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Within- and among-population variation in oviposition preference for urea-supplemented food in *Drosophila melanogaster*

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Abstract. **Oviposition preference for urea-supplemented food was assayed by simultaneous choice trials on five pairs of closely related laboratory populations of *Drosophila melanogaster*. Each pair of populations had been derived from a separate ancestral population about 85 generations prior to this study. One population in each pair had been subjected to selection for larval tolerance to the toxic effects of urea; the other population served as a control. Considerable variation in oviposition preference was seen both within and among populations, with four of the ten populations showing a significant mean preference for urea-supplemented food. The degree of specificity shown by individual females was surprisingly high, leading to a bi-modal distribution of oviposition preference in some populations. Overall, selection for larval tolerance to urea did not significantly affect oviposition preference. However, the data indicated that pair-wise comparisons between randomly selected populations from the two larval selection regimes would lead to a range of possible outcomes, suggesting, in several cases, that selection for larval urea tolerance had led to significant differentiation of adult oviposition preference for urea in one or the other direction. The results, therefore, highlight the importance of population level replication and caution against the practice, common in ecological studies, of assaying oviposition preference in two populations that utilize different hosts in nature, and then drawing broad evolutionary inferences from the results.**

Keywords. **Egg-laying behaviour; oviposition preference; specificity; urea; population differentiation; host specialization; *Drosophila melanogaster*.**

1. Introduction

Oviposition behaviour is one of the key components of the evolutionary ecology of host and habitat specialization in insects, and, over the last two decades, has received much attention from scientists interested in a variety of issues such as the origin of host shifts, insect-plant coevolution, the maintenance of genetic variation within populations, the causes of host specificity and the potential for sympatric speciation (reviewed by Futuyma and Peterson 1985; Gould 1988; Thompson 1988a, 1990; Jaenike 1990; Via 1990; Courtney and Kibota 1990; Futuyma 1991; Thompson and Pellmyr 1991). Many studies of oviposition preference, spanning a variety of insect species, have shown that populations often harbour at least moderate levels of variation for preferring to lay eggs on hosts or substrates that are clearly non-optimal for their

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offspring (e.g., Moreteau *et al* 1994); substantial variation is also seen for preferring hosts that the population normally does not encounter (e.g., Thompson 1988b; Fox *et al* 1994). Moreover, oviposition or feeding preferences for particular substrates have often been shown to be elicited by specific chemical compounds present in those substrates (Honda 1986; Nishida *et al* 1987; Feeny 1991; Rank 1992; Baur *et al* 1993; Higa and Fuyama 1993; Moreteau *et al* 1994; Renwick and Chew 1994).

In recent years, ecologists have increasingly begun to appreciate the evolutionary significance of the patterns of variation among populations, at a level of biological organization intermediate between the species, taken as a monolithic whole, and the local population (McCauley 1991; Brodie and Brodie 1991; Jarosz and Burdon 1991; Thompson 1993, 1994a,b; Burdon and Thompson 1995; Joshi and Thompson 1995a). Nevertheless, the majority of studies on oviposition preference in insects have not provided information about the variation among individual populations that have similar habitats and hosts available to them, and how this variation relates to that occurring within such populations (reviewed in Thompson 1994b).

