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THE ROLE OF IRIDOID GLYCOSIDES IN HOST-PLANT SPECIFICITY OF CHECKERSPOT BUTTERFLIES

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Abstract—The potential role of iridoid glycosides as feeding stimulants for *Euphydryas chalcedona* larvae was examined in three laboratory experiments. The first experiment examined larval behavior in choice tests between an artificial diet with no additives (AD) and an artificial diet with the iridoid glycoside, catalpol, added (AD + I) in one group; and AD and AD plus a crude extract from which the iridoid glycoside catalpol was crystallized (AD + Ex) in the second group. The larvae were found more often on AD + I or AD + Ex. The second experiment quantified larval consumption of artificial diets when given a choice of AD or AD + I, and AD or AD + Ex, and showed that larvae ate significantly more AD + I or AD + Ex than AD. The third experiment compared growth and survival on six diets: AD; AD + I; artificial diet with dried, ground up *Scrophularia californica* leaves (AD + S); artificial diet with dried, ground up *Plantago lanceolata* leaves (AD + P); *S. californica* leaves (S); and *P. lanceolata* leaves (P). Growth was best on *S. californica* leaves, and survival was highest on *S. californica* and *P. lanceolata* leaves. There were no differences in growth rate or survival between AD and AD + I. Thus, iridoid glycosides serve as feeding attractants and stimulants for larvae of *Euphydryas chalcedona* and are suggested as the basis of radiation in butterflies of the genus *Euphydryas*.

Key Words—Iridoid glycoside, catalpol, Scrophulariaceae, *Euphydryas*, checkerspot, host-plant specificity, Lepidoptera, Nymphalidae, coevolution, insect–plant interaction, chemical ecology.

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INTRODUCTION

Host-plant specificity in insects has been described as closely tied to the secondary chemistry of the plants utilized (e.g., Vershaffelt, 1911; Dethier, 1941, 1954; Fraenkel, 1959, 1969; Brower and Brower, 1964; Ehrlich and Raven, 1964; Feeny, 1975). More specifically, in some cases certain compounds typical of host-plants have been shown to be feeding or oviposition stimulants to insects specializing on those plants (e.g., Dethier, 1941; David and Gardner, 1966a,b; Schoonhoven, 1972; Ma and Kubo, 1977; Stanton, 1979). The Lepidoptera in particular have been used in efforts to pinpoint the chemical factors responsible for producing specificity in feeding habits. Although mistakes do occur (e.g., Straatman, 1962; Sevastopolo, 1964; Chew, 1977), female butterflies are generally quite discriminating about where they will lay their eggs (e.g., Chew, 1977; Tabashnik, 1981; Wiklund, 1981; Rausher, 1982; Singer, 1982), and this discriminatory ability is important in ensuring the survival of offspring, as most newly hatched larvae cannot go far in search of food. Larval feeding preferences, however, may be more catholic; larvae may exhaust the food supply and in later instars have to search for additional resources. The ranges of plants utilized by larvae, particularly later instars, may be much larger than those acceptable to ovipositing females, although females may become less discriminating the longer they are prevented from ovipositing (e.g., Singer, 1981). Thus, larvae may respond to a more general range of stimuli than ovipositing females.

In North America, butterflies of the genus *Euphydryas* Scudder (Nymphalidae) utilize plants in four families: Scrophulariaceae, Plantaginaceae, Caprifoliaceae, and Oleaceae (Clark, 1927; Klots, 1958; Masters, 1969; Bowe, 1972; Tietz, 1972; Ehrlich et al., 1975) and in one instance the Labiatae (D. Wiernasz, personal communication). These plant families have in common the presence of a group of plant secondary compounds known as iridoid glycosides (Table 6) (Kooiman, 1972; Jensen et al., 1975). A variety of iridoid glycosides occur in *Euphydryas* food plants, and no one single iridoid is characteristic of all the host-plants (Jirawongse, 1964; Kooiman, 1972; Jensen et al., 1975). However, all of the plant genera fed on by *Euphydryas* that have been tested contain iridoid glycosides (Table 6).

Feeding behavior in another lepidopteran, *Ceratomia catalpae* (Sphingidae) (the catalpa sphinx) was shown by Nayar and Fraenkel (1963) to be elicited by an artificial diet containing a mixture of "catalposides" (i.e., iridoid glycosides) isolated from its food plant, *Catalpa bignonioides* (Bignoniaceae). In contrast, Bernays and de Luca (1981) found that another iridoid glycoside, ipolamiide [isolated from *Stachytarpheta mutabilis* (Verbenaceae)] was a feeding deterrent for three generalist insects: *Spodoptera littoralis* (Lepidoptera: Noctuidae), *Schistocerca gregaria*, and *Locusta migratoria* (both

Orthoptera: Acrididae). Thus iridoid glycosides seem to deter generalist insects, protecting the plants containing them against all but a few specialist species which have circumvented this defense.

