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Between-Site Variation in Suitability of *Salix Cordata* as a Host for *Altica Subplicata* (Coleoptera: Chrysomelidae)

Dennis J. Milanowski
Washington University

Catherine E. Bach
Eastern Michigan University

BETWEEN-SITE VARIATION IN SUITABILITY OF *SALIX CORDATA* AS A HOST FOR *ALTICA SUBPLICATA* (COLEOPTERA: CHRYSOMELIDAE)

Dennis J. Milanowski[1] and Catherine E. Bach[2]

ABSTRACT

To investigate local adaptation of insect herbivore populations to host plant populations, willow flea beetles (*Altica subplicata*) were collected from two distant sites in northern Michigan (Grass Bay, GB; Pte. Aux Chenes, PAC) and reared on host plants (*Salix cordata*) collected from each of the sites. Larval development (measured by molt frequency and length of larval stage) was significantly faster on PAC plants than on GB plants but did not differ for the two beetle populations. For both populations of beetles, mean pupal weight was also greater on PAC plants than on GB plants. Thus, there was no evidence for adaptation of beetle populations to local host plant populations. The greater performance of *A. subplicata* on PAC plants most likely resulted from a lower trichome density on leaves of plants from that site.

Considerable attention in the field of insect/plant interactions has focused on genetic variation in both plant and insect populations. Plant populations differ in secondary chemistry and many other physiological and morphological characteristics (Sturgeon 1979, Waser et al. 1982, Silander 1985). Population differentiation has also been reported for many insect herbivores (Futuyma and Peterson 1985, Rank 1992). Many studies have found significant differences between populations of herbivores in performance on different populations of host plants (Scriber 1983, Rowell-Rahier 1984, Hare and Kennedy 1986, Feder et al. 1988). For example, geographic races of swallowtail butterflies are better adapted to sympatric host species (Nitao et al. 1988), and the Colorado potato beetle has "host-adapted populations" (Hsiao 1978). Local adaptation can also occur at the individual plant level. Edmunds and Alstad (1978) showed that demes of scale insects were adapted to individual conifer trees, and Karban (1989) found that thrips were adapted to individual plant genotypes.

Herbivore performance is often affected by plant morphological characteristics, such as leaf pubescence (Schillinger and Gallun 1968, Rowell-Rahier 1984, Woodman and Fernandes 1991) and cuticular morphology (Stork 1980). Some species of willows show a high degree of variation in leaf pubescence, both within and between populations. Cassin (1989) found strong within-population variation in pubescence of *Salix exigua*, and resultant effects on preference and development of a willow flea beetle, *Altica subplicata* LeC. Observations of *Salix cordata* reveal large differences in leaf pubescence between populations, but effects on herbivore development have not been studied.

[1]Department of Biology, Washington University, St. Louis, MO 63130.
[2]Department of Biology, Eastern Michigan University, Ypsilanti, MI 48197.

The purpose of this study was to examine population differentiation in host-herbivore dynamics between a willow flea beetle, *Altica subplicata* (Coleoptera: Chrysomelidae: Alticinae) and sand-dune willow, *Salix cordata* (Salicaceae). The study addressed the following questions: (1) Do two beetle populations differ in performance (larval development and pupation success) on two different plant populations?, (2) Is there local adaptation of beetle populations to plant populations?, and (3) Are there between-population differences in leaf pubescence that are consistent with differences in herbivore performance?

MATERIALS AND METHODS

Salix spp. are dominant woody plants of developing sand dunes along the shores of the Great Lakes. *Altica subplicata* is a specialist herbivore that feeds predominantly on *Salix cordata* and *S. exigua*. In northern Michigan, *A. subplicata* has one or two generations per year and overwinters as adults (Bach 1990). Larvae are leaf skeletonizers; adults are foliovores that consume entire leaves. After the third instar, prepupae burrow into the sand and pupate.