A commonly used approach in these studies has been to record patterns of oviposition preference and offspring performance in a single population, and use those data to assess their relationship (Wiklund 1975, 1981; Courtney 1981; Williams 1983; Penz and Araujo 1990; Valladares and Lawton 1991; Rank 1992; Fox and Eisenbach 1992; Hamilton and Zalucki 1993; Hanks *et al* 1993; Leddy *et al* 1993; Nylin and Janz 1993; Janz *et al* 1994). Despite its widespread use, this approach is not very useful if the ultimate purpose is to draw general evolutionary conclusions; explicitly estimating the within-population correlation between maternal oviposition preference and offspring performance on alternative hosts or substrates (e.g., Via 1986; Ng 1988; Singer *et al* 1988; Jaenike 1989; Fox 1993) provides much more evolutionarily meaningful information about the relationship between preference and performance. Another frequently used approach in studies of oviposition preference has been to look at the pattern of oviposition (or host) preference across various species, or populations of a species, that differ in the pattern of host use in their natural habitat. Very often in such studies, however, each species, or host-specific population type, is represented by only one experimental population (Singer 1983; Tabashnik 1983; Nylin 1988; Thompson 1988b,c; Roininen and Tahvanainen 1989; Krebs *et al* 1992; Lederhouse *et al* 1992; Singer *et al* 1992; Craig *et al* 1993; Huang and Renwick 1993; Roininen *et al* 1993; Fox *et al* 1994; Lu and Logan 1994; Moreteau *et al* 1994). Consequently, these studies, though pertinent to the ecology of specific populations, do not permit the drawing of clear conclusions about the role of selection in moulding oviposition preferences.

The few detailed studies that have yielded information on variation in oviposition preference both within and among populations, provide empirical evidence for several different patterns of within- and among-population partitioning of variation in oviposition preference. Populations of the butterfly *Euphydryas editha* in the western United States exhibit fairly high levels of variation in oviposition preference both within and among populations, although some strictly monophagous populations of this species seem to have low levels of variation within populations (Singer 1982, 1983; Thomas *et al* 1987; Singer *et al* 1989, 1991, 1992). In contrast, allopatric populations of the swallowtail butterfly *Papilio zelicaon* that differ in patterns of both host availability and host use, show a highly conserved preference hierarchy, with relatively low levels of variation in oviposition preference both within and among populations (Thompson 1993). A third pattern, one of high levels of variation within populations but very little

variation among populations, has been observed in the generalist fruit and mushroom feeding *Drosophila tripunctata* (Jaenike 1987, 1989), as well as the cactophilic species *Drosophila buzzatii* and *Drosophila aldrichi* (Barker 1992). A similar pattern was observed in the cowpea weevil *Callosobruchus maculatus*, with allopatric strains exhibiting considerable variation in preference hierarchies within populations, but little genetic differentiation among populations (Wasserman 1986). The combination of low within-population variation and high among-population variation for oviposition preference has been seen only in comparisons of what appear to be distinct host races, rather than different populations with a similar host use pattern (Craig *et al* 1993; Roininen *et al* 1993), and in a comparison of four mutant lines of *Drosophila simulans* that are very likely to have been inbred (Moreteau *et al* 1994). Thompson (1994b) has suggested that this type of partitioning of variation among and within ecologically similar populations of the same species is likely to be transient evolutionarily and, consequently, should rarely be observed; it may, however, give rise to host-race formation and speciation if gene flow among populations is very low.

Despite the diversity of observed patterns in the distribution of oviposition preference within- and among-populations, there are two general results that emerge. In several studies in which females were assayed individually, specificity of preference was seen to be more variable within populations, as compared to the overall preference hierarchy (Tabashnik *et al* 1981; Wiklund 1981; Wasserman 1986; Thompson 1988b; Singer *et al* 1991). Unfortunately, oviposition preference and specificity are often defined and measured in diverse ways, making direct comparisons of studies somewhat difficult (Singer 1986; Thompson 1990; Thompson and Pellmyr 1991). In general, what is implied by specificity, as distinct from the order in which hosts are ranked from the most to the least preferred, is some measure of the strength of the tendency of females to restrict egg laying to one or a few preferred hosts. Clearly, at the individual level, preference and specificity are related to each other, in that a female that has no preference cannot exhibit specificity, and a female that has some preference must, by definition, exhibit some degree of specificity. Nevertheless, within the confines of a given hierarchy of preference, there is considerable scope for individual females to vary in the degree of their specificity. Moreover, at the population level, the average specificity can be high even though the average preference for any one host is low; taken together, both measures convey a fuller picture of how oviposition behaviour varies in the population.