Different *Euphydryas* species feed on different plants and have different feeding strategies: *E. gilletii*, for example, is monophagous on *Lonicera involucrata* (Caprifoliaceae) (Williams et al., 1983), while in *E. editha*, different populations use different host-plants, some populations being confined to a single plant species and others using multiple hosts (Ehrlich et al., 1975; Singer and Ehrlich, 1979). Strong circumstantial evidence, such as *Euphydryas* larvae feeding on "non-host" plants which contain iridoid glycosides (Bowers, 1981, and unpublished) coupled with the ubiquity of these compounds in the host-plants, suggested that iridoid glycosides might provide the chemical basis for host-plant specificity in the genus *Euphydryas*.

To determine the role of iridoid glycosides in *Euphydryas* foodplant specialization, I undertook a series of experiments using artificial diet, into which I could incorporate plant material or iridoid glycosides. The first experiment examined the behavioral response of larvae to artificial diets with or without iridoid glycosides; and the second quantified larval consumption of artificial diets with and without iridoid glycosides. The third experiment compared the growth and survival of larvae reared on fresh leaves and on artificial diets with a variety of additives.

METHODS AND MATERIALS

Butterflies and Plants. Larvae of *Euphydryas chalcedona* (Doubleday) were reared from the eggs of females from two populations. Larvae of this species diapause in the fourth instar and thus all experiments were conducted on prediapause larvae. Caterpillars used in the behavior and consumption tests were from Echo Lake, El Dorado County, California, where the foodplant is *Penstemon newberryi* Gray (Scrophulariaceae) (D. Murphy, personal observation). Those used for measuring growth and survival on different diets were taken from Jasper Ridge Biological Preserve, San Mateo County, California, where the food plants are two members of the Scrophulariaceae, *Diplacus aurantiacus* (Curtis) Jeps. and *Scrophularia californica* Cham. & Schlecht.

Scrophularia californica, *P. newberryi*, and *Plantago lanceolata* L. (Plantaginaceae) were used for rearing larvae and incorporation into the artificial diets. *Scrophularia californica* and *P. newberryi* were collected from the native populations, potted, and maintained in a greenhouse. *Plantago lanceolata* leaves were collected on the Stanford campus.

All larvae were reared in plastic Petri dishes with a piece of damp paper

towel taped to the lid to prevent desiccation. The food was placed in the center of the dish. The Petri dishes were kept in an environmental chamber with 16 hr of light and 8 hr of dark, and a day temperature of 25°C and a night temperature of 15°C.

Extraction of the Iridoid Glycoside, Catalpol. Catalpol was extracted from fresh *P. lanceolata* leaves using the charcoal adsorption method of Trim and Hill (1952). This involved a water extraction of the plant material followed by charcoal adsorption of the iridoid glycosides. The adsorbed iridoid glycoside was eluted using 50:50 EtOH–H_2O, and the eluate was concentrated by evaporation. The catalpol crystallized out of the resulting liquor and was purified by recrystallization three times. Thin-layer chromatography showed the compound to be catalpol: the single spot had an R_f of 0.3, and a brown color reaction with H_2SO_4 in MeOH (Wieffering, 1966; Bobbitt and Segebarth, 1969), and an orange color reaction with a *p*-anisidine phosphate reagent (Kooiman, 1967).

Artificial Diet. The artificial diets were made using a slight modification of the recipe of Lincoln et al. (1982) (Table 1). To this basic diet (total dry

TABLE 1. COMPONENTS OF BASIC ARTIFICIAL DIET[a]

Ingredient	Amount
Starch	3.00 g
Sucrose	4.95 g
Wheat germ	2.40 g
Wesson salts	1.38 g
Vandersandt, vitamin mix	2.67 g
Brewer's yeast	0.60 g
Choline chloride	0.135 g
Methyl parabenzoate	0.21 g
Cholesterol	0.09 g
Casein	7.29 g
Ascorbic acid	0.90 g
Tetracycline	0.15 g
Agar	4.00 g
Safflower oil	0.67 ml
Formaldehyde	0.30 ml
KOH	0.72 ml
H_2O	149.00 ml

[a]From Lincoln et al., 1982. Experimental diets contained one of the following in addition to the ingredients above: 1.00 g dried plant material, 0.05 g catalpol, 0.02 g catalpol, or 1 ml crude plant extract.

weight 28.4 g) could be added one of the following: 1 g dried plant material (40.0 mg plant/g diet), 0.02 g of the iridoid glycoside catalpol (0.70 mg catalpol/g diet), 0.05 g catalpol (1.76 mg catalpol/g diet), 1 ml of the crude extract from which the catalpol was crystallized, or nothing. Although relatively little quantitative work has been done on iridoid plant constituents, the amount of iridoid glycoside added to the diet is within the range found in 1 g of plant material (e.g., Trim, 1952; Bobbitt and Segebarth, 1969; Takino et al., 1980). Thus the amount of catalpol found in artificial diets with catalpol corresponds to the amount in artificial diets plus leaf material. The amount of catalpol in the crude extract was not quantified.

