat 41.81°N, long 86.08°W) near Edwardsburg, Michigan (south Michigan population) (Fig. 1) and another set of macrophytes were collected. In 1995, this population of *Galerucella* used only *P. amphibium* as a host plant in the field, even though other macrophyte spe-

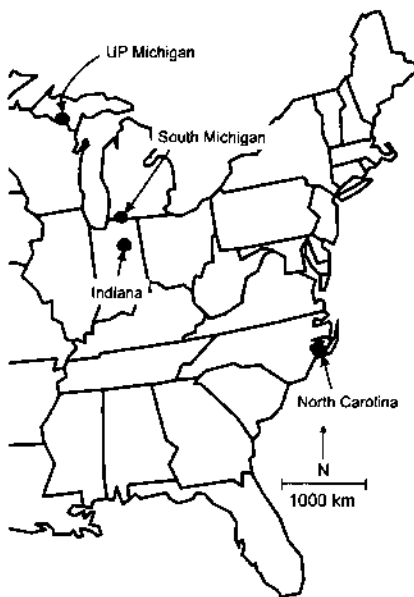


FIG. 1. The eastern USA showing the location of *Galerucella nymphaeae* populations used in this study. UP = Upper Peninsula.

cies, including *N. advena*, were available. However, beetles at this site were found on *Brasenia* in 1996 (see below).

The plants and animals collected on 25–26 June were used in feeding assays that ran from 27–30 June (62 h) and the organisms collected on 23 August were used in a feeding assay performed on 23–25 August (42 h). Macrophytes used in this study are named following Voss (1972, 1985). For aquatic and semiaquatic plants, only floating or emergent leaves were offered to these semiterrestrial beetles. A *natural host plant* is the macrophyte species that the beetle was found on in the field, and a *surrogate host plant* was an alternative (i.e., non-natural) host plant that an animal developed on in the laboratory.

We then determined the feeding preferences of different *Galerucella* populations. Square portions of leaves (22 × 22 mm) were cut from 16 different macrophytes and arranged in a randomized 4 × 4 array on moist paper towels in covered plastic trays to prevent desiccation. For narrow leaves, 2–3 pieces of leaves were used to form the square, but all 4 edges of the square were always cut to avoid introducing a systematic bias. Cutting of the leaves did not appear to alter the feeding pattern of the beetles (e.g., beetles did not initiate or concentrate feeding

near the cut edges). The beetles made grazing scars on the surface of the squares that were identical to feeding scars on whole leaves in the field. Only 1 square from any leaf was used per feeding assay. Generally, 2 animals were added to each tray, but because adults were rare at the UP Michigan site, only 1 animal per replicate was used for this adult assay. After an assay was terminated, the leaf area consumed was visually estimated independently by 2 people; these 2 estimates were averaged to produce 1 datum per macrophyte per replicate. Visual estimates by an experienced observer allowed rapid, accurate measurement of leaf damage (visual estimate vs digitized measure: $r^2 = 0.917$, $n = 31$ representative values >0 and <100%, linear regression). Consumption data, expressed as mm² of leaf area damaged per animal per day, were analyzed with a nonparametric Friedman test (Conover 1980) because multiple choice feeding assays violate the assumption of independence among treatments, precluding the use of standard methods for multiple comparisons (Peterson and Renaud 1989). Leaf squares were neither damaged nor changed area in the absence of beetles; hence, controls for changes in leaf squares not caused by herbivory were not required (Peterson and Renaud 1989).

In August 1995, eggs, 2nd- and 3rd-instar larvae, and adults were collected from Gray Lake (south Michigan) and from Lake Papakeechee (Indiana) to assess feeding preferences for *P. amphibium* and *N. advena* collected from the beetles' respective habitats. Egg masses were carefully removed from the host plants and placed in the center between 22 × 22 mm squares cut from *Polygonum* and *Nuphar*. When the eggs hatched, the naive larvae were allowed to feed on the 2 macrophyte species. Second and 3rd-instar (i.e., experienced) larvae and adults from the same collection were offered the same choice using the leaf-square method. Data from each of these assays were analyzed with paired-sample *t*-tests.

In August 1996, larval and adult *Galerucella* were collected from *B. schreberi* at Gray Lake (the same lake where we observed *Galerucella* on *P. amphibium* in 1995 and 1996). These beetles were offered a choice of leaf squares cut from *Brasenia*, *Polygonum*, *Nuphar*, and *Nymphaea* collected from Gray Lake using methods described above. These data were analyzed with a Friedman test.

Performance of Galerucella on different host plants

Galerucella nymphaeae were raised on various macrophytes (without choice) to determine 1) if they could survive on alternative hosts, 2) how well they grew on alternative plants, 3) if the rearing plant influenced feeding preferences, and 4) if there were performance differences among different populations of *Galerucella*.

Performance experiment I.—In July 1995, *G. nymphaeae* from North Carolina and Indiana were raised on *N. luteum* from Galerucella Pond (North Carolina) or *N. advena* from Lake Papakeechee (Indiana). Egg masses removed from leaves collected on 26 June 1995 were kept in a warm (~28°C) humid environment until they hatched. Six newly hatched larvae were used from each clutch of eggs: 3 larvae were placed in a covered container with a 22 × 22 mm square of *N. luteum* and 3 were placed in a separate container with *N. advena* in the same manner. Each clutch represented a single replicate from its population. A total of 240 larvae (3 larvae/container × 2 *Nuphar* spp. × 2 *Galerucella* populations × 20 replicates/population) from 40 clutches were used. If a container had no larvae surviving to the 2nd instar, it was excluded from the experiment because it was difficult to determine if early mortality was caused by an unsuitable host plant or handling of the small larvae; this criterion resulted in a sample size of 15 for North Carolina beetles and of 18 for Indiana beetles. Food was replaced as required; if food was replaced in a container, the food of the clutchmates was also replaced. Because 1 of our field sites (North Carolina) was ~3000 km from our laboratory, *Nuphar* used in this experiment was collected every 1.5 wk on the same day at both field sites, placed in plastic bags, and stored (or shipped) in coolers at ~5°C until used. This procedure assured that the 2 *Nuphar* species offered to the beetles had been treated similarly.

