

Effect of Selection on Oviposition Site Preference in *Drosophila melanogaster*

Gladys Ruiz Dubreuil and Eduardo del Solar

Universidad Austral de Chile, Casilla 567, Valdivia, Chile.

Abstract

A divergent selection program for gregarious oviposition behaviour was carried out for 112 generations on cage population of *D. melanogaster*. Gregarious oviposition was measured using five different indices: number of occupied tubes, percentage of eggs in the preferred tube, mean variance ratio, Charlier coefficient of disturbance and k parameter of the negative binomial distribution. All these statistics showed similar tendencies, but k seemed to be the most sensitive indicator of intensity of aggregation. The high aggregation line showed a rapid initial response but little change later. In contrast, the low aggregation line showed a steady but slow response to selection. The realized heritability for the low line in generations 67-73 was 0.11 ± 0.04 .

Introduction

Gregarious behaviour is important for the regulation of population density, resource utilization, stimulation and synchronization of mating and protection against environmental injuries (del Solar *et al.* 1977; Richmond and Jerking 1979; Budnik 1980; del Solar 1982). Gregarious behaviour has been assessed in experimental populations of *Drosophila melanogaster* in terms of the spatial distribution of adults and the oviposition sites of females in various population cages (del Solar and Palomino 1966; Frías and del Solar 1974; Navarro and del Solar 1975).

With respect to oviposition several reports have demonstrated that female *D. melanogaster* lay preferentially in sites used previously for oviposition (del Solar and Palomino 1966; Mainardi 1968; Gress and Nickla 1973). Furthermore, de Jong (1979, 1982) found that such aggregation resulted in greater numerical stability in a single-species population. As far as competition between species is concerned Shorrocks *et al.* (1979) and Atkinson and Shorrocks (1981, 1984) have suggested that two species of insects breeding in discrete and ephemeral sites could coexist, despite strong competition between the larvae, as long as their eggs were aggregated and independently distributed.

The primary aim of the present investigation is to examine the negative basis of gregarious behaviour in *D. melanogaster*. A second aim is to identify the most sensitive statistical method for estimating aggregation intensities among flies whose distributions are restricted to discrete areas.

Material and Methods

In all, 250 fertilized females were taken at random from a stock of *D. melanogaster*, Valdivia, which had been maintained in our laboratory by mass mating for over 4 years since its capture. Ten groups

of 25 females were each placed for 24 h in a population cage, containing 25 tubes (2.0 cm diameter) of Obha culture medium (Obha 1961) arranged in 5 by 5 rows. After 24 h, the number of eggs in each tube was counted. On average a total of 129 eggs were laid in each cage and these eggs were restricted to 14 of the 25 tubes. The average number of eggs in the most utilized tube of each cage was 24, with seven tubes containing from 6 to 23 eggs and six from 1 to 5. From the total of 140 tubes containing any eggs from the 10 cages approximately 200 eggs from tubes with the highest number of eggs were selected to establish the high aggregation line. The low aggregation line was formed by selecting 350 eggs from tubes with 1-5 eggs.

The selected individuals were reared at a density not exceeding 50 eggs per tube. When the adults emerged, the 100 most aggregated females (born from the tubes with the highest number of eggs) were selected as parents for the next generation of the high line. They were tested in four population cages with 25 females in each. The 150 least aggregated females (born from the tubes with the lowest number of eggs) were used as parents for the next generation of the low line. These females were tested in six population cages of 25 females each.

The selection procedure was applied to the high and low line for 112 consecutive generations. Females were all 3-5 days old at testing. Testing and rearing were carried out in a darkened incubation chamber at $21 \pm 1^\circ\text{C}$, with a relative humidity of 60%.

