Bimodal distribution of oviposition preference for a novel food medium in *Drosophila melanogaster*

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Bimodal distributions of oviposition preference within populations are of interest as they may represent an early step towards host-race formation in nature. Oviposition preference for a novel food medium was assayed in 20 full-sib families of a laboratory population of *Drosophila melanogaster*. Significantly greater variation in preference for the novel food was observed among families, compared to within families, indicating that the observed variation is, at least partly, under genetic control. The distribution of oviposition preference of individual females in the total population was highly bimodal; resulting in a low mean oviposition preference value as a consequence of high specificity (a measure of the strength of oviposition preference) coupled with extensive variation in preference. The results confirm earlier reports of such bimodal distributions of preference in laboratory populations of *Drosophila* and provide evidence for a genetic basis to this variation, suggesting that this pattern of distribution of preference may be an evolved response to some aspect(s) of the laboratory ecology of these *Drosophila* cultures. Such an evolved response results in a partial delinking of oviposition preference and specificity at the populational level, and provides an opportunity to study the genetic architecture of preference and specificity in these populations.

THE focus of studies on the evolution of host specialization in insects is now shifting to oviposition preference (henceforth, preference), rather than larval performance, as a potential factor affecting host range expansion[1–8]. Insect populations typically harbour at least moderate phenotypic variation for preference, even on novel host plants or substrates that are often non-optimal for larval development[9–13]. Unfortunately, clear evidence of a genetic basis for this variation is often not available, although ultimately it is the pattern of genetic variation and covariation in fitness-related traits within and among populations that largely determines the course of evolutionary change[5,13–15].

Phenotypic variation in preference in insects is distributed within and among populations in different patterns[16–26] (Table 1). The distribution of relatively high levels of variation within populations need not necessarily be uniform. Populations could, in principle, exhibit intermediate mean preference due to large variation in preference, coupled with high specificity (i.e. different females exercising strong preference for alternative substrates for oviposition). Such bimodal distributions of preference within populations could represent an early step toward host-race formation, especially if coupled with ecological factors conducive to isolation between divergent preference genotypes.

Strongly bimodal distributions of preference for a novel versus a familiar food medium were first seen in a study on laboratory populations of *D. melanogaster*[13]. Here, four out of ten populations showed a bimodal distribution of oviposition preference for urea supplemented food medium as a result of high specificity for alternative media. It was not clear whether the bimodality was an artefact of the population maintenance regime and, moreover, a genetic basis for the observed variation was not demonstrated. We have since observed bimodal distributions of oviposition preference for normal versus novel food in four other laboratory populations of *D. melanogaster*[21], that shared common ancestors with the 10 populations used by Joshi *et al.*[13], but had been maintained on a different regime for 120 generations. Thus, it would seem that the observed bimodality was an evolved response to some aspect of the laboratory ecology of these populations rather than an artefact of the maintenance regime. A bimodal distribution of preference has recently been reported in the butterfly *Junonia coenia*, in conjunction with a recent host shift from *Plantago lanceolata* (Plantaginaceae) to *Kickxia elatine* (Scrophulariaceae)[27].

In this study, we assayed within- and among-family variation in oviposition preference for familiar (banana) food versus a novel (tomato) food medium in one of the large, outbred populations of *D. melanogaster* (population JB-1) used by Sheeba *et al.*[21]. These populations exhibited extensive phenotypic variation in preference for tomato food[21], and our purpose here was to determine if there was a genetic component to this variation.

The population was maintained on banana food at moderate larval densities, on a 21-day discrete-generation cycle at 25°C, under constant light. This population, and its ancestors, have been maintained on this regime for

Table 1. Major patterns in the distribution of variation in oviposition preference within and among populations of various insect species

	High variation among populations	Low variation among populations
High variation within populations	*Euphydryas editha*[16,17]	*Drosophila tripunctata*[18,19], *D. buzzati* and *D. aldrichii*[20], *D. melanogaster*: laboratory populations[14,21], *Callosobruchus maculatus*[22]
Low variation within populations	*Drosophila simulans*: mutant lines[11], *Euura atra*[23], *Eurosta solidaginis*[24]	*Papilio zelicaon*[25], *P. polyxenes* and *P. machaon*[14], *P. glaucus* and *P. canadensis*[26].

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over 600 generations. In every generation the adults were allowed to oviposit for up to 18 h on petri plates of fresh banana food in a Plexiglass cage and ~ 60–80 eggs were collected in each of 40 vials in which larvae would develop. On day 18 after egg lay, adult flies were transferred into Plexiglass cages and supplied with banana foods supplemented with live yeast paste for two days prior to egg collection as described above.