The amount of macrophyte consumed (estimated by surface area of leaf damaged by the larvae), life stage, and the number of surviving animals were monitored 10–11 times throughout their development. The size of larvae (length, thorax width, and head width) was measured periodically with a stereomicroscope equipped with an ocular micrometer. All 3 measures gave similar results and we only report thorax width. Newly emerged adults were

weighed to the nearest 0.1 mg. One to five days elapsed between monitoring periods. Animals from the same clutch were always monitored on the same day. Data for surviving clutchmates in each treatment were averaged because we did not track individual clutchmates, and to avoid pseudoreplication within independent replicates. Sample sizes for some measurements varied because of mortality or because some animals completed a life stage between monitoring intervals.

The same individuals were measured numerous times during this experiment, so data are not strictly independent. However, we performed ANOVA rather than a repeated-measures ANOVA for easier comparisons of the distinctive life stages. Therefore, development time and survivorship measures of later life stages are cumulative, and are dependent on the development and survivorship of earlier instars. Life-stage-specific comparisons between the 2 populations of *Galerucella* and a test for a population × host plant interaction were made with a 2-factor ANOVA with *Galerucella* populations and *Nuphar* spp. as the 2 fixed factors. When comparing the performance of 1 population of *Galerucella* on the 2 species of *Nuphar*, paired-sample *t*-tests were used to take advantage of our paired design (i.e., larvae from the same clutch were raised on 2 different species of *Nuphar*).

Adult *Galerucella* were offered a choice between leaf squares cut from *N. luteum* and *N. advena* within 2 d of emergence from their pupal case. Feeding data were analyzed with paired-sample *t*-tests (paired by herbivore).

Performance experiment II.—In the final rearing experiment (August 1995), south Michigan *Galerucella* from Gray Lake were raised on *P. amphibium* (their natural host plant, $n = 7$) or *N. advena* ($n = 6$) collected from Gray Lake, and Indiana *Galerucella* from Lake Papakeechee were raised on *P. amphibium* ($n = 3$) or *N. advena* (their natural host plant, $n = 3$) collected from Lake Papakeechee. Whole clutches of eggs were carefully removed from leaves and placed on either *Polygonum* or *Nuphar* in a covered plastic container with a moist paper towel. The development, survivorship, and feeding preference of animals were monitored after the eggs hatched. Fresh leaves were added ad libitum and changed every 2–3 d.

Third-instar larvae raised on the different

macrophyte species were offered a choice between *Polygonum* and *Nuphar* collected from their natal site using methods described above. Animals used in this assay were not used in further experiments. South Michigan adults raised on the different macrophytes were also offered a choice between *Polygonum* and *Nuphar*. These assays allowed us to determine if rearing host influenced feeding preferences, and to compare the host preferences of laboratory-reared animals with field-collected animals of the same populations.

Data from the 2-choice feeding assays were analyzed with paired-sample *t*-tests. Life-stage-specific comparisons between the 2 populations of *Galerucella* and a test for a population \times host plant interaction were made with a 2-factor ANOVA with *Galerucella* populations and host species as the 2 fixed factors.

Results

Feeding preferences of field-collected *Galerucella*

Galerucella nymphaeae larvae and adults from all sites usually preferred species of *Nuphar* and *Polygonum* over all other macrophytes in 1995, although this pattern was not always statistically significant (Figs 2 and 3). However, marked differences in feeding preferences existed among the 4 populations of *Galerucella*, and to a lesser extent, between larvae and adults within populations.

Larval North Carolina *G. nymphaeae* preferred *Polygonum* sp., *N. advena*, and their natural host plant *N. luteum*, and consumed very little of the remaining 12 food choices (Fig. 2A). Similarly, North Carolina adults consumed *Polygonum* sp., *N. advena*, and *N. luteum* (Fig. 2B). Although not statistically different, adults consumed $<1/2$ the amount of their natural host plant *N. luteum* than of *Polygonum* sp. or 1 population of *N. advena* (Fig. 2B).

The means in Fig. 2C show that larval UP Michigan *Galerucella* were willing to consume *Polygonum* sp., *N. luteum*, *N. advena* collected from Papakeechee (but not *N. advena* collected from Gray Lake), and some *Alnus rugosa*, but they did not eat much, if any, of the remaining species, including their natural host plant *N. variegata*. Adult UP Michigan *Galerucella* consumed significant amounts of *Polygonum* sp., *N. advena*, *Lythrum salicaria*, and *A. rugosa* (Fig. 2D). Similar

to the larvae, the adults treated their natural host plant *N. variegata* and the remaining macrophytes as low-preference food items.

Adult Indiana *Galerucella* collected from *N. advena* ate large amounts of *Polygonum* sp. and *N. advena*, small amounts of *N. luteum* and *L. salicaria*, and very little, if any, of the remaining macrophytes (Fig. 2E).

Larval south Michigan *Galerucella* (1995) fed almost exclusively on their host *P. amphibium* and *P. hydropiperoides* during a feeding assay (Fig. 3). They largely ignored the remaining macrophytes, although the Friedman test did not detect a difference between the amount consumed of *Polygonum* spp. collected from Gray Lake and *L. salicaria* collected from Painter Lake (Fig. 3).

The different populations of *G. nymphaeae* usually preferred *N. advena* from Lake Papakeechee over the same species growing in Gray Lake. Consumption of *N. advena* from Papakeechee Lake was significantly greater than consumption of *N. advena* from Gray Lake ($p = 0.039$, $n = 5$) when the mean consumption of *N. advena* from Papakeechee vs Gray from each of the assays shown in Fig. 2 (appreciable amounts of *N. advena* were not consumed by south Michigan *Galerucella*, Fig. 3) were compared using a paired-sample *t*-test. In addition to this different response to different populations of *N. advena*, *Galerucella* also discriminated among species of *Nuphar*. For example, *N. variegata* was consumed little (Fig. 2), even by the UP Michigan animals, whose natural host plant it is (Figs 2C and 2D).