Table 1. Comparison by Student's *t*-tests of the mean values for five statistical indices over 112 generations of selection between the high and low lines of *D. melanogaster*
** $P < 0.01$

| Statistical index | High line | Low line | <i>t</i> |
|--|--------------------|-------------------|----------|
| Number of tubes used | 14.00 ± 3.18 | 19.55 ± 3.84 | 11.75** |
| Percentage of eggs in the preferred tube | 28.85 ± 10.60 | 19.84 ± 6.64 | 7.63** |
| Variance-mean ratio | 31.83 ± 11.24 | 10.26 ± 4.79 | 14.95** |
| Charlier coefficient of disturbance | 178.70 ± 47.98 | 117.70 ± 35.5 | 10.81** |
| Reciprocal of <i>k</i> | 6.49 ± 2.74 | 1.10 ± 0.90 | 18.68** |

Aggregation was assessed using five different statistics:

- (1) Number of tubes containing eggs as a proportion of the total of 25 tubes in the cage. As the aggregation increases the number of tubes with eggs decreases.
- (2) Percentage of eggs in the most utilized tube as a proportion of the total found in the cage. In a relatively highly aggregated population, one of the 25 tubes generally contains more than 50% of the eggs laid during a 24-h period (del Solar and Palomino 1966).
- (3) Variance-mean ratio, s^2/\bar{x} . In an aggregated distribution, the population variance exceeds the population mean (Pielou 1977).
- (4) Charlier coefficient of disturbance, $100(s^2 - \bar{x})^{-1/2}/\bar{x}$. Values greater than 1 indicated aggregation (Cole 1946).
- (5) *k* parameter of the negative binomial distribution, calculated by maximum likelihood from

$$N \log_e[1 + (\bar{x}/k)] - [\sum Ax/(k+x)] = 0,$$

where *N* is the number of tubes of the population cage (25) and *Ax* is the sum of the observed numbers of tubes containing more than *x* eggs. Smaller values of *k* indicate greater aggregation (Poole 1974). The reciprocal of *k* was used in some analyses where it was preferable to have values that increased with an increase in gregariousness.

Realized heritability was estimated from parent-offspring regression (Falconer 1981) in the low line between generations 66 to 73.

Results

Table 1 shows the mean values of the five test statistics over the 112 generations of selection in both the high and low lines. (The complete data have been lodged as Accessory Publication and are available on request from the Editor-in-Chief,

Editorial and Publications Section, CSIRO, 314 Albert St, East Melbourne, Vic. 3002.) Student's *t*-test values comparing the high and low lines are also given in Table 1. Each aggregation index shows a significant statistical difference between the lines in the expected direction. Selection was thus effective in both directions, although asymmetrical and irregular (Fig. 1).

According to these results, the most sensitive statistical method for measuring aggregation appears to be parameter *k*, which gave the largest *t* values and least overlap between lines.

