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QUINOLIZIDINE ALKALOIDS OBTAINED BY *Pedicularis semibarbata* (SCROPHULARIACEAE) FROM *Lupinus fulcratus* (LEGUMINOSAE) FAIL TO INFLUENCE THE SPECIALIST HERBIVORE *Euphydryas editha* (LEPIDOPTERA)

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Abstract—*Pedicularis semibarbata* is apparently an obligate hemiparasite of coniferous trees. It is also a facultative parasite of *Lupinus fulcratus* from which we find that it obtains quinolizidine alkaloids, principally α-isolupanine. As a result, a single population of *P. semibarbata* contains both alkaloid-rich and alkaloid-free plants. The butterfly *Euphydryas editha* naturally oviposits on both plant types. This butterfly population, which is the principal herbivore attacking *P. semibarbata* at this site, is known to contain two morphs. Individuals of a specialist morph discriminate when ovipositing among individual *P. semibarbata* plants and produce offspring that survive better on accepted than on rejected plants. Those of a generalist morph accept all *P. semibarbata* plants and produce offspring that survive equally well on plants accepted or rejected by the discriminating morph. Because of the existence of this complex variation among the butterflies, the presence of naturally laid eggs on alkaloid-containing plants still leaves the possibility that the alkaloids may defend the plants against the specialist morph. In experiments on both oviposition preference and larval performance in early instars, we failed to detect any correlation between alkaloid content of a plant and either its acceptability to or suitability for the discriminating morph of the insect. Alkaloid presence in the host-plant population, achieved through root parasitism, is currently neither subject to strong insect-mediated selection nor a major cause of selection on the insects.

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Key Words—*Pedicularis semibarbata*, Scrophulariaceae, *Lupinus fulcratus*, Fabaceae, *Euphydryas editha*, Lepidoptera, Nymphalidae, herbivory, quinolizidine alkaloids, isolupanine, oviposition, parasitic plants.

INTRODUCTION

Harris and Stermitz (1987) showed that root parasitic *Castilleja* species acquire quinolizidine alkaloids from their hosts. This class of alkaloid has been implicated several times in the allelochemical defense of plants (Dolinger et al., 1973; Bentley et al., 1984; Wink, 1985; for example). A single population of parasitic *Castilleja* was found to contain both plants with alkaloids and plants without them. Here, we report a similar finding for a population of the hemiparasite *Pedicularis semibarbata* Gray (Scrophulariaceae) that also contains both alkaloid-rich and alkaloid-free individuals. Such plant populations provide natural systems in which correlations between alkaloid concentration and herbivore preference can be studied, with implications for the importance of secondary utilization by hemiparasites of host defensive compounds (Atsatt, 1977). For these purposes we explored the interaction between *P. semibarbata* and *Euphydryas editha* Bdv (Lepidoptera: Nymphalidae).

METHODS AND MATERIALS

Study Site. Our study site was at Rabbit Meadow along the Generals' Highway above Fresno in Sequoia National Forest, California. At this site the distribution of *E. editha* eggs among four host-plant species has been described by Singer (1983), who found that *P. semibarbata* received about 70% of the eggs deposited by the insect population. Search behavior of the insects for *P. semibarbata* has been studied by Rausher et al. (1981) and by Mackay (1985).

Plant Chemistry. Lupinus fulcratus Greene was identified by D. Wilken, Department of Biology, Colorado State University and a voucher (FRS 326) deposited in the Colorado State University Herbarium. The alkaloid content of *L. fulcratus* and its parasitic *P. semibarbata* was determined as follows.

For a typical alkaloid isolation, 1.3 g of *L. fulcratus* was crushed, wetted with 5% $NaHCO_3$ and stirred in 55 ml of 1 : 1 butanol–toluene for 48 hr. The mixture was filtered and extracted three times with 10 ml of 1 M H_2SO_4. The combined acid layers were extracted with $CHCl_3$ and made basic to pH 9 with NaOH pellets. The basic solution was extracted three times with equal volumes of $CHCl_3$, the $CHCl_3$ layers combined, and evaporated to yield 34 mg of alkaloid mixture.