When naive larvae, experienced larvae, and adults were offered a choice between *Polygonum* and *Nuphar* (the 2 most palatable plants), all stages of south Michigan beetles collected from *Polygonum* preferred *Polygonum*, whereas all stages of Indiana beetles collected from *Nuphar* preferred *Nuphar* (Fig. 4).

Larval and adult south Michigan *Galerucella* collected from *Brasenia* (a plant that has never been listed as a host for *Galerucella*) in 1996 preferred *Brasenia* over *Polygonum*, *Nuphar*, and *Nymphaea* (Fig. 5). Intermediate amounts of *Polygonum* were also eaten, but no *Nuphar* or *Nymphaea* were eaten.

Performance of *Galerucella* on different host plants

Performance experiment 1.—North Carolina and Indiana populations of *Galerucella* raised on *N.*

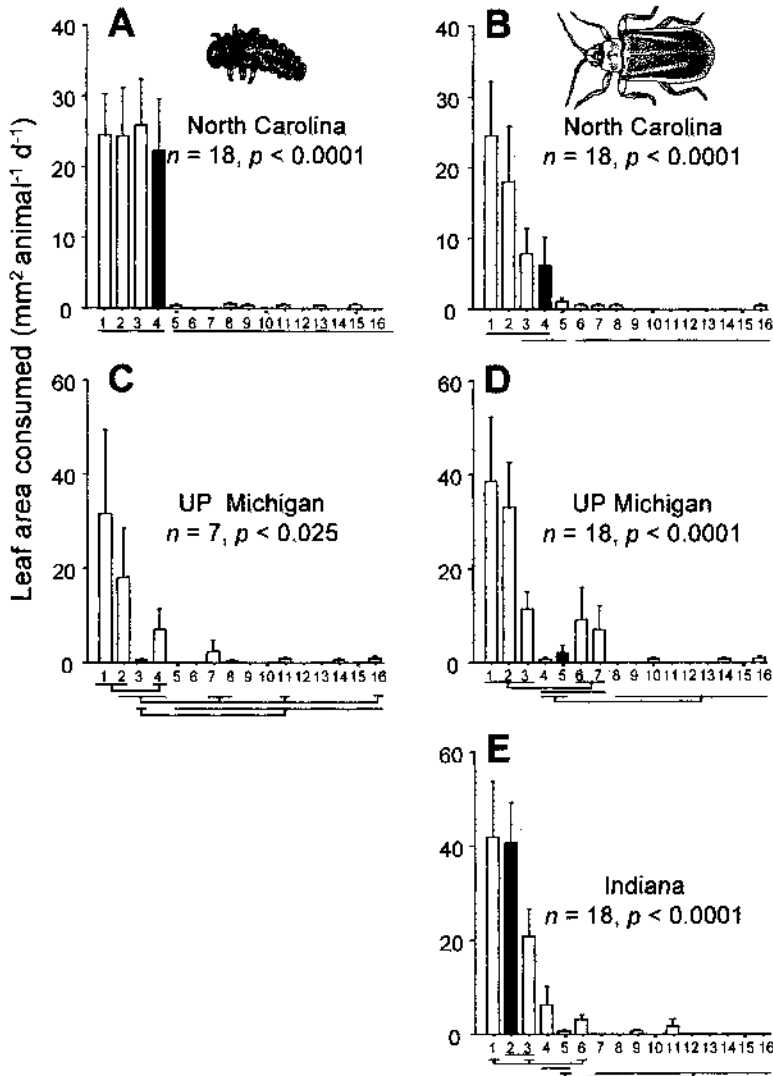


FIG. 2. Leaf area consumed by field-collected larval (A, C) and adult (B, D, E) *Galerucella nymphalae* collected from Galerucella Pond, North Carolina (A and B), Morris Lake, Upper Peninsula (UP) Michigan (C and D), and Lake Papakeechee, Indiana (E). Bars represent the mean (+1 SE) area of each macrophyte type consumed. Shaded bar represents the natural host of the beetles (for C, host plant is *Nuphar variegata*). Numbers below each bar correspond to the numbered macrophyte in the key below. Lines below bars connect means that are not statistically different ($\alpha = 0.05$, Friedman test). Macrophyte codes, species, and collection sites are: 1—*Polygonum* sp., Kickapoo Lake, UP Michigan; 2—*N. advena*, Lake Papakeechee, Indiana; 3—*N. advena*, Gray Lake, south Michigan; 4—*N. luteum*, Galerucella Pond, North Carolina; 5—*N. variegata*, Morris Lake, UP Michigan; 6—*Lythrum salicaria*, Painter Lake, south Michigan; 7—*Alnus rugosa*, Tenderfoot Lake, UP Michigan; 8—*Ulmus americana*, Notre Dame, Indiana; 9—*Nymphaea odorata*, Lake Papakeechee; 10—*N. odorata*, Gray Lake; 11—*Brasenia schreberi*, Gray Lake; 12—*Potamogeton amplifolius*, Gray Lake; 13—*Typha latifolia*, Granger Pond, Indiana; 14—*Peltandra virginica*, Painter Lake; 15—*Pontederia cordata*, Lake Papakeechee; 16—*Salix longifolia*, Lake Papakeechee.