The diet was stored until use in covered plastic boxes in the refrigerator, at 3–5°C, and fed to larvae in chunks about 1 × 2 × 10 mm. Larvae in all experiments were given fresh food every two or three days.

Choice Test—Behavior. Larvae in this experiment hatched from eggs obtained from eight Echo Lake females. The eggs from females ovipositing on one day were combined, and all replicates were begun on the same day. Two groups, each containing five replicates of ten larvae, were used. One group was offered a choice of artificial diet with catalpol or artificial diet with no additive; while the second group was offered a choice of artificial diet with the crude extract or artificial diet with no additive. The diets were placed about 2 cm apart in the dish and the dishes oriented randomly to control for position effects (see e.g., Chew, 1980).

Twice a day, once in the morning between 0930 and 1100, and once in the afternoon, between 1500 and 1630, the positions of the larvae were noted as follows: number on AD, number on AD + I or AD + Ex, and number off diet. This was continued for 30 days, when the larvae began to enter diapause.

Choice Test—Consumption. Preliminary experiments had indicated that for prediapause larvae only the third instars ate enough food to be detectable on a weight basis and that field-collected larvae did not initially treat artificial diet with catalpol added as food. If, however, field-collected larvae were fed on artificial diet plus ground *P. newberryi* leaves (the natural host plant), which they ate, the larvae then accepted AD + I or AD + Ex as food. So the experiment was designed using field-collected third instar *E chalcedona* larvae which had fed on AD + *P. newberryi* for two days. One hundred thirty larvae were used in the experiment, five groups of 13 larvae were offered a choice of AD or AD + I and five groups of 13 larvae were offered a choice of AD or AD + Ex.

The larvae were given weighed pieces of diet and allowed to feed for 48 hr (trial 1), the diet collected, fresh diet given, and the larvae allowed to feed for another 48 hr (trial 2). Consumption during each trial was determined on a dry weight basis using the gravimetric techniques of Waldbauer (1968).

While in the refrigerator during the course of the experiment, artificial diets gradually dried out, so a separate regression was used to calculate the

appropriate wet weight-to-dry weight conversion factor, for a particular day of the experiment, for each of the three diets: AD, AD+ I, and AD + Ex. The equations for the lines used to calculate the conversion factors were: AD: $y = 0.2014 - 0.00121x + 0.00005x^2$; AD + I: $y = 0.1838 - 0.0064x + 0.0006x^2$; AD + Ex: $y = 0.1137 + 0.00437x$.

Estimates of the amount of diet eaten in each of the ten groups of larvae included some negative values. These negative numbers reflect the small amounts eaten of the artificial diet with no iridoid glycosides (AD), as well as the necessity of using a wet weight-to-dry weight conversion method. They may also be a function of nonhomogeneity of the diet. The amounts eaten of AD + I and AD + Ex were never negative. To correct for the few negative numbers, the amounts eaten were adjusted by adding 4.0 mg to each (the lowest "negative" amount eaten was −4.00 mg). All statistics and the figures (Figures 3 and 4) reflect these recalculated values.

Growth Rate and Survival. Euphydryas chalcedona eggs were obtained from 12 Jasper Ridge females over a period of one week. Batches of eggs from all females ovipositing on a given day were pooled, so that larvae were not from a single female. Each day that at least 150 larvae hatched, a new replicate of the experiment was set up, for a total of six replicates of six treatments, with 25 larvae in each treatment.

Groups of 25 larvae were reared on one of six diets: (1) S, *Scrophularia californica* leaves; (2) P, *Plantago lanceolata* leaves; (3) AD + S, artificial diet with 1 g dried, ground *S. californica* leaves; (4) AD + P, artificial diet with 1 g dried ground *P. lanceolata* leaves; (5) AD + I, artificial diet with 0.02 g catalpol; (6) AD, artificial diet with nothing added.

This experiment compared the larval growth rate and survival on the various diets, in particular to see how AD + I compared with the others. Every five days, for 25 days, the larvae in each replicate of each treatment were weighed as a group, and the number of surviving larvae was counted. Using these numbers, the mean weight per larva for each replicate of each treatment was calculated.

RESULTS

Choice Experiment—Behavior. When given a choice of AD versus AD + I or AD versus AD + Ex, the pooled results (AM and PM combined) showed that larvae were found significantly more often on AD + I and AD + Ex (Figures 1 and 2). When the AM and PM observations are considered separately, the differences are significant in each case except the PM observations of larvae offered AD + I (Figure 1).

Within a few days after hatching, the larvae constructed a web on one side of the dish and left this only to feed. This resulted in the larvae spending most of their time off the diet (see legends, Figures 1 and 2).