Essentially the same procedure was used for semiquantitative alkaloid analysis of individual *P. semibarbata* plants. Samples of either 100 or 50 mg

of dry *P. semibarbata* plants from a 1986 collection were extracted and the final $CHCl_3$ solutions evaporated in test tubes. To each tube was added 1.0 ml of $CHCl_3$. Then, 0.10 ml for the 100-mg samples or 0.20 ml for the 50-mg samples was applied to Si gel (0.25 mm, Merck 60) TLC plates. The plates were developed in 9:1 $CHCl_3$–MeOH and spots visualized with iodoplatinic acid spray. Spot sizes and intensities were visually compared. Five 100-mg samples of individual *L. fulcratus* plants were treated and analyzed similarly. Alkaloid presence was readily detected qualitatively using 5–10 mg of dry small individual *P. semibarbata* leaf samples with a modified procedure. Here, the leaf was macerated in a drop or two of 1 M Na_2CO_3 solution in the bottom of a small test tube and about 0.5 ml of 2:1 $CHCl_3$–MeOH added. The mixture was agitated with a small stirring rod for a few minutes, allowed to stand briefly, and the $CHCl_3$ was drawn off with a disposable pipet into a depression in a spot plate. The $CHCl_3$ was allowed to evaporate to a small volume and one tenth to one half spotted on a TLC plate, then developed and visualized as above. This procedure is easily adapted to use in the field.

Alkaloid residues from the 1986 1.3 g *L. fulcratus* extraction and the combined *P. semibarbata* extractions were analyzed by NH_3 chemical ionization mass spectrometry, [^{13}C]NMR spectroscopy, and TLC.

Insect Preferences. Insect preference was tested by placing field-caught insects on undisturbed plants still growing in their natural positions, using the technique of Singer (1986). If deprived of the opportunity to oviposit, discriminating insects will consistently accept some individual plants and reject others.

We performed two experiments in separate years, 1986 and 1988. In 1986 we tested randomly selected morphologically similar plants with no initial knowledge of their alkaloid content. By this means we obtained pairs of accepted and rejected plants. We then analyzed the plants for alkaloids. Only in those cases where alkaloid concentrations were different in accepted and rejected members of a pair could we obtain information about the correlation between alkaloid content and acceptability. In order to increase our efficiency in the second experiment in 1988, we assessed alkaloid content first, then paired each plant that contained alkaloids with a morphologically similar plant that was alkaloid-free. Persons who performed preference trials to determine which (if any) member of the plant pair was more acceptable were ignorant of the identities of the plants that actually contained alkaloids.

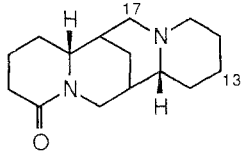
Insect Performance. Eggs of *E. editha* are laid in clusters and larvae are gregarious, occurring naturally in groups of 5–100. As each egg cluster hatched, two groups of 20 newly hatched larvae were isolated. One group of 20 was placed on each member of a plant pair (still growing *in situ*) and allowed to feed for 10 days, after which the remaining larvae were gathered and counted. Again, two experiments were performed in separate years, but with some differences between the years. In 1986, we used eggs gathered at random and

performed alkaloid analyses after larval feeding, while in 1988 we used offspring of discriminating butterflies and performed the analyses before the experiment.