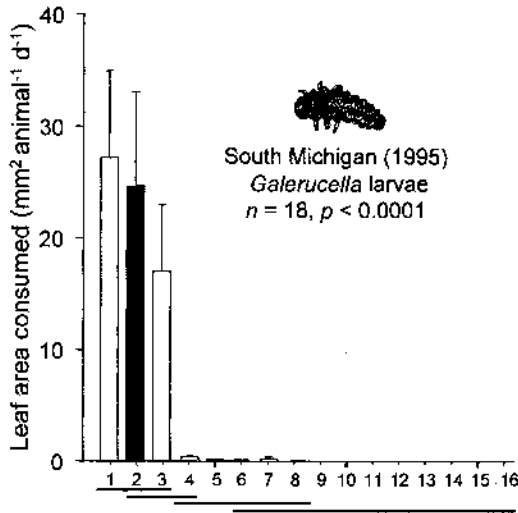


FIG. 3. Feeding preferences of larval *Galerucella nymphaeae* collected from *Polygonum* at Gray Lake, south Michigan in 1995. Bars represent the mean (± 1 SE) area of each macrophyte type consumed. Shaded bar represents the natural host of the beetles, *Polygonum amphibium*. Numbers below each bar correspond to the numbered macrophyte in the key below. Lines below bars connect means that are not statistically different ($\alpha = 0.05$, Friedman test). Macrophyte codes, species, and collection sites are: 1—*P. amphibium*, Lake Papakeechee, Indiana; 2—*P. amphibium*, Gray Lake, south Michigan; 3—*P. hydropiperoides*, Gray Lake; 4—*Lythrum salicaria*, Painter Lake, south Michigan; 5—*Nuphar advena*, Lake Papakeechee; 6—*N. advena*, Gray Lake; 7—*Nymphaea odorata*, Lake Papakeechee; 8—*N. odorata*, Gray Lake; 9—*Brasenia schreberi*, Gray Lake; 10—*Potamogeton amplifolius*, Gray Lake; 11—*Typha latifolia*, Lake Papakeechee; 12—*Peltandra virginica*, Painter Lake; 13—*Pontederia cordata*, Lake Papakeechee; 14—*Salix nigra*, Gray Lake; 15—*Impatiens capensis*, Gray Lake; 16—*Liriodendron tulipifera*, Notre Dame, Indiana.

luteum (from North Carolina) or *N. advena* (from Indiana) consumed similar amounts of leaf material of each species of *Nuphar*. There were no significant differences in the amount of food eaten between 2 populations of beetles ($p > 0.08$), between the 2 species of *Nuphar* ($p > 0.30$), or for a population \times *Nuphar* species interaction ($p > 0.13$). However, there was a significant difference in the amount of food consumed by each instar ($p < 0.0001$): instars 1, 2, and 3 consumed 3.2 ± 0.3 , 16.7 ± 1.4 , and 62.8 ± 2.9 mm² leaf area per larva per day, respectively. Early development of the larvae of both *Galerucella* populations was significantly faster on *N. advena*

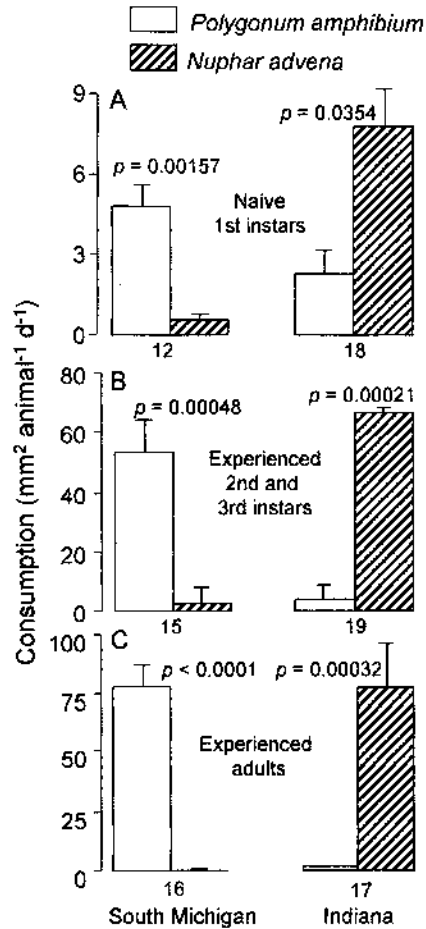


FIG. 4. Feeding preference of (A) naive larvae, (B) experienced larvae, and (C) experienced adults from Gray Lake (south Michigan; natural host *Polygonum*) and Lake Papakeechee (Indiana; natural host *Nuphar*) when offered a choice between *Polygonum amphibium* and *Nuphar advena* collected from the same lake from which the beetles were collected. The sample size of each assay is at the base of each pair of means (± 1 SE). Paired bars were compared with paired-sample *t*-tests.

than on *N. luteum*, but both populations reached adulthood in similar time regardless of rearing host (Fig. 6A). There was a nonsignificant ($p = 0.056$) trend towards early instars from North Carolina surviving better on *N. advena*, but this trend was not apparent for adults (Fig. 6B). For all measures, there was never a significant population \times host plant interaction.

Both populations of *Galerucella* attained a greater adult mass when raised on *N. luteum*

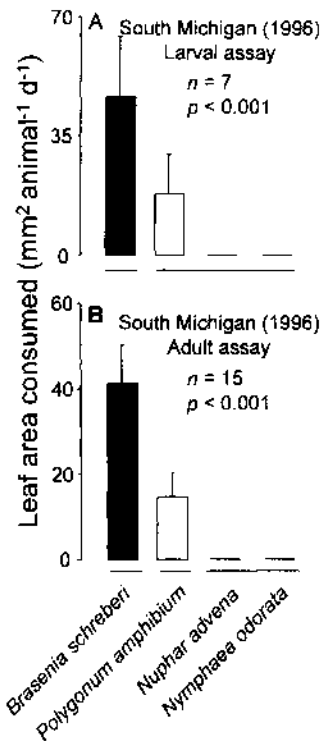


FIG. 5. Feeding preferences of (A) larval and (B) adult *Galerucella nymphaeae* collected from *Brasenia* at Gray Lake, south Michigan in 1996. Bars represent the mean (+1 SE) area of each macrophyte type consumed. Shaded bar represents the natural host of the beetles. Lines below bars connect means that are not statistically different ($\alpha = 0.05$, Friedman test).

than when raised on *N. advena*, although the size of instars did not differ significantly with respect to rearing host (Fig. 6C). The size of instars 1 and 2 did not differ with respect to population. However, instar 3 and adults of the Indiana *Galerucella* were significantly larger than conspecifics from North Carolina (Fig. 6C).