From the running culture, ~ 60–80 eggs per vial were collected in 10 vials containing approximately 6 ml of banana food. Upon eclosion, virgin females were collected in vials with banana food, supplemented with live yeast paste. Virgin males and females that had eclosed on the same day were placed, one male and one female, in a vial of banana food (48 such mating pairs were set up). For the next three days, the mating pairs were shifted to a fresh vial every 24 h. The vials with eggs laid in them were incubated and the adults discarded. Sterilized plastic sleeves were inserted into the vials after egg lay such that larvae developed in the food and then pupated on the plastic sleeves. After peak pupation had occurred, the plastic sleeves were removed from the vials, cleaned of any food sticking to their surface, and then inserted into fresh vials containing non-nutritive agar medium (1.3% agar). This was done to ensure that adult flies were not exposed to either banana or tomato foods prior to being assayed for preference on these two food media, as oviposition preference in *Drosophila* is influenced by prior exposure to a particular substrate[28,29]. When the pupae on the plastic sleeves were close to eclosion, live yeast paste was added to the walls of the vial. Eclosing adults were transferred to fresh yeasted agar vials two days after eclosion. Thus, we obtained 48 full-sib families, of which 20 families, each consisting of more than 10 females collected over a span of three days was assayed.

Four-day-old female flies (n = 13–20; Table 2) from each of 20 full-sib families were assayed for oviposition preference when given a choice between banana and tomato foods. Following Joshi *et al.*[13], one male and one female were left in an arena consisting of one vial each of tomato and banana food, taped together at their open ends for 24 h under constant light. The adults were then discarded and the number of eggs in each vial counted. The orientation of the two types of vials was alternated so as to avoid possible confounding effects of directionality due to light or temperature gradients.

For each female assayed, we obtained two secondary variables from the number of eggs in each vial. Following Joshi *et al.*[13], preference for banana food was computed as the fraction of eggs laid on banana food; the fraction was then subjected to an arcsine square-root transformation to induce a closer fit to normality. Fecundity was computed as the total number of eggs laid by a female on both food media. The transformed oviposition preference data were then subjected to a one way analysis of variance (ANOVA), with family as a random factor. Data for preference were not scaled with fecundity, as it was known that total fecundity in this population was not correlated with preference for either of the two foods used[21].

Considering all families together, (n = 356 females), preference for banana food showed a strikingly bimodal distribution (mean = 0.4859, s.d = 0.3969) (Figure 1). Sixteen out of twenty families showed no significant mean preference, one family showed a significant preference for banana, and three families showed a significant preference for tomato (Table 2, Figure 2). Fecundity of the females assayed was on average quite high with only 10 out of 356 flies laying less than 20 eggs in the oviposition preference

Table 2. Mean fecundity (total number of eggs laid on both foods in the oviposition preference trial) and mean oviposition preference for banana (fraction of eggs laid on banana food) in the 20 full-sib families used in the study. Only families yielding > 10 females were assayed out of an original total of 48 families

Family number	Number of females assayed	Mean fecundity (± s.d.)	Mean preference for banana (± s.d.)
4	20	40.3 (14.01)	0.273 (0.352)***
5	19	48.0 (14.94)	0.358 (0.406)
8	18	44.1 (12.69)	0.254 (0.382)**
9	13	57.5 (13.84)	0.529 (0.398)
11	18	46.2 (32.27)	0.557 (0.450)
13	17	58.0 (11.73)	0.389 (0.431)
15	16	53.5 (21.46)	0.307 (0.386)
16	14	44.9 (12.04)	0.590 (0.378)
18	19	67.1 (26.68)	0.707 (0.372)*
19	17	62.2 (21.68)	0.475 (0.332)
22	17	44.0 (10.4)	0.414 (0.405)
23	17	67.24 (17.17)	0.561 (0.395)
24	20	38.8 (15.71)	0.472 (0.461)
31	20	72.6 (11.49)	0.648 (0.350)
32	19	59.0 (13.19)	0.624 (0.360)
33	20	64.2 (15.24)	0.329 (0.361)*
35	19	51.7 (22.62)	0.510 (0.400)
36	19	63.7 (9.79)	0.510 (0.407)
40	20	71.3 (11.36)	0.526 (0.445)
47	18	51.9 (11.23)	0.477 (0.421)

Significance levels (t test): * = $P < 0.05$; ** = $P < 0.02$; *** = $P < 0.01$.