RESULTS

Alkaloid Content. TLC on *L. fulcratus* and 16 of 40 individual *P. semibarbata* plants from the 1986 experiment showed the presence of one major alkaloid at R_f 0.40 along with a small amount of a second at R_f 0.05. NH_3 chemical ionization mass spectra of the alkaloid fractions from both plants were nearly identical and showed a very large M+1 ion at *m/z* 249 and a very small one at 263, indicating the presence of one major and one very minor alkaloid of mol wt 248 and 262, respectively. The mass and [^{13}C]NMR spectra and TLC of the *L. fulcratus* extract corresponded to data for α-isolupanine (Scheme 1), which we had previously isolated (Harris and Stermitz, 1987; Harris, 1987) and identified from *Castilleja miniata* (GH 167, Emerald Lake; Harris, 1987) and its host, *L. argenteus* subp. *spathulatus*. The molecular weight 262 corresponds to that of 17-oxoisolupanine (or an isomer; Kinghorn and Balandrin, 1984), but no attempt was made to further identify this minor component.

Alkaloid Content and Butterfly Oviposition Behavior. In the 1986 experiment, alkaloid analyses were performed on 20 previously identified pairs of accepted and rejected plants. Table 1 shows that eight pairs contained no alkaloids in either plant and three contained alkaloids in both members. This left nine pairs in which one member contained alkaloids while the other did not. Of these, six pairs had alkaloids in the rejected plant and three in the accepted plant. Among the three pairs that both contained alkaloids, the concentrations were about equal in both plants of one pair and greater in the rejected members of the other two pairs. So, in total, we found eight pairs with more alkaloids in the rejected than in the accepted plant and three with more in the accepted than in the rejected. If alkaloid content is independent of acceptability, the probability of observing pairs in which the accepted plant contained more alkaloids than the rejected plant should be equal to the probability of observing pairs in



α-isolupanine

SCHEME 1.

TABLE 1. SURVIVAL OF TWENTY *Euphydryas editha* LARVA ON ALKALOID-CONTAINING AND ALKALOID-FREE PLANTS OF *Pedicularis semibarbata* TESTED FOR OVIPOSITION.

Plant pair:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Accepted:	7[a]	**0**[b]	9	16	12	7	**14**	14	**17**	11	0	11	0	**0**	2	17	17	**10**	18	**13**
Rejected:	14	**0**	14	**18**	13	10	11	**12**	0	7	**0**	8	**6**	0	**9**	10	4	**3**	**8**	**13**

Larval survival on 15 plants with alkaloids: $X = 8.2$; S.D. = 6.39
Larval survival on 25 plants without alkaloids: $X = 9.28$; S.D. = 5.7

[a] Normal type: alkaloid-free.
[b] Bold face: alkaloid-containing.

which the accepted plant contained less alkaloids than the rejected plant (0.50 each). With the finding of eight pairs of plants with more alkaloids in the rejected member and three pairs of plants with more alkaloids in the accepted member, we cannot reject this null hypothesis (Sign test, $Z = 1.21$, $P > 0.10$). In the 1988 experiment, where the alkaloid content of the plants was identified first, the acceptabilities of 15 pairs of plants with and without alkaloids were compared. No difference in acceptability was found between alkaloid-containing and alkaloid-free members in two of the 15 pairs. The alkaloid-containing member was preferred in eight pairs, while the alkaloid-free member was preferred in five pairs (Sign test, $Z = 0.55$, $P > 0.10$).

Alkaloid Content and Early Larval Survival. The number of larvae, out of each grouping of 20, that survived to 10 days is also given in Table 1. Overall, there is no significant difference in larval survival on alkaloid-containing versus alkaloid-free plants ($t = 0.554$, $df = 38$, $P > 0.25$). In this and other studies at Generals' Highway, a number of cases are always observed where there is no survival. Small larval webs are set up initially, but the larvae disappear after only one or two days. From observations, such young larvae are not able to survive away from web or the plant, so their disappearance is considered as mortality. We cannot, however, attribute this mortality specifically to a plant effect since the actual cause is not known. If the cases with zero larval survival are eliminated, the average number of larvae surviving (out of 20 placed) is 11.8 (SD = 4.53, $N = 11$) on alkaloid-containing plants, and 10.1 (SD = 4.97, $N = 21$) on alkaloid-free plants (not significantly different; $t = 0.61$, $df = 30$, $P > 0.25$). There is also no significant difference in larval survival between alkaloid-containing and alkaloid-free members of paired plants (average paired difference -2.09, SD = 6.66, $t = 1.04$, $df = 1$, $P > 0.25$).