Laboratory-reared adult Indiana beetles preferred *N. luteum* over their natural host plant *N. advena* regardless of the *Nuphar* species on which they were raised (Fig. 7). This preference differed from the preference observed when field-collected adults were offered *N. luteum*, *N. advena*, and 14 other macrophyte types (Fig. 2E). Adults from North Carolina also preferred *N. luteum* over *N. advena* when they were raised on *N. luteum* (Fig. 7), a preference that differed from field-collected adults (from *N. luteum*) when offered 16 macrophytes (Fig. 2B). Adults from North Carolina raised on the surrogate

host *N. advena* showed no significant preference between the species of *Nuphar* (Fig. 7). Overall, the preferred foodplant *N. luteum* was also the plant on which animals attained the greater adult mass (Fig. 6C).

Performance experiment II.—*Galerucella* collected from south Michigan and Indiana that were subsequently raised on *N. advena* or *P. amphibium* showed more differences in larval performance than did Indiana and North Carolina *Galerucella* raised on different *Nuphar* species (Performance experiment I). South Michigan *Galerucella* developed more rapidly (Fig. 8A) and survived better (Fig. 8B) on *Polygonum* than on *Nuphar*. In contrast, development rate and survivorship of Indiana *Galerucella*, which were raised on *Polygonum* vs *Nuphar*, did not differ significantly (Fig. 8), but their development was monitored only through the 2nd instar. Survivorship to instar 2 was higher for Indiana beetles than for south Michigan beetles ($p = 0.006$) mostly because of the poor performance of the latter on *Nuphar*. Survivorship to instar 2 varied with respect to rearing host differently for the 2 populations of *Galerucella* as indicated by a significant population \times host plant interaction ($p = 0.00002$): south Michigan larvae survived better on *Polygonum* and Indiana larvae tended to survive better on *Nuphar* (Fig. 8B).

Larval and adult *Galerucella* from south Michigan preferred their natural host *Polygonum* to *Nuphar* when raised on *Polygonum* (Fig. 9). Poor survivorship of the beetles raised on *Nuphar* (Fig. 8B) limited the sample size and statistical power for feeding assays. Yet, south Michigan larvae that were raised on *Nuphar* showed a nonsignificant preference for *Nuphar* over their natural host *Polygonum* (Fig. 9A). This trend was completely reversed with metamorphosis because the 4 surviving adults raised on *Nuphar* ate only *Polygonum* (Fig. 9B), but different animals were used in these 2 assays. Larval *Galerucella* from Indiana showed no statistically significant preference for either *Polygonum* or *Nuphar*, but trends suggested that these larvae ate more *Nuphar* than *Polygonum* (Fig. 9A). The feeding preferences of field-collected *Galerucella* from south Michigan (on *Polygonum*) and Indiana *Galerucella* (on *Nuphar*) were similar to the preferences of animals reared in the lab on their respective natural hosts. All stages of *Galerucella* from both sites preferred their natural host (Fig. 4).