Two sites were selected for sampling of *A. subplicata* and *S. cordata*: Pte. Aux Chenes (PAC) on Lake Michigan in the upper peninsula of Michigan (Mackinac Co.) and Grass Bay (GB) on Lake Huron in the lower peninsula of Michigan (Cheboygan Co.). These sites are approximately 45 km apart and were considered to contain distinct (non-interbreeding) populations of both beetles and plants. Mark-recapture studies have shown that vagility of beetles is low (Bach, unpublished data).

Adults were collected from PAC on 3 June 1992, and from GB on 4 June 1992. Mating pairs were placed individually in 100 mm petri dishes lined with moist filter paper and containing fresh shoots of *S. cordata* collected from the same site as the adult beetles. Eggs were removed daily and placed in 60 mm petri dishes on moist filter paper until hatching. Hatched larvae were not allowed to feed before the onset of the experiment to prevent the possibility of larval conditioning to *S. cordata* from a particular site (although this experimental design cannot rule out the possibility of conditioning due to maternal diet). Larvae were pooled from all mating pairs obtained at a given site.

Salix cordata leaves were collected from each site once per week for the duration of the study. At each collection, a random point was selected 5–10 m from the lake shore and plants nearest to that point were collected by clipping 10–15 cm shoots. Host plant age and degree of feeding damage were similar among the plants used from the two sites. Leaves were stripped from the shoots and refrigerated in plastic bags until needed. Bags were shaken prior to use, to provide a random mixture of leaves for each dish.

Using a 2x2 factorial design, each population of beetles (GB and PAC) was reared on *S. cordata* from each of the sites, resulting in four treatments. There were ten replicates of the two treatments with PAC beetles and eight replicates of the two treatments with GB beetles. Each replicate consisted of a 60 mm petri dish lined with moist filter paper, sufficient *S. cordata* leaves to cover the bottom of the dish, and five larvae. Larvae were added to each dish at the onset of the study, 16 June 1992. Dishes were placed in an environmental chamber in a randomized block design with one replicate of each treatment in each row; position of dishes within rows was re-randomized every two days. Larvae were incubated at 22° C with a 16:8 h light:dark cycle for the duration of the experiment (Cassin 1989).

On days 2, 4, 6, 8, and 11 after the onset of the experiment, the number of molts (determined by counting the number of head capsules) and number of

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dead larvae in each dish were recorded. Molt frequency was calculated as the number of molts divided by the number of larvae present. On each sampling date, *S. cordata* leaves were replaced, and dead larvae, frass, and head capsules were removed.

On day 11, remaining larvae were transferred to screen-topped pupation dishes (pint plastic dishes half-filled with moistened sand). *Salix cordata* leaves were placed on the sand and replaced every 2–3 days until all larvae either burrowed into the sand to pupate or died. The date that each larva burrowed into the sand to pupate was recorded. Four to five days after the last larva in each dish pupated, all pupae were removed, dried at 45° C for 72 h, and weighed. The 4–5 day period was required to allow sufficient time for transformation of the prepupae. Larvae that burrowed into the sand, but died without pupating were recorded but not weighed. Mean pupal dry weight was determined for each dish.

Larval developmental rate (molt frequency; mean date of burrowing into sand to pupate) and pupation success (proportion burrowing into sand to pupate; proportion dying after burrowing; mean pupal weight) were analyzed using 2-way ANOVA, testing for effects of beetle population, plant population, and an interaction between beetle and plant populations. A significant interaction term may indicate adaptation of herbivore populations to local plant populations. Separate analyses were conducted on molt frequency for each successive time period. All proportions were arcsine transformed prior to analysis.

To quantify possible differences in densities of trichomes on leaves from the two sites, 5 leaves were haphazardly selected from the bags of leaves from each site and prepared for electron microscopy. Leaves were fixed in 3% glutaraldehyde in phosphate buffer, washed twice in buffer, and dehydrated to 100% ethanol. Leaves were critical-point dried, affixed to stubs with carbon paint, and sputter-coated with gold. All specimens were examined at 10 kV on an Amray scanning electron microscope. Micrographs were taken at 100X of the upper and lower surfaces of each leaf in the center of the leaf to the right of the mid-vein, and were enlarged to 136X. Trichome densities were counted using a 6.35 mm grid, and were calculated as the number of grid intersections that fell directly over a trichome divided by the total number of grid intersections (N=160–216). Because trichome densities were not normally distributed, they were compared with a Mann-Whitney U test.

