

## CONDITIONING AND GENETIC VARIATION AS CAUSES OF INDIVIDUAL VARIATION IN THE OVIPOSITION BEHAVIOUR OF THE TORTOISE BEETLE, *DELOYALA GUTTATA*

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**Abstract.** Individuals of the tortoise beetle (*Deloyala guttata*) from sites containing different host plants differ genetically in performance on different hosts. Because individual variation in habitat (host) preference could contribute to the maintenance of these genetic differences, this study was undertaken to find out whether *D. guttata* exhibits such variation, and if so, to determine the causes of that variation. No evidence for either larval conditioning or genetic variation in oviposition preference was obtained. An effect of adult experience on oviposition preference was detected, but the magnitude of the effect was so small that it probably has little influence on genetic divergence in traits affecting growth and fecundity. It is concluded that behavioural variation does not contribute to maintenance of the observed genetic divergence.

Habitat-selection behaviour may have a profound influence on the degree to which individuals living in different habitats diverge genetically. In particular, variation among individuals in habitat preference can facilitate genetic divergence under some conditions. Maynard Smith (1966), for example, demonstrated theoretically that variation in behaviour caused by conditioning can have such an effect: a tendency by females to place offspring in the same habitat in which they developed facilitates divergence. Genetic variation in habitat preference may be expected to have similar effects, since heritable variation in habitat preference implies that there will be a correlation between the habitat preference of a female and the habitat preference of her mother (Falconer 1960).

The tortoise beetle, *Deloyala guttata* (Coleoptera: Cassidinae), exhibits genetic variation in traits related to growth and fecundity on two different species of morning glory (*Ipomoea*: Convolvulaceae) (Rausher, in preparation). In general, beetles collected from sites containing only *I. pandurata* have higher fecundities and more rapid growth when reared on *I. pandurata* than beetles collected from sites containing only *I. purpurea*: the opposite is true when the beetles are reared on *I. purpurea*. It seems likely that these differences between sites are due largely to divergent selection, though no direct evidence is yet available on this point. However, divergent selection by itself is not necessarily sufficient to maintain genetic variation in the local metapopulation, particularly if gene flow between sites is high (Christiansen &

Feldman 1975; Felsenstein 1976). If selection is weak and gene flow high, some sort of differential habitat selection by individuals originating from different sites may be required to maintain the observed variation.

The objective of this investigation was to determine whether female *D. guttata* behave in a way that facilitates genetic divergence between sites. In particular, I attempted to determine whether females exhibit any sort of conditioning of oviposition behaviour, or whether individuals vary genetically in host preference. I reasoned that by showing that beetles do not exhibit either conditioning or genetic variation in preference, I could rule out these factors as being necessary for the observed genetic divergence. Both conditioning and genetic variation were examined because previous studies with other phytophagous insects have revealed that individual variation in oviposition preference can be caused by either mechanism (Tabashnik et al. 1981; Jaenike 1982, and unpublished manuscript; Prokopy et al. 1982; Papaj & Rausher in press).

### Methods

#### Study Organisms

The beetle *Deloyala guttata* feeds and oviposits on several species of morning glory (genus *Ipomoea*, Convolvulaceae) in Orange and Durham Counties, North Carolina. Adult beetles were collected from two locations within Orange County. One location was an old field, owned by Duke University, bordered on three sides by forest and on one side by a road and a cultivated field and containing many

*Ipomoea pandurata* plants. This species is a trailing perennial vine which is a common inhabitant of disturbed areas. The population in the old field, designated DF (Duke Field), is at least several years old, probably at least 10. A second species, *I. purpurea*, occurs at the DF site but is rare. It is restricted to the edge of the road and is not found within the field itself. All beetles were collected as late-instar larvae from *I. pandurata* and reared to the adult stage in the laboratory.

The second location from which beetles were collected, WW (Wilbur Way), was a cornfield approximately 6.5 km north of the DF site. At WW, *I. purpurea* was very common, but *I. pandurata* was very rare if not absent. All beetles were collected as late-instar larvae from *I. purpurea* and reared to the adult stage in the laboratory. Foliage for rearing and for behavioural assays was collected from these two sites approximately every 2 days.