In 1988 we observed higher larval survival on the alkaloid-containing plant in five pairs, higher survival on the alkaloid-free member in five pairs, and equal survival on both plants in a single pair. Because there is no trend at all in these data, statistical analysis is not necessary.

DISCUSSION

Plant Parasitism and Alkaloid Transfer. With this work, the transfer of alkaloids from a host plant to a normally alkaloid-free root parasite has now been established for *Pedicularis*, extending the results we found for *Castilleja* (Harris and Stermitz, 1987). As in the *Castilleja* case, root parasitism on a *Lupinus* species has resulted in quinolizidine alkaloid transfer. The quinolizidine alkaloid *N*-methycytisine was previously reported (Ubaev et al., 1963) to be present in *P. olgae*. Our finding of isolupanine as a transferred alkaloid in *P. semibarbata* adds weight to the suggestion (Harris and Stermitz, 1987) that

the *N*-methycytisine in *P. olgae* was probably also the result of root parasitism on a quinolizidine-containing host plant.

P. semibarbata appears from its distribution to be an obligate associate of conifers. This hypothesis is supported by the observation that, whenever trees are removed by clear-cutting, the *Pedicularis* invariably die in one to two years, even though many of them must also be parasitizing *L. fulcratus*, which is not killed by logging. The deaths of *P. semibarbata* after logging do not result from increased insolation, since this plant often grows in open habitats, provided that conifers are close by. In contrast to the obligate association with conifers, many *P. semibarbata* are much too distant from *L. fulcratus* for the possibility of root contact, so the association of the parasite with this plant must be facultative. It appears, then, that *P. semibarbata* requires conifers for survival, but cannot survive solely as a parasite of *L. fulcratus*, from which it nonetheless acquires alkaloids.

Oviposition Behavior. Ng (1988) showed that, with respect to *P. semibarbata*, all discriminating female *E. editha* accept and reject the same plants. Individual plants retain their accepted or rejected status from year to year. Plants that are accepted in preference trials receive more naturally laid eggs than rejected plants (Ng, 1987). We were interested in learning whether or not the acquisition of alkaloids was relevant to the correlated variation of insect adult preference and larval performance shown by Ng (1988). With respect to preference, nine pairs of accepted/rejected plants in our 1986 experiment showed no difference within the pair in alkaloid concentration. For pairs in which there was such a difference, the nonsignificant correlation between alkaloids and host acceptability found in 1986 was in the opposite direction to the even less significant trend in 1988. We conclude that any correlation between alkaloid content and acceptability that may exist could at best explain only a small proportion of the variation in plant acceptability. It could not be the basis of the discrimination phenomena demonstrated by Ng, the chemical basis of which remains unsolved. Ng (1987) showed that variation of water content and nitrogen content were likewise not correlated with acceptability of *P. semibarbata* in the field.

Early Larval Survival. In 1986 we found a nonsignificant trend for better survival on the alkaloid-containing plants and in 1988 found no trend at all in the data. Our conclusion is that the quinolizidine alkaloid content (principally α-isolupanine) of *P. semibarbata* does not render it toxic to young *E. editha* larvae at General's Highway even if they are the offspring of discriminating mothers. We do not think it likely that toxic effects would have been found if we had used later development stages, since early instar lepidopteran larvae are usually more sensitive than later instars to host-plant quality (e.g. White, 1978; Harrison and Karban, 1986; Johnson and Bentley, 1988).