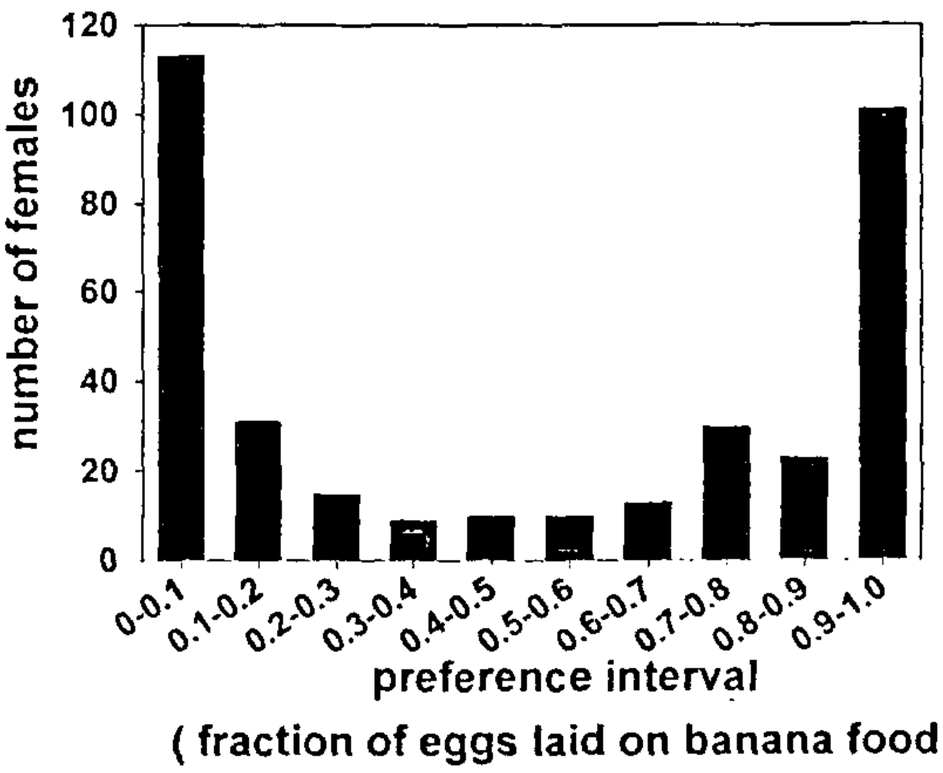

Figure 1. Distribution of oviposition preference for banana food among the 356 females assayed, pooled across 20 full-sib families.

rials. Among the families with non-significant mean preference, seven families showed distinctly bimodal distributions of preference (some representative families are shown in Figure 3). The ANOVA on preference data revealed significant among-family variation ($F = 2.17$, $P = 0.0035$), clearly indicating a genetic basis for the observed variation in preference.

The distribution of oviposition preference for banana versus tomato food in this laboratory population of *D. melanogaster* is clearly bimodal, and the observed variation is, at least in part, genetic. Indeed the degree of bimodality in the pooled data (Figure 1) is much higher than that previously observed by Joshi *et al.*[13] and Sheeba *et al.*[21], which is likely to be due to smaller sample sizes per population ($n \sim 18$–20 females) in those studies. The population used in this study differs from the MX and MC populations used by Joshi *et al.*[13] in the crucial issue of the duration of egg laying time-window. The MX and MC populations were given 6 h to lay eggs that would be used to start the next generation; the JB populations used in this study, and by Sheeba *et al.*[21] were given 18 h. Joshi *et al.*[13] had speculated that the observed bimodality in preference was due to a combination of a selectively neutral polymorphism for a tendency to lay eggs on a chosen food medium, coupled with a high specificity, induced by the reluctance of females to move around too much once they had chosen a substrate for oviposition. Such a tendency may have been inadvertently selected for because of the relatively short 6 h window of time given for oviposition during routine population maintenance[13]. The repeated observations of bimodal distributions of preference in populations under different maintenance regimes however suggest that this is more than just an artefact of maintenance regime. It also appears that high specificity in these populations has evolved independently of preference, thereby causing a partial delinking of specificity and preference at the populational level. Such delinking could prove to be a valuable asset in further studies on these two components of oviposition behaviour.

