A diallel analysis of gregarious oviposition in *Drosophila melanogaster*

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Drosophila females exhibit gregarious oviposition. This behaviour was analysed by a diallel cross. The trait shows considerable additive genetic variation and a significant dominant effect. A variance and covariance analysis suggests that *ebony* and *taxi* strains contain the most dominant alleles. Some maternal effect may also be present. This genetical architecture is in line with previous studies of selection and hybridization of the lines selected for high and low gregarious oviposition in *Drosophila melanogaster* females.

Keywords: diallel analysis, *Drosophila melanogaster*, gregarious oviposition.

Introduction

The choice of an oviposition site is important to our understanding of adaptation by *Drosophila* species. Carson (1971) pointed out that *Drosophila* females are generally more selective in choosing an oviposition site than a feeding site. On the other hand, de Jong (1982) has suggested that the ability of *Drosophila* females to select an appropriate oviposition site, which already contains eggs, is an important behaviour that could affect the viability of progeny, increasing the probability of survival of their descendents.

Several studies have dealt with oviposition site preferences in different species of *Drosophila*. Extrinsic factors such as substrate conditions (David, 1970; Atkinson, 1983; Takamura, 1984), humidity (Sameoto & Miller, 1966), density of females (Barker, 1973), temperature (Fogelman, 1979) and illumination conditions (Seiger & Khamus, 1987) affect the choice of oviposition site in *Drosophila*. Few studies have concentrated on the genetic basis of oviposition behaviour. In this connection, del Solar (1968) demonstrated that aggregation behaviour of females can be modified by selection in *Drosophila pseudoobscura*. Takamura & Fuyama (1980) detected a genetic basis for the choice of oviposition site in *Drosophila melanogaster* either on medium or on a paper surface. Albornoz & Dominguez (1987), and Kamping & van Delden (1990) have described a quantitative genetic analysis of egg insertion behaviour of *Drosophila melanogaster*.

A divergent selection programme for high- and low-egg aggregation intensity, carried out for 112 generations in *Drosophila melanogaster*, showed that this behaviour is under genetic control (Ruiz & del Solar, 1986). In a further study using hybridization of the selected lines, we demonstrated that aggregated oviposition has an additive polygenic basis (Ruiz & del Solar, 1991).

This paper is a continuation of the studies concerning genetic components underlying oviposition behaviour in *Drosophila melanogaster*. Here we analyse the genetic components of phenotypic variation by using the complete diallel mating design.

Materials and methods

Several inbred strains of *D. melanogaster* were obtained and the degree of gregarious oviposition shown by each was measured before performing the diallel crosses.

The strains used were: *Bar* (*B*), *Curly* (*Cy*), *dumpy* (*dp*), *ebony* (*eII*), *taxi* (*tx*), *vestigial* (*vg*) and *white* (*w*). The aggregation rate of each strain was measured 30 times by using groups of 25 females of 3–5 days old. The aggregation intensity was assessed using the variance–mean ratio, $s^2/\bar{x}$. In an aggregated distribution, the population variance exceeds the population mean (Pielou, 1977).

Of the seven strains studied, four showed significantly different aggregation indices, according to the Least Significant Difference (Sokal & Rohlf, 1981). These were used to construct a 4 × 4 diallel. Of the 16 F₁ hybrid classes, obtained from crossing each strain in

all possible combinations, 10 samples of six females each were examined. The females of each group, aged 3–5 days and already fertilized, were left to oviposit for 24 h in transparent acrylic population cages of $30 \times 20 \times 10$ cm. Each cage contained six tubes arranged in two columns of three rows. Each tube was a cylinder, 2.5 cm in diameter and 2.5 cm high, containing 5 ml of Burdick culture medium (Burdick, 1954). The population cages with the females were maintained in the same conditions as those in which the strains were evaluated, that is, in complete darkness in an incubator room at $21 \pm 1^\circ\text{C}$ and 60 per cent RH.

The number of eggs per tube found in each population cage was analysed by the variance–mean statistic, in order to obtain an index of gregariousness.

The 16 crosses made up separately five times and data were analysed following the methods of Hayman (1954) and Crusio *et al.* (1984).