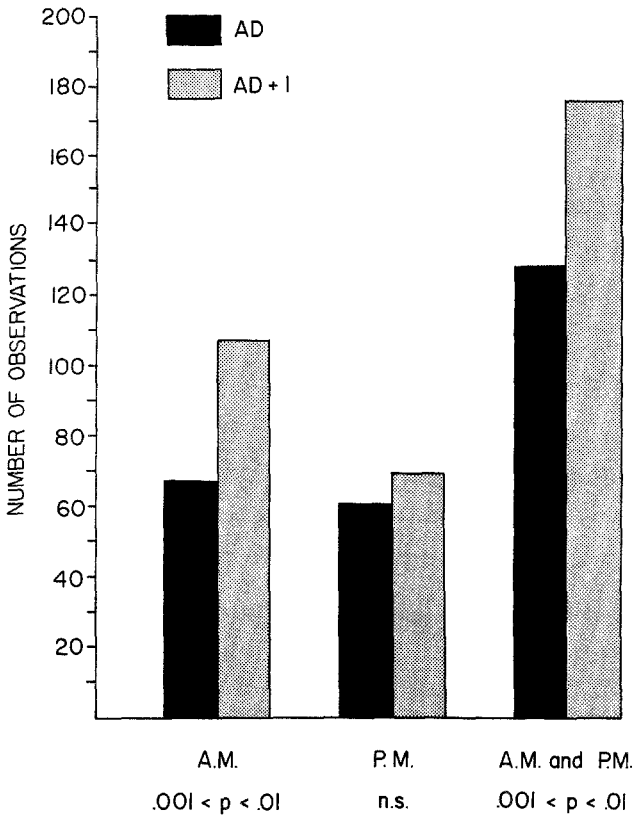

FIG. 1. Behavior of larvae when offered a choice of AD or AD + I, showing the number of larvae observed on each diet. The number of larvae not on either diet was as follows: AM, 750/924 = 81.2%; PM, 784/914 = 85.8%.

Mortality of larvae was high and almost equal in the two groups: group 1 (offered a choice of AD or AD + I), 60%; group 2 (offered a choice of AD or AD + Ex), 62%.

Choice Experiment—Consumption. The results show that much more of the two diets containing iridoid glycosides (AD + I and AD + Ex) were eaten than the artificial diet with nothing added (AD) (Figures 3 and 4). Two-way analyses of variance comparing the amounts eaten of AD and AD + I, and AD and AD + Ex for each of the two trials showed that in every case, more of the artificial diets containing iridoid glycosides (AD + I and AD + Ex) were eaten (Tables 2 and 3, Figure 3 and 4). There were no significant differences among the five dishes of larvae in each trial ($P > 0.10$ in each case).

Growth Rate and Survival. Growth (as measured by weight gain) was greater on *Scrophularia* leaves than on any of the other foods (Table 4, Figure

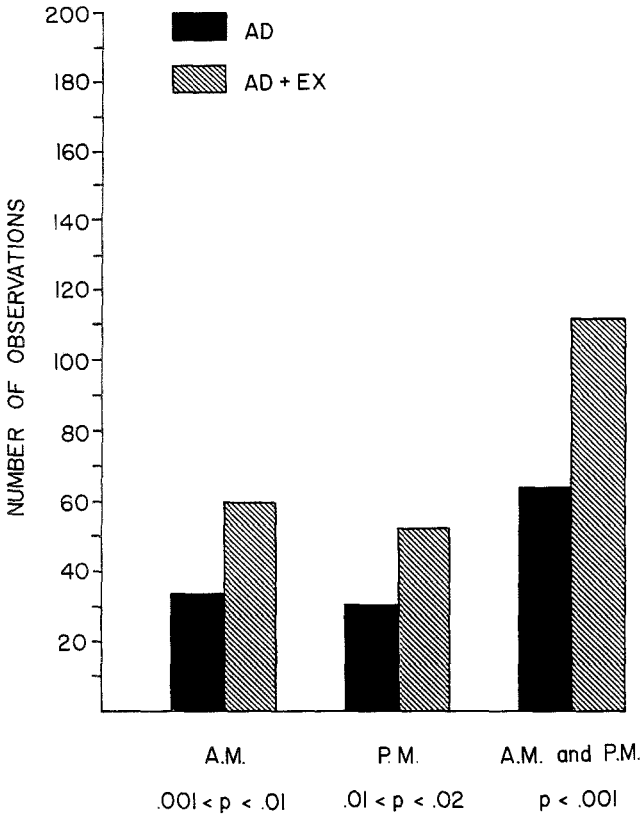

FIG. 2. Behavior of larvae when offered a choice of AD or AD + Ex, showing the number of larvae observed on each diet. The number of larvae not on either diet was as follows: AM, 917/1007 = 90.9%; PM, 920/1002 = 91.8%.

5), while there were no differences between AD and AD + I. Although there were no significant differences in mean larval weight among treatments P, AD + S, AD + P, AD + I, and AD, the relative rankings of these means did change. In particular, the ranking of the larvae fed AD moved from sixth to second. This, as well as the relatively high weight of the AD + I-fed larvae, is due to several of the larvae in these groups bypassing diapause and molting to fifth instar. Larvae in the other treatments, however, were eating less by day 25, preparatory to entering diapause. For all other days, larvae fed AD + I were relatively low in weight, similar to those fed AD.