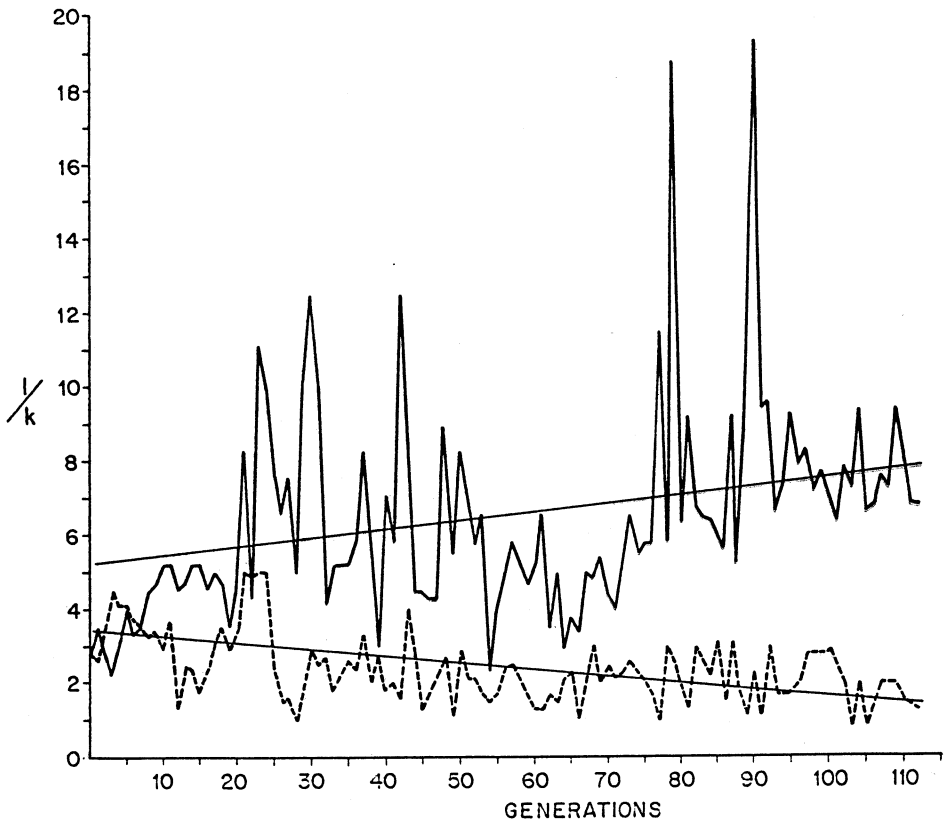


Fig. 1. Index of aggregation (reciprocal of *k* parameter of the negative binomial distribution) for the high line (—, $y = 5.18 + 0.024x$) and low line (---, $y = 3.02 - 0.019x$) during 112 generations of selection, in *D. melanogaster*.

Fig. 1 shows the course of bidirectional selection over the 112 generations, using the *k* parameter of the negative binomial distribution. The responses in the two selected lines differed. The low line showed a constant although slow response. There was a significant decrease ($P < 0.01$) in the percentage of eggs in the preferred tube, in the variance-mean ratio, in the Charlier coefficient of disturbance and in the reciprocal of *k*. The coefficients of linear regression for these four parameters were -0.107 , -0.094 , -0.651 and 0.010 respectively. There was also a significant increase in the number of tubes occupied ($b = 0.065$; $P < 0.01$). The response in the high line was only significant for two statistics: variance-mean ratio ($b = 0.072$, $P < 0.05$) and the reciprocal of *k* ($b = 0.023$; $P < 0.01$).

Regression analysis shows a significant tendency to decrease in the phenotypic variance of each index in each line (Table 2). Realized heritability for the reciprocal of k in the low line from generations 67 to 73, calculated from the regression of the response to the selection over the cumulated selection differential, was 0.11 ± 0.04 (Fig. 2).

Table 2. Coefficient of regression and its standard error of the phenotypic variance of gregarious oviposition of *D. melanogaster* against generations

| Statistical index | Line | $b \pm \text{S.E.}$ | P |
|--|------|---------------------|-------|
| Number of tubes used | High | -0.008 ± 0.005 | 0.10 |
| | Low | -0.021 ± 0.004 | 0.001 |
| Percentage of eggs in the preferred tube | High | -0.051 ± 0.021 | 0.01 |
| | Low | -0.058 ± 0.013 | 0.001 |
| Variance-mean ratio | High | -0.197 ± 0.089 | 0.03 |
| | Low | -0.248 ± 0.052 | 0.001 |
| Charlier coefficient of disturbance | High | -0.113 ± 0.030 | 0.001 |
| | Low | -0.063 ± 0.014 | 0.001 |
| Reciprocal of k | High | -0.021 ± 0.004 | 0.001 |
| | Low | -0.005 ± 0.001 | 0.001 |

The average number of eggs laid per population cage was very variable. Nevertheless, the data suggested that the fecundity (number of eggs per population cage during 24 h) was similar in both lines. Mean number of eggs over 112 generations for the high line was 209.6 ± 95.2 and for the low line, 194.5 ± 95.2 .