Results

The seven strains evaluated showed substantial differences in aggregation intensity, as depicted by the *F*-ratio in the ANOVA ($F = 15.45$; $P < 0.001$).

A posteriori statistical analysis, using the Least Significant Difference (LSD) method, revealed that the *ebony* (39.7 ± 2.7), *dumpy* (29.0 ± 0.8) and *taxi* (18.0 ± 1.9) strains are significantly different from each other. These strains also show important differences in gregarious oviposition with respect to *Bar* (10.7 ± 0.8), *Curly* (12.8 ± 1.7) and *vestigial* (15.4 ± 2.3). Consequently we used *ebony*, *dumpy*, *taxi* and *Bar* in the diallel crosses reported here.

Table 1 shows the weighted average of five replicates for the aggregation indices for each of the 16 F_1 s

Table 1 Aggregation mean rate and standard errors ($n = 5$ replicates) in the F_1 s obtained when crossing *Bar*, *dumpy*, *ebony* and *taxi*, in all possible combinations. The data transformed are given in parentheses

Female	Male			
	<i>B</i>	<i>dp</i>	<i>e</i>	<i>tx</i>
<i>B</i>	7.8 ± 1.5 (2.8 ± 0.3)	6.4 ± 0.8 (2.5 ± 0.2)	22.2 ± 3.6 (4.6 ± 0.4)	9.3 ± 0.9 (3.0 ± 0.2)
<i>dp</i>	9.6 ± 2.4 (3.0 ± 0.4)	8.0 ± 1.1 (2.8 ± 0.3)	19.6 ± 2.4 (4.4 ± 0.3)	14.6 ± 1.3 (3.8 ± 0.3)
<i>e</i>	34.1 ± 5.1 (5.8 ± 0.4)	18.6 ± 3.3 (4.3 ± 0.4)	18.6 ± 3.8 (4.2 ± 0.4)	14.5 ± 1.3 (3.8 ± 0.2)
<i>tx</i>	10.7 ± 0.5 (3.3 ± 0.1)	13.6 ± 3.1 (3.6 ± 0.4)	16.8 ± 2.3 (4.1 ± 0.3)	9.7 ± 1.1 (3.1 ± 0.2)

obtained by crossing the *Bar*, *dumpy*, *ebony* and *taxi* strains.

The F_1 hybrids with higher aggregation rates correspond to those obtained when crossing the *ebony* strain with the rest of the strains used. The *ebony* strain seems to be the most gregarious, suggesting dominance for high aggregation behaviour in the females. Furthermore, when F_1 hybrids obtained by crossing the *ebony* (high aggregation behaviour) and *Bar* (less gregarious strain) strains are compared with the parental strains, it is possible to note that they show a greater aggregation behaviour than their parental strain. This heterotic effect is also found in a lesser degree in the other hybrids, except in the cross between *Bar* females and *dumpy* males.

Due to the lack of homoscedasticity among variances, a transformation of data using $\sqrt{x + 0.5}$ was applied prior to the ANOVA test. A $\chi^2_{[15]} = 1.78$ for the Bartlett test was obtained after the data transformation.

Analysis of the variance and covariance for the F_1 hybrids showed that the *ebony* and *taxi* strains were the ones that contained the greater proportion of dominant genes, producing progeny with a low phenotypic variation, besides a smaller covariation between F_1 and its progenitors carrying recessive alleles. Conversely, the *Bar* strain was found to have the highest variance and covariance values, behaving as recessive (Fig. 1).

Components of the phenotypic variance are shown in Table 2. The principle effects of each item (a, b, c and d) were tested against their own interaction with the repetitions, $B \times a$, $B \times b$, $B \times c$ and $B \times d$ (Hayman, 1954). The results show that there is considerable

Fig. 1 Regressions of W_r on V_r of F_1 obtained by crossing the strains *Bar* (*B*), *dumpy* (*dp*), *ebony* (*e11*) and *taxi* (*tx*). $W_r = -0.06 + 0.66 V_r$.