Survival of larvae was significantly higher on S and P than on AD + I and AD on all days (Table 5, Figure 6), although survival was never

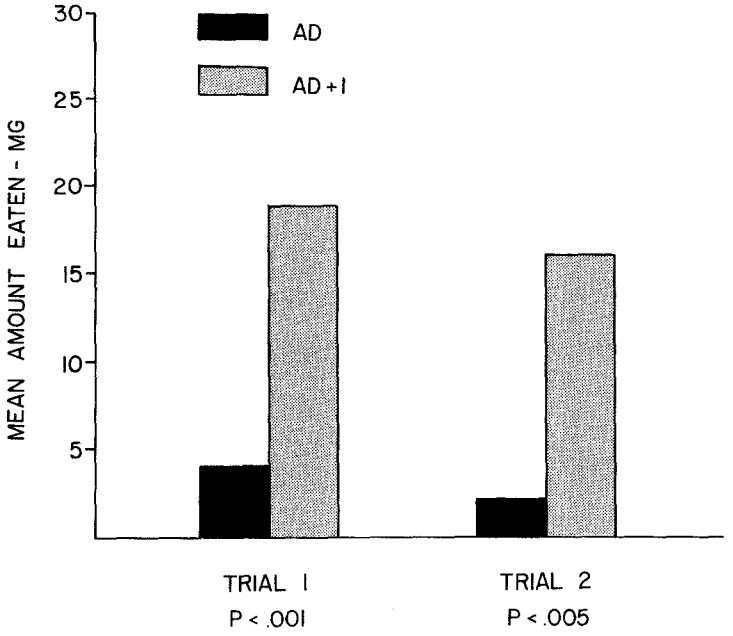

FIG. 3. Consumption by larvae when offered a choice of AD and AD + I: mean amounts eaten in two 48-hr trials.

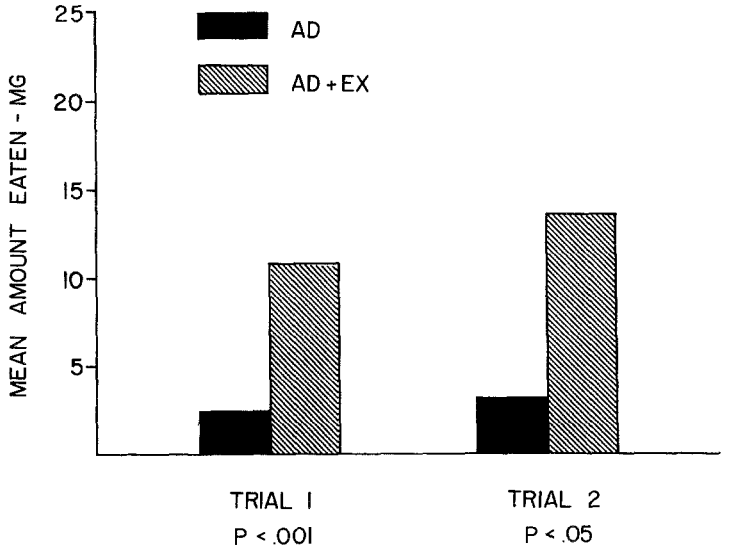

FIG. 4. Consumption by larvae when offered a choice of AD and AD + Ex: mean amounts eaten in two 48-hr trials.

TABLE 2. TWO-WAY ANALYSIS OF VARIANCE COMPARING AMOUNTS EATEN OF AD AND AD + I IN 2 TRIALS, USING 5 DISHES OF 10 LARVAE

Source	Sum of squares	*df*	Mean square	F	*P*
A. Trial 1					
Diet	552.05	1	552.05	111.38	<0.001
Dish	2.71	4	0.68	0.14	>0.10
Error	19.38	4	4.96		
B. Trial 2					
Diet	481.64	1	481.64	569.31	<0.001
Dish	5.14	4	1.29	1.52	>0.10
Error	3.38	4	0.85		

significantly different among larvae fed on S, P, and AD + S, nor among larvae fed on AD + P, AD + I, and AD (Table 5, Figure 6).

DISCUSSION

Bowers (1981) suggested that iridoid glycosides might play a role in determining patterns of hostplant utilization in *Euphydryas*. A literature survey of the genera of food plants of *Euphydryas* butterflies (refs. in Bowers, 1981) showed that they all contained iridoid glycosides, except *Diplacus* and *Besseya*, both in the Scrophulariaceae, which had not been tested (see Table 6). Laboratory tests however, revealed that *Diplacus aurantiacus* and *Besseya alpina* do indeed contain iridoid glycosides (Bowers, unpublished) as

TABLE 3. TWO-WAY ANALYSIS OF VARIANCE COMPARING AMOUNTS EATEN OF AD AND AD + EX IN 2 TRIALS, USING 5 DISHES OF 10 LARVAE

Source	Sum of squares	*df*	Mean square	F	*P*
A. Trial 1					
Diet	176.40	1	176.40	32.15	<0.005
Dish	13.91	4	3.48	0.63	>0.10
Error	21.95	4	5.49		
B. Trial 2					
Diet	201.60	1	201.60	7.92	<0.05
Dish	161.95	4	40.49	1.59	>0.10
Error	101.77	4	25.44		