Another fairly consistent pattern observed in populations in which mean oviposition preference for one of two hosts or substrates is intermediate, is that the distribution of oviposition preference tends to be symmetrical and either densely concentrated around the mean (Tabashnik *et al* 1981-figure 2; Singer 1983-figure 3; Singer *et al* 1989-figure 1), or fairly uniform (Jaenike 1987-figure 2, table 2, 1989-figure 2; Lu and Logan 1994-figure 1a). In other studies, where population mean preferences are relatively extreme, the distributions, naturally, tend to be highly skewed (Thompson 1988c; Singer *et al* 1991; Lu and Logan 1994). To the best of our knowledge, no populations studied have ever exhibited intermediate mean preferences as a result of strong individual specificities for different hosts, leading to bi-modal distributions of oviposition preference.

In this paper, we report results from an experiment in which we assayed a set of ten laboratory populations of *Drosophila melanogaster* for oviposition preference for regular banana-molasses food versus urea-supplemented food. Five of

these populations had been under selection for larval tolerance to the toxic effects of urea; the other five populations were controls. None of the populations had been consciously selected for oviposition preference for either of the food media used in the assay. We were specifically interested in the distribution of phenotypic variation for oviposition preference within and among these populations, as it would be an indicator of the kind of variation for novel oviposition substrates that may be harboured by similar arrays of large, outbred populations. This study differs considerably from most other studies of oviposition preference in that we used a set of laboratory adapted populations of known ancestry, that are maintained at fairly large populations sizes (1000–2000 flies) under well characterized and uniform environmental conditions. These populations, therefore, are much more representative of the kind of large, outbred populations, reasonably close to genetic equilibrium in their environments, around which most population genetic theories are built.

2. Materials and methods

2.1 *Experimental populations*

This study was conducted on five populations of *D. melanogaster* that have been selected for larval tolerance to toxic levels of urea ($MX_1 \cdots MX_5$), along with their corresponding control populations ($MC_1 \cdots MC_5$) (Joshi *et al* 1996). These populations were derived from the five B populations of Rose (1984), with each B population being used as the ancestor of one MX and one MC population (MX_i, MC_i derived from B_i, $i = 1 \cdots 5$). At that time the five B populations had been maintained independently for ~ 200 generations since their derivation from a common ancestral population. Consequently, the five pairs of populations, MX_i and MC_i are more closely related to each other than either of MX_i and MX_j, or MC_i and MC_j ($i \neq j$; $i, j = 1 \cdots 5$), even though the pattern of ancestry need not necessarily exercise any significant effect upon the traits under study. At the time of the present study, the MX and MC populations had been maintained in our laboratory for ~ 85 generations.

All the MX and MC populations are maintained in the laboratory in a similar fashion. Every generation, adult flies are allowed to oviposit for approximately 6 h on non-nutritive agar to which live yeast has been applied to stimulate fecundity. These eggs are then placed at moderate densities (60–80 eggs per vial) into 8 dram vials containing about 5 ml of either banana-molasses food (MC populations), or banana-molasses food supplemented with 18 g/l of urea (MX populations). The following day, plastic sleeves are inserted into each vial; when they are ready to pupate, the larvae leave the food and crawl onto these sleeves. When peak pupation occurs, the plastic inserts with the pupae on them are removed from the vials and placed into plexi-glass cages ($25{\cdot}5 \times 20 \times 14{\cdot}4\,cm^3$) with Petri-dishes containing banana-molasses food with a generous dab of live yeast paste on the surface. This procedure ensures that adult flies will not be exposed to urea-supplemented food, thereby restricting selection for urea tolerance to the larval phase. The food plates are changed daily for five or six days after peak eclosion, whereupon egg collection for the next generation takes place. Both the MX and the MC populations have a generation time of about 2 weeks and all populations are maintained in incubators under 24 h light at 25° C.