Table 2 Analysis of variance of the diallel for gregarious oviposition in *Drosophila melanogaster*

Item	d.f.	MS	F
a	3	9.81	40.07***
b	6	3.57	9.84***
b1	1	5.58	5.91
b2	3	1.16	3.50*
b3	2	6.18	52.82***
c	3	0.99	3.34*
d	3	0.42	0.41
Interactions			
within blocks (<i>t</i>)	15	3.67	
Block interactions			
(<i>B</i>)	4	0.62	1.34
<i>B</i> × a	12	0.24	
<i>B</i> × b	24	0.36	
<i>B</i> × b1	4	0.94	
<i>B</i> × b2	12	0.33	
<i>B</i> × b3	8	0.11	
<i>B</i> × c	12	0.33	
<i>B</i> × d	12	1.03	
<i>B</i> × <i>t</i>	60	0.46	
Total	79		

* $P < 0.05$, *** $P < 0.001$.

genetic variation among the lines, this variation being mostly additive in origin (a item). Significant dominance components were also present (b item) and were significant at $P < 0.001$.

The dominance component is accounted for chiefly by a specific combining ability (b3 item), which would be represented by the crossing between the *Bar* and *ebony* strains. The F_1 hybrids produced by crossing these strains showed gregarious oviposition to be significantly higher than expected. The b2 item was significant at the 5 per cent level, which implies asymmetry in the distribution of dominant alleles among the parental lines. On the other hand, the plot of the variance and covariance of the hybrids and parental strains, suggests that the order of the strains used with respect to dominance effects is *ebony*, *taxi*, *dumpy* and *Bar*.

Although the component directional dominance was not significant, it does exist. There is a greater tendency to increase the gregarious oviposition for hybrids than for parental lines ($\bar{F}_1 - \bar{P} = 19.5$ per cent). The c item was significant at a 5 per cent level, which indicates the presence of maternal effects, possibly due to the cytoplasm.

Finally, the differences among the replicates (*B*) were not statistically significant. Indeed, the possible environmental differences between replicates have not caused any variation in the aggregation intensity.

Discussion

In the present study, previous reports of a genetic basis for the aggregated oviposition of *Drosophila melanogaster* have been confirmed.

The differences between the seven strains analysed verify that change in the intensity of aggregation is under genetic control. An analysis following Hayman (1954) revealed a high proportion of additive variation. These results confirm the supposition of polygenic inheritance advanced by Ruiz & del Solar (1986) after observing the greater fluctuations in the intensity of aggregation of eggs in the lines under selective pressure.

The genetic architecture of a behavioural trait may indicate the nature of the relationship between expression of the character and fitness. Characters closely associated with fitness usually show significant directional dominance (Broadhurst & Jinks, 1974). In the present case, aggregated oviposition showed a dominance component, with the direction of dominance being towards high aggregation. This finding, added to the wide variety of abiotic factors found to influence selection for oviposition site, is consistent with the view that the rate of aggregation of eggs is a behaviour of ecological importance for the females.

The food resources exploited by *Drosophila* are divided into small, discrete and ephemeral patches, such as fruit, flowers and fungi. Frequently, such sites support large numbers of species, each with a clear tendency for an aggregated oviposition (Shorrocks, 1977; 1982; Brncic & Valente, 1978; Brncic, 1987; Atkinson & Shorrocks, 1984). Aggregation of eggs may assist the larvae in exploitation of the resources, or protection from toxic effects of infection and desiccation of single eggs. In certain *Sophophora* species and others of the mesophragmatica group, there exists a positive relationship between density and survival from egg to adult. This may be because each larva releases biotic waste and salivary secretion into the substrate and this may cause larvae to cooperate in mobilizing food (Dawood & Strickberger, 1969; Budnik *et al.*, 1971; Budnik & Brncic, 1975).

On the other hand, where there is restriction of resources, the more gregarious phenotypes may spend less time and energy searching for adequate sites for the oviposition. The same applies to social facilitation. For these reasons natural selection has led to dominance for aggregated oviposition behaviour. Heterosis was observed in 75 per cent of the 12 hybrid crosses between different strains. This phenomenon is expected to occur on crossing inbred lines which have been subjected to directional selection. Heterosis is not unusual in those traits that contribute to high fitness (Bruell, 1964).

In summary, we conclude that gregarious oviposition behaviour in *Drosophila melanogaster* females is under polygenic control. The genetic organization of this trait involves genes with additive and dominant effects. A substantial maternal effect may also be present.

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