TABLE 4. COMPARISON OF MEAN WEIGHT PER LARVA (6 REPLICATES PER MEAN) OF LARVAE REARED ON SIX DIFFERENT DIETS (EXPERIMENT 3), OVER 25 DAYS[a]

Day	Mean weight per larva (mg) on various diets					
5	S	P	AD + S	AD + I	AD + P	AD
	1.63	1.41	.894	.799	.649	.619
10	S	AD + S	P	AD + I	AD	AD + P
	6.22	3.87	3.65	2.31	2.19	2.09
15	S	P	AD + S	AD + I	AD	AD + P
	16.49	7.07	5.70	4.26	4.13	3.77
20	S	P	AD + S	AD	AD + P	AD + I
	22.48	12.67	10.38	8.47	7.84	7.70
25	S	AD	AD + I	P	AD + S	AD + P
	26.84	15.22	14.07	12.12	10.97	9.07

[a]According to the Student-Newman-Keuls range test (Sokal and Rohlf, 1969) those means connected by a line are not significantly different at the 1% level.

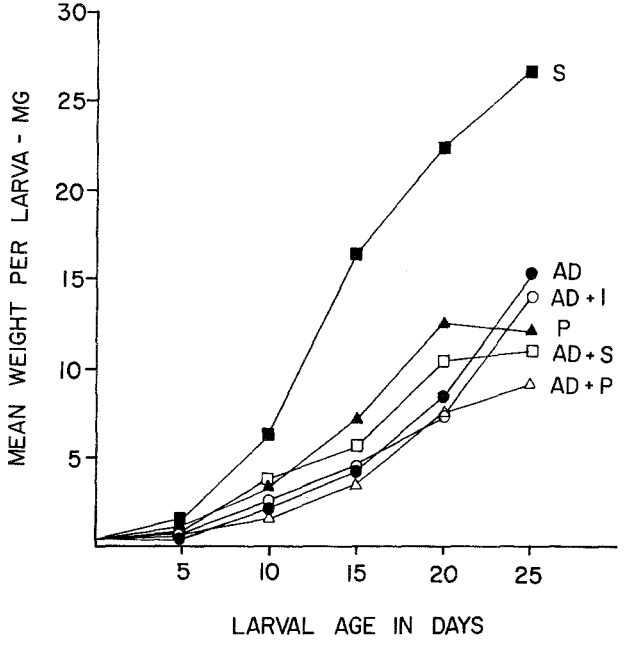

FIG. 5. Mean weight per larva of larvae fed on 6 diets: S, P, AD + S, AD + P, AD + I, and AD; over the 25 days of the experiment.

TABLE 5. COMPARISON OF MEAN SURVIVAL (6 REPLICATES PER MEAN) OF LARVAE REARED ON SIX DIFFERENT DIETS (EXPERIMENT 3), OVER 25 DAYS[a]

Day	Mean Survival (out of 25) of Larvae on Various Diets					
5	S	P	AD + S	AD + P	AD + I	AD
	22.7	22.2	21.0	17.7	14.7	12.5
10	S	P	AD + S	AD + P	AD + I	AD
	22.2	21.5	17.0	12.7	8.3	7.2
15	S	P	AD + S	AD + P	AD + I	AD
	21.0	19.2	15.2	10.7	6.0	5.7
20	S	P	AD + S	AD + P	AD + I	AD
	17.5	17.3	14.3	9.5	5.5	4.8
25	S	P	AD + S	AD + P	AD + I	AD
	16.2	15.0	12.8	8.0	4.5	3.8

[a]According to the Student-Newman-Keuls range test (Sokal and Rohlf, 1969) those means connected by a line are not significantly different at the 1% level.

determined by the Wieffering field test (Wieffering, 1966) and thin-layer chromatography. In addition, *E. chalcedona* larvae will feed and develop on *Aucuba japonica* (Cornaceae), a nonnative shrub which contains the iridoid glycoside, aucubin. Circumstantial evidence thus supports the hypothesis that iridoid glycosides are feeding cues for *Euphydryas* larvae.

The results of the behavior and consumption experiments show that *E. chalcedona* larvae were attracted to and ate much more of the two artificial diets containing iridoid glycosides (AD + I and AD + Ex) than the artificial diet with no additives (AD) (Figures 1–4).