### Experiment 1

The purpose of this experiment was to determine whether genetic variation for host preference exists within the DF population, whether larval experience influences adult oviposition preference, and whether there exists genetic variation in the degree of such larval conditioning of adult behaviour.

Newly eclosed first-generation adults from the DF collection were allowed to mate and 12 females were placed individually in petri dishes with leaves of *I. pandurata* to obtain eggs. The larvae hatching from eggs obtained from a particular female were randomly assigned to one of two treatments: rearing on *I. pandurata* or rearing on *I. purpurea*. These experimental larvae were reared in plastic petri dishes in a growth chamber set at a photoperiod regime of L:D = 18.6 h, a temperature regime of 29 ( $\pm 1$ )°C day and 21°C night and a constant relative humidity of approximately 85%. Foliage was replaced every 2 days. Upon eclosion, the first-generation adults were allowed to mate and the females were placed individually in plastic petri dishes containing one piece of an *I. purpurea* leaf and one piece of an *I. pandurata* leaf. To eliminate effects of leaf area on preference, both leaf pieces were cut to the same standard size of approximately 2 × 1 cm. Every 2 days the number of eggs laid on each leaf piece was counted and the old leaf pieces were replaced with fresh ones. The experiment was terminated after 10 days for each female.

The results of this experiment were analysed statistically using the GLM procedure of the SAS statistical package (Barr et al. 1979). The statistical model used was that of a two-way ANOVA, with full-sib family and larval host as the two main effects (Searle 1971). Because repeated measurements were made on the same individual, the existence of variability among individuals was examined statistically by examining the effect of individual nested within family × larval host treatments. In this analysis the dependent variable, fraction of eggs laid on *I. pandurata*, was transformed using the arcsin-square root transformation (Sokal & Rohlf 1969). Family and individual were treated as random effects, while larval host was treated as a fixed effect. Heritability of oviposition preference was estimated as  $h^2 = 2(\sigma_f^2 / \sigma_e^2 + \sigma_f^2)$  where  $\sigma_f^2$  is the between-family component of variance in proportion of eggs laid on *I. pandurata* and  $\sigma_e^2$  is the within-family component. Since full-sib families were used in this analysis, this value represents a maximum value for  $h^2$ . Dominance, epistasis and maternal effects would tend to cause  $h^2$  to overestimate the true heritability by some unknown amount (Falconer 1960).

### Experiment 2

The purpose of this experiment was to determine whether genetic variation for host preference exists within or between the DF and WW populations, whether adult experience influences adult oviposition preference, and whether there exists genetic variation in the magnitude of such a conditioning effect.

Newly-eclosed second-generation adults were obtained from the stock of each population and allowed to mate, and 12 females were placed individually in petri dishes with leaves of *I. purpurea* to obtain eggs. Larvae from each female (representing full-sib families) were randomly assigned to rearing on either *I. pandurata* or *I. purpurea*. These larvae were reared on potted plants in the growth chamber, each larva being restricted to an individual leaf by coating the petiole with Tanglefoot. Each larva was removed from its leaf after it had cleared its gut in preparation for pupation and was placed in a plastic petri dish for pupation. Upon eclosion, females were allowed to mate and placed in a petri dish with foliage from the same host species on which they were reared as larvae. After a 2-week conditioning period, females were then offered a choice of the two host

plants on which to oviposit, as in experiment 1. The 2-week conditioning period was incorporated in order to minimize the possibility of failing to detect a conditioning effect because adults underwent too short a training period.

Statistical analysis of this experiment was again by ANOVA. The statistical model used was slightly more complicated than that used in experiment 1. Families were nested within populations and each of these effects was crossed with host to which adults were conditioned. This latter factor is confounded with the effect of the host larvae were reared on, but, as explained in the Discussion, this confounding does not cause problems in interpreting the results. In this experiment the number of eggs laid per female in each 2-day census period was considerably smaller than in experiment 1, probably because females had exhausted most of their egg supply during the conditioning period. Consequently, data from all egg counts were pooled to yield one value of proportion of eggs laid on *I. pandurata* for each female, which precluded testing for the significance of differences among individual females. All values of the dependent variable were again transformed prior to analysis. Family was treated as a random effect, whereas population and host were treated as fixed effects. Heritability of oviposition preference was estimated as in experiment 1.