2.2 *Oviposition preference assay*

Prior to the initiation of this assay, all experimental populations ($MX_1 \cdots MX_5$, $MC_1 \cdots MC_5$) were reared under identical environmental conditions (similar to the MC maintenance regime) for two generations to ensure that any differences observed between the MX and MC populations were due solely to genetic effects, and not environmental or maternal effects. During the second generation of identical rearing conditions, plastic sleeves were inserted into the larval rearing vials and, upon pupation, the sleeves were placed into cages containing Petri-dishes with yeasted non-nutritive agar. This procedure ensured that adult flies were not exposed to either urea-supplemented food, or regular banana-molasses food, before being assayed for oviposition preference on those two types of food. In many insects, including *Drosophila*, oviposition preference is affected by the food to which the adults are exposed (Jaenike 1982; Szentesi and Jermy 1990).

Oviposition preference was assayed on these flies on the 14th day after egg collection; this corresponds to the time at which egg collection occurs in these populations under their usual selection regimes. Twenty females from each MX and MC population were assayed by placing individual pairs of one male and one female into an arena made up of two 8 dram vials taped together at their open ends. One vial contained 5 ml of banana-molasses food (henceforth regular food) and the other contained 5 ml of banana-molasses food supplemented with 18 g/l of urea (henceforth urea food). The females were allowed to lay eggs in these two-vial arenas, which were kept horizontally under continuous light from above, for 24 h at $\sim 25\ (\pm 2)^\circ$ C. The position of the urea food vial in each arena was alternated to avoid any possible position effects that could confound oviposition preference with a tendency to move in a particular direction. After 24 h, the taped vials were separated, the flies removed, and the number of eggs laid by each female in each of the two vials was recorded.

2.3 *Statistical analysis*

From the primary data, we obtained values of three variables for each female that were then used for the statistical analyses. Oviposition preference for urea was measured as the fraction of eggs a female laid on the urea food; this fraction was then subjected to arcsin square-root transformation to induce a closer fit to normality (Freeman and Tukey 1950). Each female was also assigned a specificity rank from 1–5 according to the following scheme. Females laying 50–60% of their eggs on one of the food media were given a specificity of 1. Those laying 60–70% of their eggs on one food medium were given a specificity of 2, and so on, through a specificity rank of 5 for females that laid 90–100% of their eggs on one food medium. The total fecundity for each female was measured as the sum of the eggs laid on both food media.

All analyses were performed using SAS for Windows version 6·08. Due to the pattern of relatedness among the MX and MC populations, pairs of MX and MC populations, matched by subscripted indices, were treated as random blocks in the analysis of variance (ANOVA) on oviposition preference. Selection regime (MX or MC) was treated as a fixed factor crossed with block. Multiple comparisons among populations were done using *t*-tests on the least-squares estimates of cell means, using MS(block × selection) as the error term. As the data, at least for some populations, were extremely non-normal even after transformation, we also did a series of non-parametric

Table 1. The correlation between total number of eggs laid by a female and the fraction of eggs laid on urea supplemented food.

	Selection regime	
Replicate population	MX	MC
1	−0·1535 ($P = 0·52$)	−0·1971 ($P = 0·42$)
2	−0·1278 ($P = 0·60$)	+0·2512 ($P = 0·29$)
3	−0·0183 ($P = 0·94$)	−0·0249 ($P = 0·92$)
4	−0·0917 ($P = 0·71$)	−0·1421 ($P = 0·55$)
5	−0·2049 ($P = 0·43$)	+0·0003 ($P = 0·99$)

The entries are estimated Pearson product-moment correlation coefficients (r), with the probability that the true correlation coefficient equals zero in parentheses. The sample size per population ranged from 17–20 females.

Table 2. The correlation between total number of eggs laid by a female and her specificity rank.