The growth rate and survival experiment showed that larvae fed on artificial diet containing the iridoid glycoside catalpol had no better survival or growth than larvae fed artificial diet with nothing added (Figure 5 and 6). This suggests that larvae were not eating much of either of these diets. Several factors may have contributed to this result: first, the iridoid glycoside used in the AD + I was catalpol, which is only a minor constituent (if present at all) of various *Scrophularia* species (Kooiman, 1972). *Scrophularia californica* is a normal food plant of the *E. chalcedona* used in this experiment. Second, the amount of catalpol used was 0.02 g/28.4 g diet (dry weight), while experiments 1 and 2 used 0.05 g catalpol/28.4 g diet; 0.02 g may be too low a concentration to stimulate much feeding. Third, although catalpol is a larval feeding

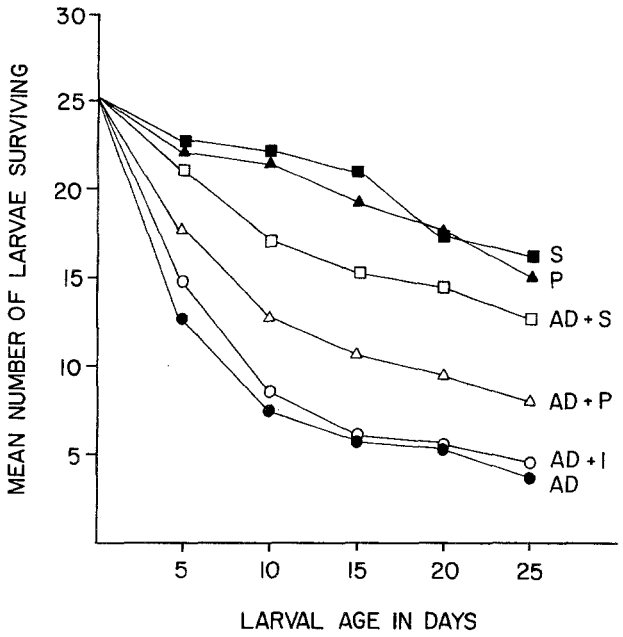

FIG. 6. Mean survival of larvae fed on 6 diets: S, P, AD + S, AD + P, AD + I, and AD; over the 25 days of the experiment.

attractant and stimulant for *E. chalcedona* as shown by the behavior and consumption experiments, other components may be involved as well, which were not present in the AD + I.

The possibility that the demonstrated larval preference in the consumption experiment for iridoid glycoside-containing diets was a conditioned response (Jermy et al., 1968), due to their feeding on an iridoid glycoside-containing food (host plant and artificial diet containing host plant), is belied by the behavior of the newly hatched larvae in the behavior experiment. The newly hatched larvae were given two diets to choose from, one containing iridoid glycosides (AD + I or AD + Ex) and the other not (AD). For the first 4 days, larvae were about equally divided between the two diets, but after this time, larvae clearly preferred the iridoid glycoside-containing diets (Figures 1 and 2). Thus unconditioned larvae also preferred the iridoid glycoside-containing diets.

The AD + I contained only catalpol in addition to the standard dietary components (Table 1); thus, catalpol is a feeding attractant and stimulant for larvae of *E. chalcedona*. The crude extract used to make the AD + Ex was from *Plantago lanceolata*, not a normal host plant of the *E. chalcedona* population that provided these larvae, and both the extract and the plant

TABLE 6. OCCURRENCE OF IRIDOID GLYCOSIDES IN FOOD PLANTS OF NORTH AMERICAN *Euphydryas*[a]

Butterfly	Food-plant genus	Family	Presence of iridoids	Reference
E. phaeton	*Chelone*	Scrophulariaceae	+	Kooiman, 1970
	Aureolaria	Scrophulariaceae	+	Kooiman, 1970
	Penstemon	Scrophulariaceae	+	Kooiman, 1970
	Plantago	Plantaginaceae	+	Duff et al., 1965; Bobbitt and Segebarth, 1969
	Lonicera	Caprifoliaceae	+	Bobbitt and Segebarth, 1969; Souzu and Mitsuhashi, 1969
	Viburnum	Caprifoliaceae	+	Jensen et al., 1975
	Fraxinus	Oleaceae	+	Jensen et al., 1975
E. chalcedona	*Castilleja*	Scrophulariaceae	+	Kooiman, 1970
	Diplacus	Scrophulariaceae	+	Bowers, unpublished
	Pedicularis	Scrophulariaceae	+	Kooiman, 1970
	Penstemon	Scrophulariaceae	+	Kooiman, 1970
	Scrophularia	Scrophulariaceae	+	Kooiman, 1970
	Symphoricarpus	Caprifoliaceae	+	Jensen et al., 1975
E. anicia	*Besseya*	Scrophulariaceae	+	Bowers, unpublished
	Castilleja	Scrophulariaceae	+	Kooiman, 1970
	Penstemon	Scrophulariaceae	+	Kooiman, 1970
	Symphoricarpus	Caprifoliaceae	+	Jensen et al., 1975
E. colon	*Penstemon*	Scrophulariaceae	+	Kooiman, 1970
	Symphoricarpus	Caprifoliaceae	+	Jensen et al., 1975
E. editha	*Castilleja*	Scrophulariaceae	+	Kooiman, 1970
	Collinsia	Scrophulariaceae	+	Kooiman, 1970
	Orthocarpus	Scrophulariaceae	+	Kooiman, 1970
	Pedicularis	Scrophulariaceae	+	Kooiman, 1970
	Penstemon	Scrophulariaceae	+	Kooiman, 1970
	Plantago	Plantaginaceae	+	Kooiman, 1970
	Lonicera	Caprifoliaceae	+	Bobbitt and Segebarth, 1969; Souzu and Mitsuhashi, 1969
E. gilletii	*Lonicera*	Caprifoliaceae	+	Bobbitt and Segebarth, 1969; Souzu and Mitsuhashi, 1969

[a]Parts of this table, in a slightly different form, were published in a previous article: Bowers, 1981.

contain an additional iridoid glycoside, aucubin, not found in the AD + I (Kooiman, 1972, and refs. therein; Bowers, unpublished), as well as other unknown, noniridoid components. Thus, despite containing constituents of a non-host plant, the crude extract was still very attractive to larvae.