## Results

### Experiment 1

Significant phenotypic variation in oviposition preference existed among the 94 individual females tested in this experiment ( $F_{93,371} = 1.97$ ,  $P < 0.0001$ ). The proportion of eggs laid on *I. pandurata* varied from 0.32 to 1.0 and exhibited a roughly Gaussian distribution.

The contribution of genetic differences and larval experience to this phenotypic variation

is shown in Table I. The effect of larval host was not significant; there was thus no detectable effect of larval experience with a particular host species on adult preference. Similarly, there was no significant family effect, indicating no detectable genetic variation among individuals collected from the DF site. This conclusion is confirmed by the very low heritability of oviposition preference:  $h^2 \leq 0.052$  ( $\pm 0.134$ ). Finally, there was no significant interaction between the effect of family and that of larval host. There is thus no genetic variability in the degree to which adult preferences are conditioned by larval experience. This result is not surprising, since the effect of larval experience was not significant. There remains a significant amount of between-individual variation in preference that is not explained by any of these factors (Table I).

### Experiment 2

Beetles exposed initially as adults to *I. pandurata* laid an average of 55.9 ( $\pm 2.6$ )% of their eggs on *I. pandurata*. By contrast, females exposed initially to *I. purpurea* laid an average of only 46.4 ( $\pm 3.4$ )% of their eggs on *I. pandurata*, the difference being significant at the  $P < 0.025$  level (Table II). Consequently, it appears that early adult experience with a host species biases adult preference slightly but perceptibly in favour of that species.

As in experiment 1, there is no evidence of differences among families in oviposition preference (family effect, Table II). There is thus no detectable within-population genetic variation contributing to phenotypic variation among individuals. This conclusion is again confirmed by the extremely low estimate of heritability:  $h^2 \leq 0.016$  ( $\pm 0.139$ ). It is conceivable, however, that genetic variation could exist between patches of different host species

Table I. Analysis of Variance of Proportion of Eggs Laid on *I. pandurata* in Experiment 1

Source	df	SS	MS	F-ratio*	F	P
Individual females	93	11.68	M1	M1/M6	1.97	< 0.0001
Larval host	1	0.16	M2	M2/M5	1.25	NS
Family	11	1.08	M3	M3/M5	0.74	NS
Larval host $\times$ family	11	0.77	M4	M4/M5	0.51	NS
Unexplained	70	9.52	M5	M5/M6	2.13	< 0.0001
Error (repeated measurements)	371	23.71	M6			

\*The F-ratio column indicates the ratio of mean squares that would be used to test the significance of a particular effect if the data were completely balanced. Because the data were somewhat unbalanced, denominator mean squares were synthesized according to the procedure described in Sokal & Rohlf (1969).

Table II. Analysis of Variance of Proportion of Eggs Laid on *I. pandurata* in Experiment 2

Source	df	SS	MS	F-ratio*	F	P
Host	1	0.420	M1	M1/M5	8.03	< 0.025
Population	1	0.094	M2	M2/M4	1.34	NS
Host × population	1	0.635	M3	M3/M5	1.29	NS
Family	12	0.070	M4	M4/M5	1.01	NS
Host × family	11	0.575	M5	M5/M6	0.83	NS
Error	56	3.536	M6			

\*See footnote to Table I.

without such variation being present within patches. This pattern of variation could occur, for example, if two genetic variants existed in the beetle meta-population in Orange County: one that normally oviposits only on *I. pandurata* and one that normally oviposits only on *I. purpurea*. One would expect to find the first genotype only in *I. pandurata* patches, whereas the second would be found only in patches of *I. purpurea*. Such a distribution would be characterized by genetic uniformity within patches (populations or collecting sites in this investigation) but genetic variation between patches. However, the lack of a significant population effect in this experiment (Table II) indicates that patches of the two host species do not accumulate genetically different ensembles of individuals, at least with respect to oviposition preference as assayed. Consequently, this experiment indicates that little, if any, of the phenotypic variation in oviposition preference among individuals is due to underlying genetic differences.

Finally, neither the family × host nor the population × host effect was significant in this experiment (Table II). These results indicate that there was no detectable genetic variation in the magnitude of the effect of conditioning on adult preference, i.e. all beetles responded in the same way to early experience with a particular host species.