RESULTS

The frequency of molting from onset to day 2 was found to be 8 times greater for *A. subplicata* feeding on *S. cordata* from PAC than from GB (Fig. 1), indicating that larvae fed PAC plants were able to pass through the first instar more rapidly. Because larvae reared on GB plants initially developed more slowly than larvae reared on PAC plants, they had higher molt frequencies during later time periods (Fig. 1). Plant population significantly affected molt frequency for the first, second, and fifth time periods (Fig. 1, Table 1). In contrast, molt frequency was not significantly different for the two beetle populations at any time period, nor was there a significant interaction between beetle population and plant population (Table 1).

Mean date of burrowing was used as an indication of overall length of larval development. *A. subplicata* reared on GB plants burrowed into the sand 3–4 days later than beetles reared on PAC plants (Fig. 2A). Burrowing date was significantly affected by plant population, but not by beetle population or a beetle population X plant population interaction (Table 2). Thus, larval

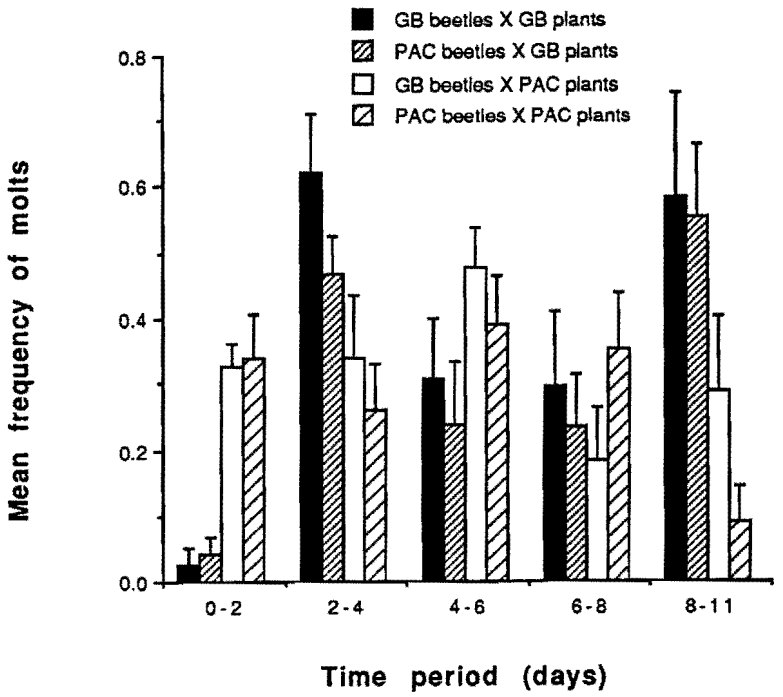

Figure 1. Mean frequency of molts per individual as a function of days from onset of experiment. Means and standard errors are presented for the replicate dishes for each treatment (N=8 for GB plants; N=10 for PAC plants).

Table 1. Results from ANOVAs of larval development, measured as the frequency of molting between successive time periods. F-values and signficance levels are presented from 2-way ANOVAs testing for effects of beetle population, plant population, and an interaction between beetle and plant population. Degrees of freedom were 1,32 for all effects.