	Selection regime	
Replicate population	MX	MC
1	−0·1219 ($P = 0·56$)	−0·3104 ($P = 0·20$)
2	−0·3341 ($P = 0·16$)	+0·2079 ($P = 0·38$)
3	+0·0755 ($P = 0·75$)	+0·3476 ($P = 0·14$)
4	+0·3150 ($P = 0·19$)	+0·0746 ($P = 0·75$)
5	−0·2440 ($P = 0·35$)	−0·2280 ($P = 0·35$)

The entries are estimated Pearson product–moment correlation coefficients (r), with the probability that the true correlation coefficient equals zero in parentheses. The sample size per population ranged from 17–20 females.

tests to ascertain whether the results were at least qualitatively similar to those from the ANOVA. To this end, we performed separate Kruskal–Wallis tests for the effects of selection regime (2 samples) and block (5 samples). For these tests, data were pooled over the five replicate populations within each selection regime, and the two populations (one MX and one MC) within each block, respectively. Individual Kruskal–Wallis two sample tests were also conducted on each pair of populations, MX_i and MC_i ($i = 1 \cdots 5$). We also estimated Pearson product-moment correlations between total fecundity and both oviposition preference and specificity over the entire data set, as well as for each indiviual population, in order to ascertain whether either preference or specificity scaled with fecundity; such an effect has been observed in some previous studies (Wasserman and Futuyma 1981; Jaenike 1989; Barker 1992), and, if present necessitates some corrective scaling.

3. Results

Total fecundity was not significantly correlated with oviposition preference or specificity, either overall ($r_{(fec,\ ovip)} = +0·04$, $P = 0·57$; $r_{(fec,\ spec)} = -0·04$, $P = 0·56$), or within each individual population (tables 1 and 2). Consequently, data for oviposition preference

and specificity did not need to be scaled with regard to total fecundity. The ANOVA on oviposition preference data revealed significant variation among populations in mean fecundity ($F_{9,182} = 3{\cdot}27$, $P = 0{\cdot}01$) which could be partitioned into significant effects of block ($F_{4,182} = 3{\cdot}05$, $P = 0{\cdot}018$) and block × selection interaction ($F_{4,182} = 4{\cdot}20$, $P = 0{\cdot}0028$); the effect of selection regime was not significant ($F_{1,4} = 0{\cdot}03$, $P = 0{\cdot}871$). Overall, about 14% of the variation in oviposition preference was due to variation among the means of individual populations ($r^2 = 0{\cdot}139$); almost all of this was due to the sums of squares for the block × selection interaction. Four of the ten populations showed a significant preference for urea food (figure 1). Mean oviposition preference in the other six populations was not significantly different from 0·5, indicating no