Our sample sizes (20 plant pairs in 1986 and 11 in 1988) are not large,

and a larger data set with more power to detect small correlations between alkaloid content and host-plant acceptability or suitability would be advantageous. Such a data set would, however, be difficult to obtain because the season is short (four weeks) and the work of establishing accepted and rejected plant pairs is labor-intensive.

Context. Previous assessments of insect herbivore interactions with quinolizidine alkaloids or quinolizidine-containing plants have encompassed a variety of situations. Most recently, the lupine alkaloids lupanine and sparteine were shown to reduce growth and survival of first instars of the generalist *Spodoptera eridamia*, but not the growth of later instars (Johnson and Bentley, 1988). Extensive work on quinolizidine alkaloids and "sweet" (alkaloid-free) or "bitter" *Lupinus* species (Wink and Römer, 1986; Wink, 1985, 1987 and references therein) has shown deterrence of leaf miner and aphid generalist feeding and the use of sequestered alkaloids as toxic defenses by specialist aphids. Others have also shown quinolizidine alkaloid deterrence to aphids (Dreyer et al., 1985) and to spruce budworm (Bentley et al., 1984) feeding. In some of these cases, it was clearly evident that there was both herbivore taxon and quinolizidine structure dependence on bioactivity, but even here consistency has not always been observed. For example, 13-acyllupanines were highly deterrent to spruce budworm (*Choristoneura fumifera*) larval feeding, but several other quinolizidines, including α-isolupanine, were not (Bentley et al., 1984), at least at the concentrations used. In contrast, *Lupinus floribundus*, the most heavily attacked of three lupine species studied at several sites (Dolinger et al., 1973) had relatively high concentrations of 13-acyllupanines, which were lacking in the less attacked species. The herbivores, *Glaucopsyche lygdamus* lycaenid butterfly larvae, were also reported to have higher survival in feeding studies on the 13-acyllupanine containing *L. floribundus* than on those species containing nonester quinolizidines.

The much-cited work of Dolinger et al. (1973) was perhaps the closest to our own work in aim. These authors were the first to suggest that interindividual variability of host-plant quinolizidine alkaloid content may have evolved in response to frequency-dependent selection exerted by insects. Working at several high elevation sites in Gunnison County, Colorado, both Dolinger et al. (1973) and Breedlove and Ehrlich (1972) searched for correlations between traits of different lupine species and oviposition by *G. lygdamus*. The plant traits investigated were different in the two studies; Breedlove and Ehrlich used pubescence and Dolinger et al. used alkaloid content. Breedlove and Ehrlich concluded that differences among attack rates on three lupine species were related to differences in pubescence among these species. Dolinger et al. (without discussing the prior results of Breedlove and Ehrlich) concluded that the same differences were related to "alkaloid configuration." Neither conclusion appears to be justified, because both the correlation shown by Breedlove and

Ehrlich between insect attack and pubescence and the correlation shown by Dolinger et al. between insect attack (or host availability) and alkaloid content fall far short of statistical significance. However, the work of Dolinger et al. does show indisputably that accumulation of high quantities of a complex mixture of quinolizidine alkaloids, some of which were subsequently shown to be highly toxic to a generalist (Bentley et al., 1984), is not sufficient to deter extensive attack by a specialist.

Some of the difficulties encountered by Dolinger et al. stemmed from attempts to make comparisons simultaneously across species and populations. In our work, we have attempted to reduce problems of data interpretation by estimating traits within, rather than among, plant and insect populations. In our *P. semibarbata* and *E. editha* system, the conclusion is that alkaloid concentration, although highly variable, is currently neither subject to strong insect-mediated selection nor a major cause of selection on the insects. We earlier identified *Castilleja* species and have now found additional *Pedicularis* species that accumulate other, perhaps more deterrent or toxic, quinolizidine or pyrrolizidine alkaloids. These populations also contain alkaloid-free plants, are attacked by insects, and should provide additional systems for study.

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