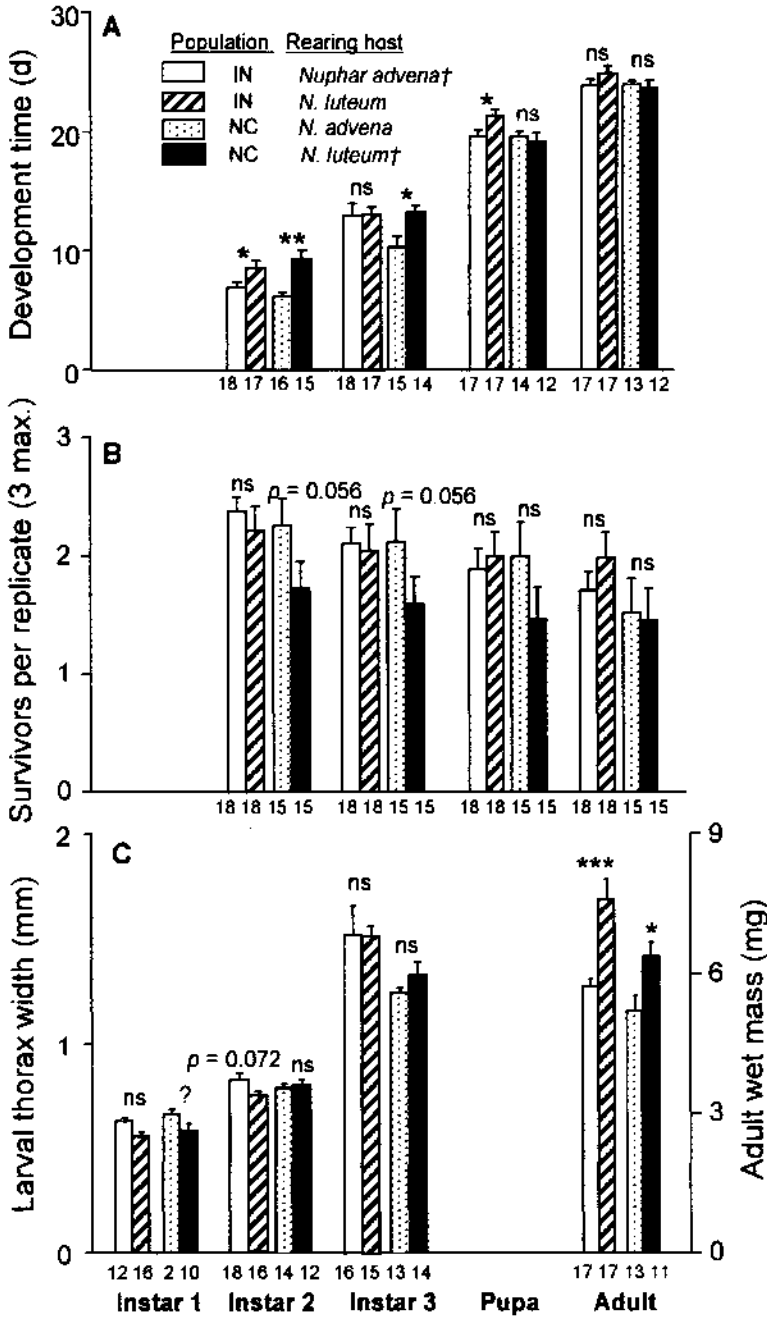


FIG. 6. Performance of Indiana (IN) and North Carolina (NC) *Galerucella* raised on *Nuphar advena* or *N. luteum*. A.—Number of days required to reach various stages of development. B.—Mean survivorship of the beetles to each stage. C.—Larval thorax width and adult wet mass. Bars are mean +1 SE of the measure. † = the natural field host. The number below each bar is the sample size. Pairs of bars (i.e., same population raised on different host plants) were compared with paired-sample *t*-tests. ns = $p > 0.1$, * = $0.01 < p < 0.05$, ** = $0.005 < p < 0.01$. *p*-values between 0.05 and 0.1 are provided. ? indicates that statistical analysis was not possible because of low sample size.

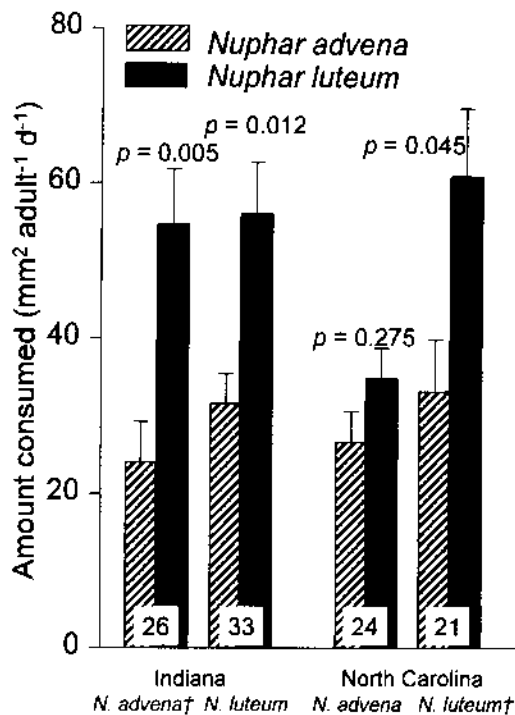


FIG. 7. Feeding preference of Indiana and North Carolina *Galerucella* adults, raised on either *Nuphar luteum* or *N. advena*, when offered a choice between the 2 species of *Nuphar*. The sample size of each assay is at the base of each pair of means (+1 SE). † = the natural field host. Paired bars were compared with paired-sample *t*-tests.

Discussion

Feeding preferences and performance of Galerucella

Our results provide the 1st experimental confirmation that North American *Galerucella* are specialized feeders, but reveal interpopulation differences in host preferences not previously described. Our populations of *G. nymphaeae* had a narrow diet breadth and strongly preferred *Polygonum*, *Nuphar*, and in 1 case, *Brasenia* (Figs 2, 3, 4, and 5). However, feeding preferences of *Galerucella* varied greatly among populations; animals from the south Michigan population even avoided *N. advena* (Figs 3, 4, and 5), a preferred food of the other populations (Figs 2 and 4). Feeding behavior of *Galerucella* was not greatly influenced by past diet within a single generation (Figs 7 and 9). Overall, our results suggest the narrow diet breadth of *G. nymphaeae* is largely genetic and non-plastic during a single generation.

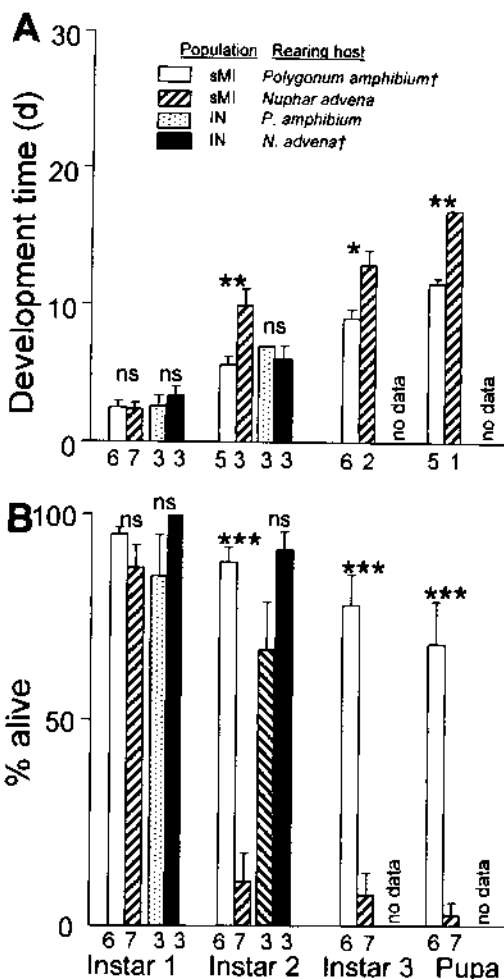


FIG. 8. Development time (A) and survivorship (B) to different life stages (e.g., survivorship to instar 1 = hatching success) of *Galerucella* collected from Gray Lake (south Michigan [sMI]; natural host *Polygonum*) and Lake Papakeechee (Indiana [IN]; natural host *Nuphar*) and raised on *Polygonum* or *Nuphar* from the beetles' respective lake. † = the natural field host. The number below each bar is the sample size. Pairs of bars (i.e., same population raised on different host plants) were compared with paired-sample *t*-tests. ns = $p > 0.1$, * = $0.01 < p < 0.05$, ** = $0.005 < p < 0.01$.