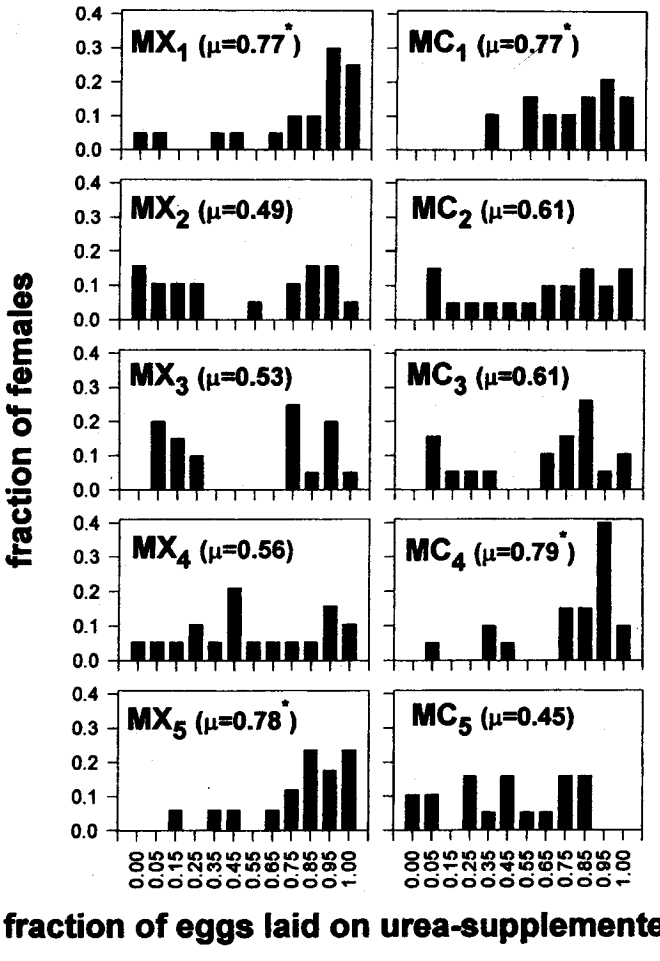

Figure 1. The frequency distribution of oviposition preference for urea (fraction of eggs laid by each female on urea-supplemented food) in the ten populations of *D. melanogaster* used in this study. The labels on the X axis designate mid-points of intervals (e.g., 0·15 represents $0{\cdot}10 < \times \leq 0{\cdot}2$), with the exception of 0·00 and 1·00 which designate single values. The mean oviposition preference for urea for each population (μ) is given in parentheses below the population designation. Asterisks are used to indicate mean preferences that differed significantly (*t*-test; $P < 0{\cdot}05$) from 0·5, or no preference for either food medium. The sample sizes per population ranged from 17–20 females.

Table 3. The range of possible outcomes had only one MX and one MC population, selected at random, been assayed for oviposition preference for urea.

	MX_1	MX_2	MX_3	MX_4	MX_5
MC_1	n.s.	MC > MX**	MC > MX*	n.s	n.s.
MC_2	n.s.	n.s.	n.s.	n.s.	n.s.
MC_3	n.s.	n.s.	n.s.	n.s.	n.s.
MC_4	n.s.	MC > MX**	MC > MX*	MC > MX*	n.s.
MC_5	MX > MC**	n.s.	n.s.	n.s.	MX > MC**

n.s.: $P > 0.05$; *$P < 0.05$; **$P < 0.01$.
The entries represent the result of comparing the fraction of eggs laid on urea-supplemented food in population pairs MX_i and MC_j ($i, j = 1 \cdots 5$) by means of a t-test.

preference either for, or against, urea food. In two of the five blocks (blocks 4 and 5), the MX and MC populations differed significantly from each other, the differences in the two blocks, however, were in opposite directions (figure 1, table 3). The matrix of all possible pair-wise comparisons between MX and MC populations shows that a range of outcomes would be possible if just one pair of populations subjected to different larval selection regimes were to be compared (table 3). Depending on which pair was used, the results could lead to the conclusion that selection for larval urea tolerance caused (i) no change, (ii) an increase, or (iii) a decrease in oviposition preference for urea.

The results from the series of non-parametric tests were essentially identical to those from the ANOVA. There was no significant overall difference between the MX and MC populations, based on pooled data from all five replicate populations within each selection regime (Kruskal–Wallis $H_{1df} = 0.0002$, $P = 0.99$). There was significant heterogeneity among blocks ($H_{4df} = 10.76$, $P = 0.029$). The results of multiple pair-wise Kruskal–Wallis tests on all possible pairs of MX and MC populations yielded a pattern of results essentially identical to that shown in table 3 for the parametric multiple comparisons. The concordance of the results from the ANOVA and the non-parametric tests suggests that, despite the non-normality of the data, the ANOVA results are robust, and do not provide evidence for a significant differentiation of populations due to selection for larval tolerance to urea.

As can be seen from figue 1, the degree of specificity in most populations was high, with some populations showing extremely bi-modal distributions (e.g., MX_2, MX_3, MC_3); in these populations, mean oviposition preference was close to 0·5 due to extreme specificity of different females for regular food or urea food, not because most females did not exercise a strong preference. This pattern of high specificity is reflected in the mean specificity ranks of populations, with seven of the ten populations showing a mean specificity rank exceeding 3·5 (table 4).