### Discussion

A companion study has shown that individuals of *Deloyala guttata* collected from sites containing *Ipomoea pandurata* differ genetically from individuals collected from sites containing *I. purpurea* (Rausher, in preparation). These differences are in traits affecting growth and reproductive success on the two host species. For example, the fecundity of females fed on *I. pandurata* is higher for beetles collected from *pandurata* sites than for beetles collected from

*I. purpurea* sites, whereas the opposite is true for females fed *I. purpurea*. Similar genetic differences exist for developmental time and possibly for pupal weight (Rausher, in preparation). Because any tendency for a female to oviposit on the plant species on which she grew up could amplify or contribute to the maintenance of these differences in fitness components (Maynard Smith 1966; Christiansen & Feldman 1975; Felsenstein 1976), this study was undertaken to determine whether such a tendency exists.

One mechanism that could cause females to oviposit on the same plant species as that on which they developed is larval conditioning, the persistence of a 'larval memory' of a food plant into the adult stage. As in virtually all previous studies of phytophagous insects (Papaj & Rausher in press), the results of this study revealed no evidence for larval conditioning.

Adult conditioning is a second mechanism that could cause females to oviposit preferentially on the plant species on which they fed as larvae. This return to the juvenile host could occur as a result of adult conditioning if (1) host plants tend to occur in monospecific stands and (2) pupation occurs on or near the larval host, since upon emergence adults would be most likely to feed first on their larval host and hence become conditioned to it. Both conditions (1) and (2) are fulfilled in *D. guttata*. Moreover, the results of this investigation indicate that oviposition preference in *D. guttata* is influenced by experience with a particular host species as an adult. Considered by themselves, the results of experiment 2 do not permit a distinction to be made between larval and adult conditioning as causes of the observed conditioning. However, experiment 1, with a larger sample size than experiment 2, failed to reveal any evidence for effects of larval experience. It seems most likely, therefore, that the conditioning effect detected in experiment 2 is due to

the effects of adult rather than of larval experience. This interpretation is consistent with what is currently known about conditioning of oviposition behaviour in phytophagous insects. As mentioned above, no evidence for larval conditioning exists, whereas several recent studies have demonstrated effects of adult experience on oviposition preference (Jaenike 1982; Prokopy et al. 1982; Papaj & Rausher in press, Stanton, unpublished MS).

Although an effect of adult conditioning was detected in this study, the effect is rather weak. Even after 2 weeks of training, the difference in proportion of eggs laid on *I. pandurata* between beetles conditioned to different hosts was less than 0.1 (0.559 versus 0.464). It is difficult to imagine that this small difference in preference could significantly influence the amount of genetic divergence between sites in characters related to growth, survival and fecundity.

A final mechanism that could cause females to tend to return to the same host species as that on which they developed is genetic variation in host preference, since if host preference is heritable there will be a correlation between the oviposition preference of a female and the preference of her mother. No evidence for genetic variation in preference was obtained in this study. The genetic analyses employed here were based on differences among full-sib families. Since differences among full-sib families could be due to either underlying genetic differences or to maternal effects, a significant family effect in an ANOVA would have to be interpreted cautiously. However, since the family effects in this study did not even approach significance and estimated heritabilities were less than 0.05 in each experiment, it seems safe to conclude that there were neither genetic nor maternal factors acting to cause differences among individuals in oviposition preference, at least as preference was assayed in these experiments. It should be emphasized, however, that only the post-alighting component of oviposition behaviour was assayed in this study. It is conceivable that genetic variability exists in some pre-alighting component of host selection behaviour, such as response to plant odours.

It thus appears that *D. guttata* individuals do not exhibit the kinds of strong differences in oviposition behaviour that would tend to maintain and/or amplify genetic differences among sites in traits directly affecting growth, survival and fecundity. I tentatively conclude, therefore,

that these observed differences are due primarily to divergent selection on subpopulations of *D. guttata* using different host species and that these differences may be enhanced by restricted migration between sites, as appears to be the case for the parthenogenetic moth *Alsophila pometaria* (Mitter et al. 1979; Futuyma et al., in press). Future studies will test this hypothesis directly.

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