In addition to the intraspecific variation in the palatability of *N. advena* collected from Lake Papakeechee vs Gray Lake (Fig. 2), temporal changes in the preferences of *Galerucella* for *N. luteum* and *N. advena* indicate temporal variation in the preferences of the beetle and/or palatability of the plants. Indiana *Galerucella* preferred *N. advena* from Lake Papakeechee over *N.*

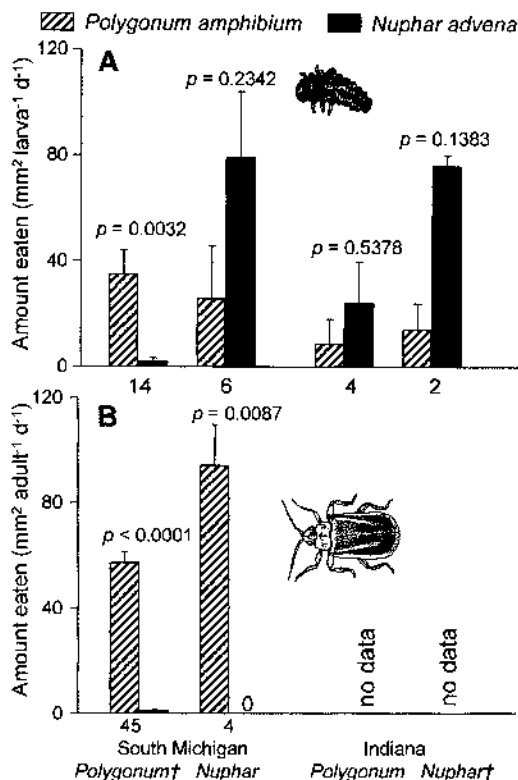


FIG. 9. Feeding preference of larval (A) and adult (B) south Michigan (from *Polygonum*) and Indiana (from *Nuphar*) *Galerucella*, raised on either *Polygonum amphibium* or *Nuphar advena*, when offered a choice between these 2 species of macrophytes. † = the natural field host. The sample size of each assay is at the base of each pair of means (+1 SE). Paired bars were compared with paired-sample *t*-tests.

luteum from North Carolina on 27–30 June (Fig. 2E), but preferred *N. luteum* over *N. advena* in late July–early August (Fig. 7). Given that populations of *Galerucella* ate on average more *N. advena* than *N. luteum* on 27–30 June (Fig. 2) and that adult mass was higher on the more preferred *N. luteum* during Performance experiment I, discrepancies in preferences in Figs 2 and 7 were most likely a result of changes in the quality of the plants.

Performance on a surrogate host ranged from dismal (Fig. 8) to similar to or better than the performance on the natural host (Fig. 6). Poor survivorship on alternative host plants was usually caused by behavioral avoidance of plants and apparent starvation. *Galerucella nymphaeae* usually performed well on *Nuphar* spp. and *P.*

amphibium, with the exception that south Michigan *Galerucella* had extremely poor survivorship on *N. advena*, again, because of avoidance and starvation (T. Schlacher, personal observation). We do not know whether the animals were physiologically capable of growing on alternative host plants had they consumed the plants. When animals consumed similar amounts of an alternative host, their development times (Fig. 6A) and survivorship to adulthood (Fig. 6B) were similar to animals raised on the natural host. Differences in the quality of different *Nuphar* spp. as a host plant is suggested by the fact that animals, even ones collected from *N. advena*, attained a heavier adult mass when raised on *N. luteum* than on *N. advena* (Fig. 6), even though similar amounts of each plant species were consumed.

Implications for host race formation

Several ecological and evolutionary theories have been proposed to account for the high diversity and host specialization of herbivorous insects (Futuyma 1983, Futuyma and Moreno 1988, Courtney and Kibota 1990), but a consensus has yet to be reached about factors that initiate narrow diet breadth. However, it is widely accepted that behavioral responses to unique plant traits (often secondary chemistry) are important in maintaining specialized insect-host relations (Futuyma 1983, Bernays and Chapman 1987, Courtney and Kibota 1990, Futuyma and Keese 1992).

Conventional wisdom holds that most host shifts occur from the original host to a closely related plant or a plant with similar traits (e.g., chemistry) because only minor physiological or behavioral modifications in the insect would be necessary (Futuyma and Keese 1992). Thus, related insects tend to use related plants (Strong et al. 1984, Farrell and Mitter 1990, Bowers et al. 1992). However, North American *G. nymphaeae* is listed as being associated with native plants in 3 unrelated genera: *Nuphar* spp. (Blatchley 1910, Böving 1929, Dillon and Dillon 1972, Wallace and O'Hop 1985); *Polygonum* spp. (Blatchley 1910, Böving 1929, Dillon and Dillon 1972, Stehr 1991, Manguin et al. 1993); and *Myrica* (Manguin et al. 1993). North American *G. nymphaeae* has also been found on purple loosestrife *L. salicaria* (Manguin et al. 1993, Nechols et al. 1996, Cronin 1997), an exotic weed introduced into

North America from Europe ~200 y ago. *Trapa natans* and *Nymphaea odorata* have also been listed as hosts (Schmidt 1985, cited in Nechols et al. 1996). Thus, although *G. nymphaeae* has a restricted diet compared to most herbivores of aquatic plants (Lodge et al. 1998), its diet is rather diverse compared to most herbivorous terrestrial insects (Bernays 1989).

Other genera of North American Nymphaeaceae are typically not listed as hosts and the beetle avoided other water lilies (*Nymphaea* and *Brasenia*) in feeding assays performed in 1995 (Figs 2 and 3). However, a population of *Galerucella* in Gray Lake that used *Brasenia* as a host was found in 1996 (S. Johnson, University of Notre Dame, personal communication). Both larvae and adults preferred *Brasenia* over *Polygonum*, *Nuphar*, and *Nymphaea* (Fig. 5). To our knowledge, the *Galerucella* population found on *Brasenia* in 1996 is the 1st known to occur on this macrophyte. All of our populations of *Galerucella* readily used *Polygonum* (Polygonaceae) as food and 2 populations (Gray Lake and some Lake Papakeechee individuals) used this genus as a host in the field, yet *Polygonum* is not closely related to *Nuphar* spp. It is not known how similar *Nuphar*, *Brasenia*, *Nymphaea*, *Trapa*, *Polygonum*, *Myrica*, and *Lythrum* (all genera are hosts for *Galerucella*) are chemically, but they all share an affinity for shallow aquatic habitats, possess floating or emergent leaves, have a widespread distribution in eastern North America, and they are often locally abundant. A shift onto a chemically dissimilar host is more likely if the plants are ecologically similar (Courtney and Kibota 1990).