4. Discussion

4.1 *Patterns of variation among- and within-populations*

Although variation in oviposition preference within-populations has often been observed (reviewed by Jaenike 1990; Thompson 1990, 1994b), bi-modal distributions of

Table 4. Mean specificity (±95% confidence interval) of the MX and MC populations.

	Selection regime	
Replicate population	MX	MC
1	4·15 (±0·65)	3·37 (±0·76)
2	4·11 (±0·53)	3·65 (±0·69)
3	4·05 (±0·44)	3·74 (±0·50)
4	3·21 (±0·81)	4·05 (±0·62)
5	3·88 (±0·65)	3·05 (±0·69)

The minimum mean for a population would be 1·0, implying that all females laid only 50–60% of their eggs on the preferred food medium. The maximum mean for a population would be 5·0, implying that all females laid 90–100% of their eggs on the preferred food medium· Confidence intervals are based on the variation in specificity rank among the 17–20 females assayed in each population, and assume population means to approximate a *t*-distribution.

oviposition preference, such as those seen in some of the populations in this study (figure 1), have rarely been recorded, especially in studies involving only two hosts or substrates. In a few instances, individual females within oligophagous populations have been observed to vary greatly in the order in which they rank hosts; most of these studies do not, however, provide unequivocal evidence for bi-modal distributions of oviposition preference (e.g., Wiklund 1975; Singer *et al* 1989; Janz *et al* 1994). Nevertheless, in situations where many hosts are actually used by the population, the possibility of polymorphism for oviposition preference being maintained by environmental heterogeneity exists, at least in principle. In the present study, however, it is difficult to envisage what forces, if any, could be responsible for the high levels of variation observed. The MX and MC populations are maintained under relatively uniform laboratory conditions, and the substrate on which they normally oviposit is agar, rather than either of the two food media used in the oviposition assay. The lack of a strong negative relationship between total fecundity and specificity in these populations (table 2) would seem to preclude the possibility that the high specificity observed is due to many females laying just a few eggs in whichever vial they happen to end up fortuitously.

It is, nevertheless, possible to speculate that the bi-modality of oviposition preference observed in some of the MX and MC populations is due to a combination of a selectively neutral polymorphism for a tendency to lay eggs on one or the other medium, coupled with high specificity induced by a reluctance of most females to move around once they have chosen a substrate and commenced egg-laying. The latter tendency may be under selection as the eggs that are collected to initiate each new generation in these populations are laid over a relatively short six hour time period. Moreover, during this time, there is just one Petri-dish containing the agar substrate in each cage. Consequently, there may be selection against females that tend to move around during the 6 h oviposition window, instead of concentrating on laying eggs. Selective neutrality, at least with regard to the choices presented here, may not be a very far-fetched idea. In *Drosophila*, adult oviposition preference is independent of the food medium experienced during the larval phase, within the same generation, even though

exposure to a particular medium early in adult life does influence subsequent oviposition preference (Jaenike 1982). Why this is so is not known, although it is possible that the development of the adult nervous system from imaginal discs, rather than from the larval nervous system, may have some role to play; a similar pattern is also seen in several other holometabolous insects. Since the populations used in this study had never before experienced urea-supplemented food as adults, selection would not have had an opportunity to act upon any alleles tending to confer a preference for urea food.