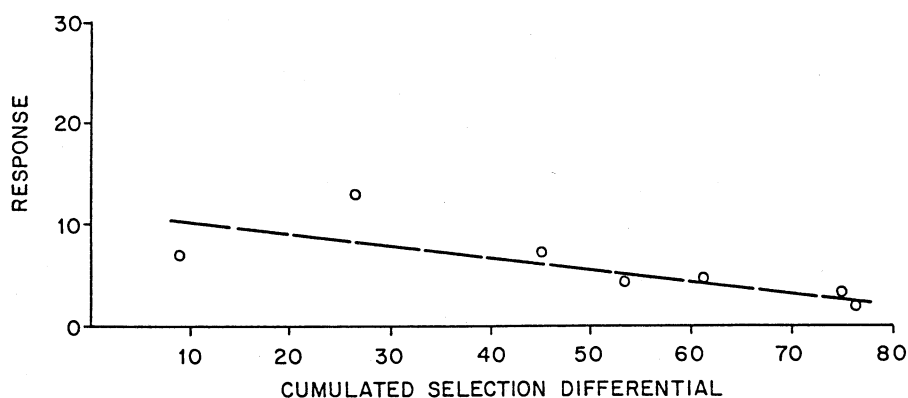


Fig. 2. Response plotted against cumulated selection differential in the low aggregation line of *D. melanogaster*. $y = 11.45 - 0.11x$. The dashed line represents a realized heritability (h^2) of 0.11 ± 0.04 .

Discussion

Many environmental and genetic factors are known to influence choice of oviposition site. The environmental factors include substrate texture (David 1970; Atkinson 1983), ethanol (Parsons and King 1977; Richmond and Jerking 1979), temperature (Navarro and del Solar 1975; Fogelman 1979), the density of females (Rockwell and Grossfield 1978) and presence of adult males (Ayala and Ayala 1969; Mainardi 1969). Regarding genetic influences, del Solar (1968) found that gregarious

egg-laying behaviour could be changed by selection in *D. pseudoobscura*. Furthermore, Takamura and Fuyama (1980) demonstrated successful divergent selection for the percentage of eggs laid by *D. melanogaster* on paper and/or medium while Pyle (1976) and Gress and Nickla (1973) observed differences in oviposition site preferences in different strains of this species.

The present results confirm that the choice of oviposition site is under partial genetic control in *D. melanogaster*. Under constant environmental conditions, directional changes in the average phenotype and decreases in phenotypic variance were obtained by selection. The response to selection was more consistent in the low selection line but was also statistically significant for at least some indices of aggregation in the high selection line.

One explanation for the lower response in the high line is that the preferred tube reached a saturation point, with an average of between 60 and 70 eggs, beyond which overcrowding worked against further selection response. The high line also showed larger random fluctuations of aggregation scores across generations than did the low line. This may simply reflect scaling problems or sampling error since on average eggs were selected from only 1 out of 25 tubes in the high line but 8 out of 25 in the low line. Alternatively, the fluctuations could be explained by the participation of genetic dominance for high aggregation. We have recorded such dominance effects in reciprocal hybridization experiments between the high and low lines (Ruiz and del Solar 1985).

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References

- Atkinson, W. D. (1983). Gregarious oviposition in *Drosophila melanogaster* is explained by surface texture. *Aust. J. Zool.* **31**, 925-9.
- Atkinson, W. D., and Shorrocks, B. (1981). Competition or a divided and ephemeral resource: a simulation model. *J. Anim. Ecol.* **50**, 161-71.
- Atkinson, W. D., and Shorrocks, B. (1984). Aggregation of larval Diptera over discrete and ephemeral breeding sites: the implications for coexistence. *Am. Nat.* **124**(3), 336-51.
- Ayala, F. J., and Ayala, M. (1969). Oviposition preferences in *D. melanogaster*. *Drosophila Inf. Serv.* **44**, 120.
- Budnik, M. (1980). Alteration wastes in different species of *Drosophila*. *Cienc. Cult. (Sao Paulo)* **32**, 1391-4.
- Cole, L. C. (1946). A study of the Cryptozoa of an Illinois woodland. *Ecol. Monogr.* **16**, 49-86.
- David, J. (1970). Oviposition chez *Drosophila melanogaster*: importance des caractéristiques physiques de la surface de ponte. *Rev. Comp. Anim.* **4**, 70-2.
- Falconer, D. S. (1981). 'Introduction to Quantitative Genetics.' (Longman: New York.)
- Fogelman, J. C. (1979). Oviposition site preference for substrate temperature in *Drosophila melanogaster*. *Behav. Genet.* **9**, 407-12.
- Frías, D., and Solar, E. del (1974). Selection for high and low dispersive behavior in *Drosophila melanogaster* females. *Boll. Zool.* **41**, 73-80.
- Gress, R. C., and Nickla, H. (1973). Choice of oviposition sites by laboratory strains of *D. melanogaster*. *Drosophila Inf. Serv.* **50**, 123.
- Jong, J. de (1979). The influence of the distribution of juveniles over patches of food on the dynamics of a population. *Neth. J. Zool.* **29**, 33-51.