	F-values		
Time period	Beetle population	Plant population	Interaction
0-2 d	0.11	44.3***	0.0
2-4 d	2.23	10.2**	0.24
4-6 d	0.90	3.81[a]	0.007
6-8 d	0.36	0.0	1.66
8-11 d	1.07	11.7**	0.56

[a] $P = 0.06$
** = $P < 0.01$
*** = $P < 0.001$

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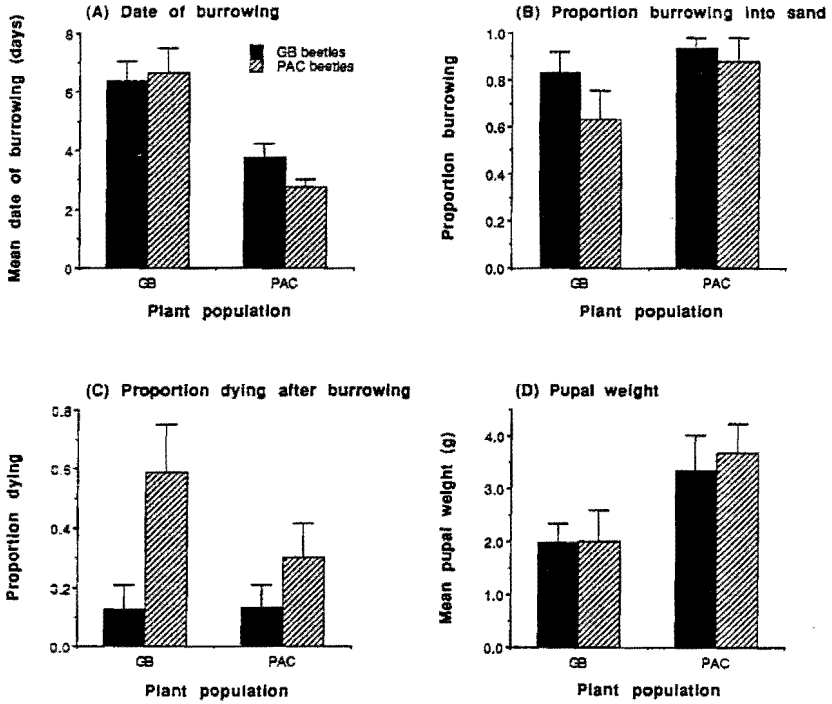

Figure 2. Mean values for parameters of larval development and pupation success for GB beetles (solid bars) and PAC beetles (hatched bars) reared on plants from GB and PAC: (A) Mean date of burrowing (days after being transferred to pupation chambers), (B) Mean proportion burrowing into sand, (C) Mean proportion dying after burrowing, and (D) Mean pupal dry weight (mg). Means and standard errors are presented for the replicate dishes (GB, N=8; PAC, N=10).

Table 2. Results from ANOVAs of length of development time (mean date of burrowing) and pupation success (proportion burrowing into sand, proportion dying after burrowing, and mean pupal weight). F-values and significance levels are presented from 2-way ANOVAs testing for effects of beetle population, plant population, and an interaction between beetle and plant populations. Degrees of freedom for all effects were: (1,29) for date of burrowing and proportion dying after burrowing, (1,32) for proportion burrowing into sand, and (1,23) for pupal weight.

	F-values		
Parameter	Beetle population	Plant population	Interaction
Mean date of burrowing	0.38	32.0***	1.23
Prop. burrowing into sand	1.08	3.47[a]	0.65
Prop. dying after burrowing	8.28**	2.14	2.25
Mean pupal weight	0.61	58.9***	0.47

[a] $P = 0.072$
** = $P < 0.01$
*** = $P < 0.001$

development appears to be more rapid on PAC foliage than on GB foliage, but does not differ for the two beetle populations.

Pupation success was also greater for beetles fed PAC plants. Beetles reared on PAC plants tended to burrow into the sand more often than beetles reared on GB plants (Fig. 2B) although the difference was not significant (Table 2). There was no effect of plant population on proportion of larvae dying after pupating; however, of those larvae that burrowed into the sand, larvae from PAC failed to pupate at a significantly higher rate than larvae from GB (Table 2; Fig. 2C). Mean pupal dry weight was very strongly influenced by host plant population (Table 2). Pupal weight was nearly 75% higher for beetles fed PAC plants than for beetles fed GB plants (3.5 vs. 2.0 mg; Fig. 2D). No measure of pupation success was significantly affected by an interaction between beetle population and plant population. Thus, both populations of beetles had greater pupation success on PAC foliage than on GB foliage.