Another, somewhat less likely possibility, is that the observed variation in oviposition preference for urea in these populations is not genetic, and is due to fortuitous environmental effects. Although the present study does not explicitly ascribe the observed variation to underlying genetic causes, we think that this explanation is unlikely to be correct, given the very uniform and controlled environmental conditions in the laboratory; the significant effect of block, representing ancestry, in the ANOVA tends to support this view. Certainly, genetic variation of this kind is not unusual and could, if present, be revealed by direct selection on oviposition preference. Populations of many insect species harbour variation, in many cases shown to be genetic, for oviposition preferences for novel, and often non-optimal, substrates (Wiklund 1975; Courtney 1981; Thompson 1988b; Roininen and Tahvanainen 1989; Thompson *et al* 1990; Fox and Eisenbach 1992; Fox *et al* 1994; Janz *et al* 1994; Moreteau *et al* 1994).

4.2 *The importance of population level replication in ecological studies*

The pattern of variation in oviposition preference among the various populations used in this study has significant implications for the design of oviposition preference experiments. Although populations varied significantly in mean oviposition preference, we were able to rule out selection for larval adaptation to urea as a cause of this variation because of the experimental design which included five replicate populations within each selection regime. Had we merely compared oviposition preference between one pair of MX and MC populations chosen at random, as is done in many studies of oviposition preference, we could have potentially seen any one of three outcomes (table 3), suggesting, respectively, that (i) MX populations had evolved a greater preference for urea food, (ii) MX populations had evolved a greater preference for normal food, or (iii) oviposition preference was conservative, with MX and MC populations not differing significantly from one another.

Although the importance of replicate populations within selection treatments has been emphasized in the context of studies on both density-dependent selection (Mueller 1995) and the evolution of ageing (Rose and Service 1985), we think it worthwhile to make this point here because many recent studies of oviposition preference do not incorporate this important level of replication in their experimental design (Fox and Eisenbach 1992; Krebs *et al* 1992; Lederhouse *et al* 1992; Singer *et al* 1992; Rank 1992; Craig *et al* 1993; Hanks *et al* 1993; Nylin and Janz 1993; Roininen *et al* 1993; Fox *et al* 1994; Janz *et al* 1994; Moreteau *et al* 1994). Moreover, the differences seen in this study among closely related laboratory populations, maintained at large population sizes and subjected to almost identical environmental conditions, suggest that similar, or even greater, differences in oviposition preference may be expected among many natural populations. Conditions in nature are likely to be much more conducive to divergence among populations because of greater temporal and spatial environmental

heterogeneity, as well as an increased likelihood of genetic drift due to population size bottle-necks and population sub-structure. Certainly, more studies that assess variation in oviposition preference among arrays of populations sharing common hosts are needed to gain a broader understanding of the role such variation may play in the evolution of host shifts.

Overall, we think that the results of this study underscore the importance of complementing field studies of oviposition preference with carefully controlled studies on replicated sets of laboratory populations. Compared to natural populations, laboratory systems have an advantage when the focus of the study is to understand how natural selection might, at least in principle, give rise to patterns of variation observed in nature. When using laboratory-adapted populations, one can avoid or, alternatively, quantify many of the confounding factors, such as ancestry effects (Travisano *et al* 1995) and population divergence due to genetic drift (Mueller 1995), that often make it difficult to draw clear evolutionary inferences from studies on natural populations. Compared to natural populations, laboratory systems also permit a much cleaner assessment of the variation among sets of populations in evolutionary responses to similar selection pressures (Joshi and Thompson 1995a, 1996); such variation is now thought to play a major role in the evolution of observed patterns of diversity in nature (Thompson 1994a,b; Burdon and Thompson 1995). Unfortunately, in studies of oviposition preference, and host shifts in general, there has been an almost exclusive focus on studying natural populations. Consequently, although much useful information about the patterns of diversity in traits relevant to these phenomena has been gathered, relatively little is known about either the genetics of these traits, or, more importantly, about the nature of the selective forces involved in moulding this immense diversity at both the within- and among-population level (Thompson 1994b; Joshi and Thompson 1995b). In our opinion, a more equitable balance between field and laboratory studies will be helpful in addressing some of these issues, especially when it comes to assessing the relative roles of drift and selection in shaping patterns of variation among populations.

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