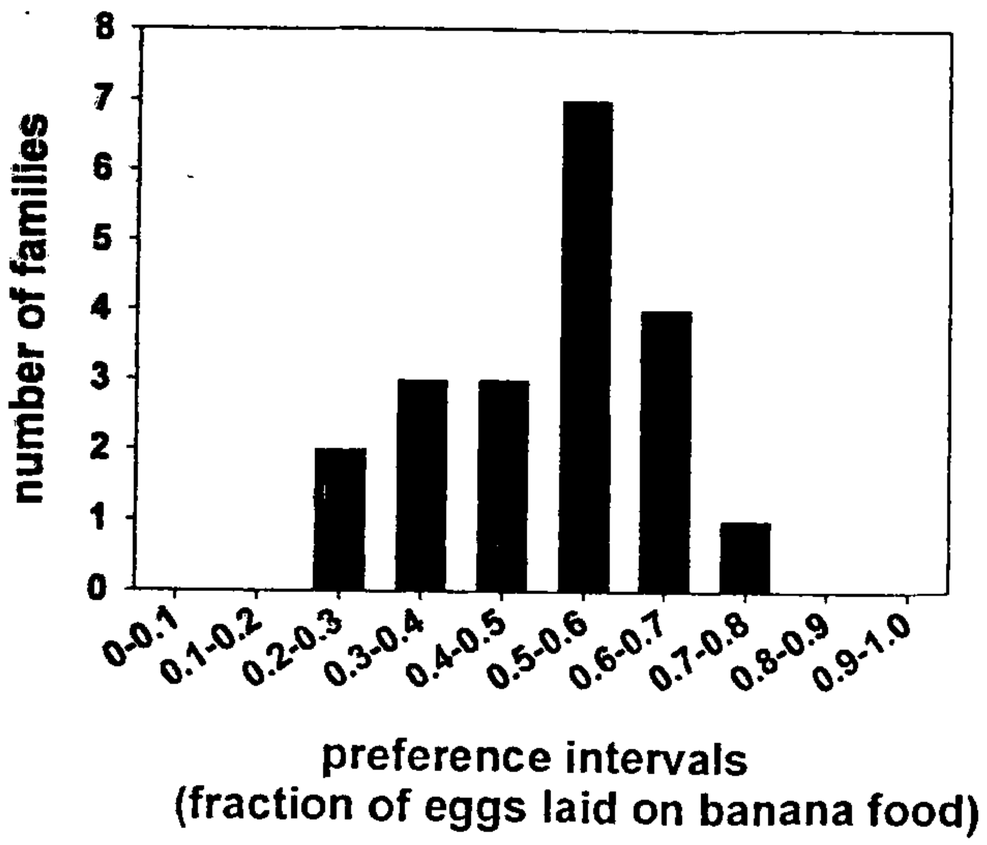

Figure 2. Distribution of family mean preference for banana food among the 20 families assayed.