GB leaves had much higher trichome densities than did PAC leaves (Fig. 3). The mean density of trichomes was significantly higher on the upper surfaces of leaves from GB (0.89 ± 0.053) than from PAC (0.25 ± 0.16) ($U=24$, $P=0.008$). In fact, 60% of the PAC leaves had trichome densities of ≤ 0.006 (less than 6 in 1,000 grid intersections overlaying a trichome), whereas the lowest trichome density on a GB leaf was two orders of magnitude greater, 0.68. Lower surfaces of leaves also had significantly higher trichome densities at GB (0.55 ± 0.18) than at PAC (0.16 ± 0.16; $U=23$, $P=0.016$); in fact, 80% of PAC leaves had no trichomes on the lower surface, whereas all GB leaves had trichomes present on the lower surface.

DISCUSSION

Our results indicate that there was a large difference between the two populations of *S. cordata* in their suitability as a larval food source. Beetles reared on plants from the PAC population had more rapid development and higher pupal weight than beetles reared on plants from the GB population. Despite pupating 3–4 days earlier, larvae reared on PAC plants were nearly double the dry weight of larvae reared on GB plants. Both shorter development and greater pupal weight would presumably lead to greater fitness because: (1) larvae would be susceptible to predation/parasitism for a shorter time (Feeny et al. 1985), and (2) pupal weight is often correlated with adult fecundity in insects (Karowe 1990).

Differences in leaf pubescence between *S. cordata* populations may account for the striking difference in suitability as a larval food source. The GB population clearly had more densely pubescent leaves than the PAC population (see Fig. 3). Leaf surface texture has been shown to affect host plant suitability by affecting attachment and microclimate (Kennedy 1986, Stork 1980). Leaf pubescence is also positively correlated with resistance to herbivory (Schillinger and Gallun 1968) and negatively correlated with host plant preference (Rowell-Rahier 1984). Woodman and Fernandes (1991) reported that leaf hairs explained within-plant patterns of insect herbivory in mullein, *Verbascum thapsus*. In a study similar to ours, Cassin (1989) found that both GB and PAC beetles consumed greater quantities of smooth than hairy *Salix exigua*. PAC beetles developed more rapidly on smooth *S. exigua*; however, pupal weight did not differ between smooth and hairy plants for either population. In addition, beetles preferred shaved over unshaved leaves of *S. cordata* (Cassin 1989).

The higher trichome densities on GB leaves than on PAC leaves could have resulted from differences in the number of trichomes per unit leaf area