We can only speculate about the ancestral host of North American *Galerucella* and what host shifts have occurred in the past. Given the terrestrial habits of the *Galerucella* clade, it is most likely that a terrestrial relative of *G. nymphaeae* moved onto aquatic plants prior to speciation. Our results suggest that *Polygonum* was colonized prior to *Nuphar*, *Brasenia*, or *Lythrum*: *Polygonum* was readily eaten by all of our populations of *Galerucella*, whereas other macrophytes were not. Additionally, *Polygonum* lies closer to the terrestrial end of the terrestrial-aquatic continuum than does *Nuphar* or *Brasenia*. Perhaps *Polygonum* served as a stepping stone for *Galerucella* to reach *Nuphar*, which typically occurs in deeper water than *Polygonum*. The shift onto *L. salicaria* has occurred within the

last 200 y in North America. The apparently recent shift onto *Brasenia* was observed at a site inhabited by a non-*Nuphar*-feeding population previously (and currently) found on *Polygonum*.

Preferences of the Indiana population were more similar to the preferences of the UP Michigan and North Carolina populations than to the south Michigan population located just 67 km away (Figs 1, 2, and 3). *Galerucella* collected from the same lake, but from different hosts in different years, had very different preferences (Fig. 3 cf. 5). Thus, there does not appear to be a cline, and geographic proximity is a poor predictor of host preferences in *Galerucella*.

The *G. nymphaeae* species complex is broadly distributed in the northern hemisphere and has been the subject of taxonomic study for 2 centuries; yet, species assignments within this group remain uncertain (Hippra and Koponen 1986, Wallace and O'Hop 1985, Manguin et al. 1993, Nokkala and Nokkala 1994, Nokkala et al. 1998). Northern European *G. nymphaeae* specializes on *N. luteum* (as does our North Carolina population). However, there is uncertainty about whether *Galerucella* that occur on *Rubus chamaemorus* (Rosaceae) or *Comarum palustre* (Rosaceae) represent conspecifics to those on *Nuphar* (i.e., different host races) or justify consideration as the separate species *G. sagittariae* (Hippra and Koponen 1986, Nokkala and Nokkala 1989a, 1989b, 1989c, 1994, Nokkala et al. 1998). These sibling species of *Galerucella* have the same number of diploid chromosomes ($2n = 34$) and successfully hybridize in the lab to produce fully viable and fertile offspring (Hippra and Koponen 1986, Nokkala and Nokkala 1994). However, they differ morphometrically (Hippra and Koponen 1986, Otto and Wallace 1989), have different frequencies of B-chromosome and large Y-chromosome carrying individuals (Nokkala and Nokkala 1989a), have fixed differences in chorion polypeptides (Nokkala and Nokkala 1989b, 1989c), and are reproductively isolated in nature. Both Hippra and Koponen (1986) and Nokkala and Nokkala (1994) concluded that *G. nymphaeae* and *G. sagittariae* are closely related, but distinct, sympatric species.

Although North American and European beetles from both locations were treated as *G. nymphaeae*, Otto and Wallace (1989) suggested that these allopatric beetles might represent different species. More recently, chorion polypeptide analysis suggests that North American *G. nym-*

phaeae are more closely related to European *G. sagittariae* than to European *G. nymphaeae* (Nokkala et al. 1998). Recent analyses of mtDNA suggest that European *G. nymphaeae* and *G. sagittariae* are more closely related to each other than either is related to North American *G. nymphaeae*, although European *G. sagittariae* and North American *G. nymphaeae* successfully breed in the lab (J. Feder and co-workers, University of Notre Dame, personal communication). Clearly much phylogenetic work remains to be done on this group of species. Whatever species the animals used in our experiments represent, preliminary allozyme analyses of our populations of *Galerucella* indicated that they were all the same species (J. Feder and U. Stolz, University of Notre Dame, personal communication). Likewise, allozyme analyses of 3 North American populations of *G. nymphaeae* examined by Manguin et al. (1993) revealed only minor among-population differences.

Not only are herbivorous insects more likely to use related than unrelated host plants, but related insects are more likely than unrelated insects to have similar host plant preferences (Futuyma 1983, Bernays and Chapman 1987, Futuyma and Keese 1992). *Lythrum salicaria* was 1 of the more commonly sampled plants during 1995 feeding assays (Figs 2 and 3). Consistent with the knowledge that related insects prefer similar plants, other species of *Galerucella*, *G. californiensis* and *G. pusilla*, are natural herbivores of *L. salicaria* in Europe. However, *G. nymphaeae* and *G. sagittariae* are not herbivores of *L. salicaria* in European habitats (C. Nokkala, University of Turku, personal communication). The US Fish and Wildlife Service has recently released *G. californiensis* and *G. pusilla* in the USA in a biocontrol effort (Malecki et al. 1993). Given that native North American *G. nymphaeae* shows some inclination to use *L. salicaria* as a host (Nechols et al. 1996, Tauber et al. 1996, Figs 2 and 3) and can grow from egg to adult on a monospecific diet of *L. salicaria* (*G. Cronin*, personal observation), it seems wiser to attempt biocontrol with a native herbivore before resorting to the use of exotic species to control another exotic species (*Cronin* 1997). Other attempts to control exotic weeds with native insects (*Creed and Sheldon* 1995, *Sheldon and Creed* 1995, *Newman et al.* 1998) or to alter the behavior of native herbivores to preferentially use exotic weeds (*Solarz and Newman* 1996) show potential for biocon-

trol of exotic nuisances without resorting to further introductions. Efforts such as these will provide opportunities to study the effects of environment on insect populations, the effects of herbivory on plant performance, the occurrence of host shifts, and possibly to observe speciation on short time scales.

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