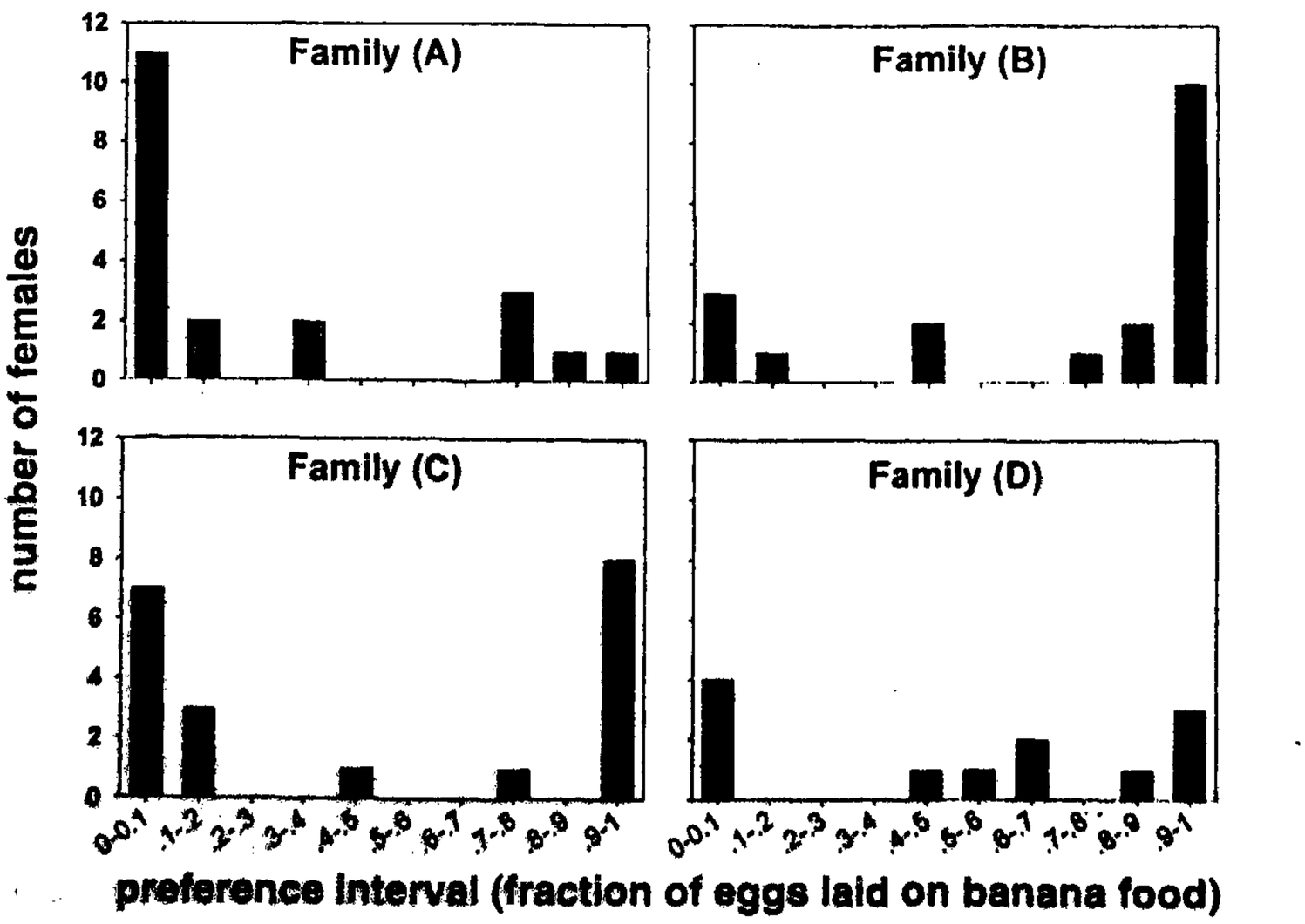

Figure 3. Distribution of oviposition preference in a representative sample of 4 out of the 20 families assayed. The families were chosen to represent (A) strong preference for tomato food, (B) strong preference for banana food, (C) a bimodal distribution of preference, and (D) a relatively uniform distribution of preference. These families exemplify the range of variation seen among families in the distribution of oviposition preference for banana food.

The only other species about which some aspects of the genetics of oviposition behaviour are known are species of *Papilio* and *Drosophila*. In the *Papilio machaon* species group, preference is largely X-linked, with some autosomal effects, and specificity and preference are under partly separate genetic control[9,10]. X-linked inheritance of oviposition preference is also suggested in the tiger swallowtails *P. glaucus* and *P. canadensis*[30], and in the moth *Heliothis virescens*[31]. In both the latter studies, the genetic relationship between preference and specificity was not addressed. In *D. tripunctata*, preference is known to be largely additive and autosomal[18,19], and long-distance attraction to oviposition sites and laboratory preference seem to have a different genetic basis[32]. The distribution of preference within populations of *D. tripunctata* is unimodal[19], although this may be a consequence of assaying preference on groups of females together which could mask the effects of high individual specificity for different food media. Preference is also known to be largely additive in the specialist *D. seychellia*[33]; in this case too, the genetic relationship of preference and specificity is not known.

Overall, we feel that it is worthwhile to further examine this pattern in *individual* oviposition behaviour, wherein a population shows a combination of high specificity and variation for preference, because such variation in nature could be the starting point for the evolution of host-race formation or host-shifts via founder effects. A bimodal distribution of preference has recently been reported from a wild population, and in conjunction with a recent host-shift[27], supporting the view that further genetic studies on the evolutionary genetic relationships between preference and specificity may help to advance our understanding of the evolution of host specialization in insects.

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Mating success in *Drosophila ananassae*: Evidence for greater variation in receptivity of females compared to male mating ability

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Mating success was studied by direct observation in Elens–Wattiaux mating chamber to test mating propensity in six mass culture wild-type strains of *Drosophila ananassae* originating from different geographic localities. The results indicate that the mean number of matings varies significantly among the stocks tested which is attributable to genetic heterogeneity among the stocks. Diallel analysis showed that the variation observed between the strains is due to differences in sexual activity of both sexes. However, females show more variation than males. In 15 pairwise comparisons between the strains, 10 comparisons showed greater variation in receptivitiy of females than in sexual activity of males. In 3 comparisons there is greater difference in male mating ability than in female

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