Dethier (1947, 1954, 1973) and others (e.g., Schoonhoven, 1972, and refs. therein) have emphasized the importance of olfaction in a larva's initial assessment of the suitability of a plant as food. Crystalline catalpol is not volatile and had no odor detectable to me; the crude extract, however, did contain volatile components and had a distinctive odor. Despite these and the other differences in the two diets, the amounts eaten of AD + I and AD + Ex as a function of the amount of AD eaten were not significantly different in trial 1 or 2 or the two trials combined.

The importance of odor as a key discriminant factor may help explain why third instar larvae in the consumption experiment would not initially accept AD + I as food—there was no appropriate odor to initiate their tasting a food presented in the unusual form of a chunk of artificial diet. However, after eating AD + *P. newberryi*, which provided the correct olfactory cues, a chunk of artificial diet was perceived as food and so tasted; the gustatory cues were correct, thus the larvae would feed.

The extensive work that has been done on the biology of the six North American species of *Euphydryas* (*E. chalcedona, E. editha, E. anicia, E. colon, E. gillettii,* and *E. phaeton*) (Ehrlich et al., 1975; Cullenward et al., 1979; Bowers, 1980, 1981; Brown and Ehrlich, 1981; Morrison et al., 1983; Williams et al., 1983) suggests that while iridoid glycosides may be the basis of host plant specialization in this group, other factors are certainly involved, such as ecological factors and other plant secondary compounds. For example, in *E. editha* two populations have the same two potential food plants present; however, in the Jasper Ridge population females oviposit on *Plantago erecta*, while the Edgewood Road population uses *Orthocarpus densiflorus* Benth. (Scrophulariaceae) (Singer, 1971). Singer (1971) suggested that this difference was due to differences in food plant quality.

In a more recent paper, Singer (1982) illustrated different specificities of individual females from different populations, suggesting a genetic component to female oviposition preference in *E. editha*. In a *Colias* species, Tabashnik et al. (1981) found intrapopulation variation in oviposition choice among females and suggested that these differences were genetically based. Rausher (1982) reared larvae of *E. editha* from two populations on their own and the other's host plants. He found that larvae from each population survived better and grew faster on their own host plant than larvae from the other population [although both groups of larvae did better overall on one of the plants, *Collinsia* (Scrophulariaceae)], suggesting that larvae from a particular population are genetically adapted to their own normal food plant.

Other secondary chemicals as well as iridoid glycosides may play an

important role in determining patterns of host plant specificity. *Diplacus aurantiacus*, for example, which has a digestibility-reducing phenolic resin covering its leaves (Lincoln, 1980; Lincoln et al., 1982), is used as a host plant by some populations of *E. chalcedona*, while individuals from other populations will die if they try to eat those leaves (N. Johnson, personal communication). Some species in the genus *Pedicularis* (Scrophulariaceae), a genus which is fed on by several *E. editha* populations, contain alkaloids (e.g., Lutfullin et al., 1965; Abdusamatov and Yusanov, 1971) which may require special adaptations in individuals from populations feeding on those plants.

The experiments described above show that one iridoid glycoside, catalpol, acts as a feeding stimulant and attractant to *E. chalcedona*. The ubiquity of these compounds among the *Euphydryas* host plants, coupled with the results from these experiments, suggest a general role for iridoid glycosides as the basic feeding attractant/stimulant for the larvae and, I would suggest, oviposition stimulant for females.

Thus, the evolution of the ability of *Euphydryas* species to utilize plants containing iridoid glycosides, and in fact to use these compounds as larval feeding stimulants (and probably adult female oviposition stimulants) may have enabled them to radiate onto a variety of plant families containing those compounds. The related European genera *Euphydryas, Mellicta,* and *Melitaea* also feed primarily, although not exclusively, on plants containing iridoid glycosides, such as *Plantago* (Plantaginaceae), *Scabiosa* (Dipsacaceae), *Gentiana* (Gentianaceae), *Lonicera* (Caprifoliaceae), and a variety of scrophulariaceous plants such as *Linaria, Veronica, Melampyrum*, and *Antirrhinum* (Jensen et al., 1975; Higgins and Riley, 1980). Thus, although many factors influence patterns of host-plant utilization in *Euphydryas*, iridoid glycosides seem to play a fundamental role in determining host-plant specificity in these species.

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