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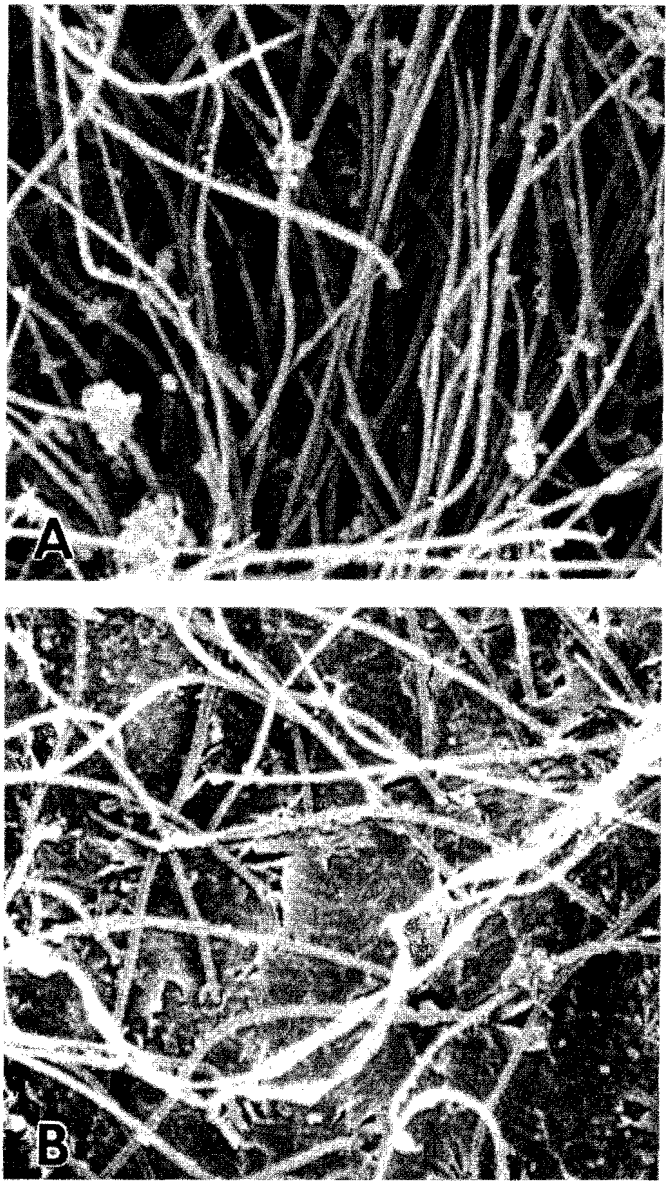

Figure 3. Scanning electron micrographs of the upper surfaces of leaves from (A) Grass Bay and (B) Pte. Aux Chenes. 136x. The two leaves chosen for this figure had the closest trichome densities to the mean for each site.

and/or trichome length; our method of estimation did not distinguish between these alternatives. However, either of these features could interfere with larval feeding. On *Salix cordata*, larvae feed by burrowing through the leaf trichomes on the upper surface of the leaf, scraping the trichomes off and forming a channel as they feed. Consequently, actively-feeding larvae are surrounded by trichome accumulations which they do not consume (Cassin 1989). We suspect that trichomes present a mechanical barrier that decreases consumption rate.

It was interesting to find that, despite strong differences in plant populations, beetle populations performed similarly. Cassin (1989) compared GB and PAC beetle populations on young *S. cordata* leaves from one site (GB) and found no difference in survivorship or developmental time, but GB beetles had signficantly greater consumption rates and pupal weights than did PAC beetles. Perhaps pupal weights of the same two populations did not differ in this study because a mixture of leaf ages was used; Cassin (1989) found no significant difference between pupal weights of GB and PAC beetles reared on old *S. cordata* leaves. Beetle populations in this study differed only in that larvae from PAC had higher mortality after burrowing, independent of host plant population. Perhaps the sand used in the pupation dishes was moister than the sand found at PAC and thus was not tolerated as well by PAC larvae. The lack of any beetle population × plant population interaction effects suggests an absence of local adaptation of *A. subplicata* to *S. cordata*. This result is in contrast to those from other studies reporting local adaptation of herbivores to plant populations (Hsiao 1978, Scriber 1983, Rowell-Rahier 1984, Futuyma and Peterson 1985, Hare and Kennedy 1986, Feder et al. 1988, Nitao et al. 1991) or individual plants (Edmunds and Alstad 1978, Karban 1989).

Overall it appears that the two beetle populations are similar in their ability to use *S. cordata* as a larval food source. It appears that the populations from GB and PAC have not diverged, despite the strong selective pressure on GB beetles for adaptations that would lead to improved performance on pubescent leaves. Perhaps gene flow between the two populations is high enough to mitigate incipient divergence or perhaps sufficient genetic variation is not present. In contrast, the results of this study suggest that there is genotypic or phenotypic differentiation between *S. cordata* populations. Waser et al. (1982) reported genetic differentiation in populations of *Mimulus guttatus* in Utah, resulting in adaptation to local variation in the physical environment. It is likely that the degree of pubescence exhibited by *S. cordata* is a response to the local environment; further study is necessary to determine the underlying causes and whether the response is mediated genotypically or phenotypically.

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