- Jong, J. de (1982). The influence of dispersal pattern on the evolution of fecundity. *Neth. J. Zool.* **32**, 1-30.
- Mainardi, M. (1968). Gregarious oviposition and pheromones in *D. melanogaster*. *Boll. Zool.* **35**, 135-6.
- Mainardi, M. (1969). Oviposition preferences in *Drosophila simulans*. *Boll. Zool.* **36**, 101-3.
- Navarro, J., and Solar, E. del (1975). Pattern of spatial distribution in *Drosophila melanogaster*. *Behav. Genet.* **5**(1), 9-16.
- Obha, S. (1961). Chromosomal polymorphism and capacity for increase under optimal condition. *Heredity* **22**, 169-85.
- Parsons, P. A., and King, S. B. (1977). Ethanol: larval discrimination between two *Drosophila* sibling species. *Experientia* **33**, 989.
- Pielou, E. C. (1977). 'Mathematical Ecology.' (John Wiley and Sons: New York.)
- Poole, R. W. (1974). 'An Introduction to Mathematical Ecology.' (John Wiley and Sons: New York.)
- Pyle, D. W. (1976). Oviposition site difference in strains of *Drosophila melanogaster* selected for divergent geotactic maze behavior. *Am. Nat.* **110**, 181-4.
- Richmond, R. C., and Jerking, J. (1979). Oviposition site preference in *Drosophila*. *Behav. Genet.* **9**(3), 233-41.
- Rockwell, R. F., and Grossfield, J. (1978). *Drosophila*: behavioral cues for oviposition. *Am. Midl. Nat.* **99**, 361-8.
- Ruíz, G., and Solar, E. del (1985). Análisis genético preliminar de la conducta gregaria en *D. melanogaster*. Resúmenes VII Congreso Latinoamericano de Genética. *Biomédica*, Suppl. 1.
- Shorrocks, B., Atkinson, W. D., and Charlesworth, P. (1979). Competition on a divided and ephemeral resource. *J. Anim. Ecol.* **48**, 899-908.
- Solar, E. del (1968). Selection for and against gregariousness in the choice of oviposition sites by *D. pseudoobscura*. *Genetics* **58**, 275-82.
- Solar, E. del (1982). Aspectos ecológicos y conductuales de la microdistribución geográfica en poblaciones de *Drosophila*. Actas Vº Congreso Latinoamericano de Genética. (Eds R. Cruz-Coke and D. Brncic.) *Reun. Anu. Soc. Genet. Chile* **14**, 247-55.
- Solar, E. del, and Palomino, H. (1966). Choice of oviposition sites in *D. melanogaster*. *Am. Nat.* **100**, 127-33.
- Solar, E. del, Ruíz, G., and Kohler, N. (1977). Conducta gregaria y microdistribución geográfica. *Medio Ambiente* **3**(1), 15-25.
- Takamura, T., and Fuyama, J. (1980). Behavior genetics of choice of oviposition sites in *Drosophila melanogaster*. I. Genetic variability and analysis of behavior. *Behav. Genet.* **10**, 105-20.