

# SELECTION FOR AND AGAINST GREGARIOUSNESS IN THE CHOICE OF OVIPOSITION SITES BY *DROSOPHILA PSEUDOOBSCURA*<sup>1</sup>

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THE distribution of individuals in space is seldom, if ever, random. Most animal species tend to have a nonrandom distribution at least during some phases of their life cycles or during some seasons of the year. TIMOFEEFF-RESSOVSKY (1940), DOBZHANSKY and EPLING (1944), DOBZHANSKY and PAVAN (1950), and BURLA *et al.* (1950) found that the individuals of several species of *Drosophila* form aggregation nuclei in their natural habitats. They ascribed these aggregations to the nonhomogeneity of the environments in which the flies live. Aggregations may also be caused by genetically conditioned features of the behavior of the flies. DEL SOLAR and PALOMINO (1966) have shown, in *D. melanogaster*, that the flies presented with several apparently similar oviposition sites do not distribute their eggs at random. Similar aggregation behavior was observed in *D. funebris* and *D. pavani* (PALOMINO and DEL SOLAR 1967). The main factor which brings about the aggregation of the eggs is the preference of the female flies for ovipositing near the sites previously used for oviposition by other females. It is possible to direct the flies to a given site simply by placing some eggs on one of the several suitable sites offered them (DEL SOLAR and PALOMINO 1967).

The aggregation behavior may play an important role in the ecology of the flies. As found by BEARDMORE, DOBZHANSKY and PAVLOVSKY (1960), LEWONTIN and MATSUO (1963), and WEISBROT (1966), the association of the larvae of different genotypes in the same medium may influence differentially the fitness of the carriers of these genotypes. The experiments described in the present article show that the tendency of the flies to distribute their eggs more or less randomly, or to concentrate them on a single or a minority of the available oviposition sites, is under genetic control. By selection over a series of generations populations of *D. pseudoobscura* have been obtained which display relatively strong or weak aggregation propensities.

## MATERIALS AND METHODS

Populations of *D. pseudoobscura* were established, two of which were monomorphic and one was polymorphic for the Chiricahua (CH) and Arrowhead (AR) gene arrangements of the third chromosomes. The stocks, descended from single females collected at Piñon Flats, California, have been maintained in the laboratory for several years previous to the beginning of the experiments.

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Flies from nine strains with each gene arrangement were allowed to mate freely in half-pint milk bottles for two generations. Ten to fifteen females from the second generation were taken and allowed to oviposit on paper spoons with OHBA's (1961) medium. Three hundred eggs collected from 10 different spoons were then washed in 72% ethanol and in a solution of penicillin, to free them from bacterial or yeast contamination. These eggs were distributed in groups of 30 per culture bottle with OHBA's sterile medium. The flies emerging from these bottles were the base populations used to start the experiment. The selection procedure was as follows. In every generation, 15 fertilized females and 5 males were placed in a population cage containing 15 cups with OHBA's culture medium. The females were allowed to oviposit for 24 hours at 25°C. To rule out the possibility that the flies may be attracted to some part of the cage by light, the cages were covered with heavy cloth, making them uniformly dark. Two selection lines, H (high aggregation) and L (low aggregation) were established from each of the three base populations, two of which were monomorphic AR/AR or CH/CH, and the third polymorphic AR + CH. In the H lines, 60 eggs were selected from the cup containing the greatest numbers of eggs. In the L lines, 60 eggs were taken from as many cups as necessary, starting with the one having fewest eggs. Six replications were made in each line in every generation. The selection pressure was increased from the eighth generation on, by selecting 30 instead of 60 eggs in each replication.

For a statistical evaluation of the experimental results, three statistics were used. The simplest is the number of cups (out of the total of 15 present in the cage) in which one or more eggs were found. Another, is the percentage of the eggs deposited in the cage which are in the cup with the largest number of eggs. Finally, the aggregation was measured by means of the Charlier coefficient, as recommended by COLE (1946).

## RESULTS

Table 1 shows the mean numbers of cups containing eggs (out of the total of

TABLE 1

*Average numbers of cups containing eggs*

Generations	CH/CH		AR/AR		CH/AR	
	Low	High	Low	High	Low	High
1	8.3 ± 1.1	6.3 ± 0.6	4.2 ± 0.4	4.1 ± 0.9	5.8 ± 0.7	7.0 ± 0.8
2	6.5 ± 0.7	4.3 ± 1.2	7.6 ± 0.6	7.5 ± 1.3	7.5 ± 0.7	4.6 ± 0.6
3	11.6 ± 0.6	6.5 ± 0.9	10.6 ± 0.7	9.3 ± 0.7	6.0 ± 1.6	7.1 ± 0.9
4	10.6 ± 0.9	7.8 ± 0.6	9.1 ± 1.1	8.6 ± 0.9	11.5 ± 0.5	6.8 ± 1.0
5	8.8 ± 0.9	8.8 ± 0.4	11.5 ± 1.1	9.1 ± 0.5	11.1 ± 0.9	8.5 ± 0.5
6	7.8 ± 1.2	5.5 ± 1.3	9.8 ± 1.1	5.0 ± 0.9	11.8 ± 0.8	5.1 ± 0.9
7	5.5 ± 1.0	6.1 ± 1.1	9.5 ± 0.7	5.5 ± 0.4	7.0 ± 1.1	5.6 ± 0.8
8	10.1 ± 1.3	4.8 ± 0.9	8.6 ± 1.0	4.6 ± 0.9	9.1 ± 1.4	4.5 ± 0.4
9	10.2 ± 0.8	4.5 ± 0.8	10.1 ± 0.8	4.3 ± 0.4	11.0 ± 1.2	4.5 ± 1.1
10	9.8 ± 0.9	3.8 ± 0.6	12.0 ± 0.7	3.8 ± 0.7	11.2 ± 0.8	4.1 ± 0.4
11	11.1 ± 0.9	2.0 ± 0.2	9.6 ± 0.9	3.6 ± 0.5	11.6 ± 0.9	3.0 ± 0.2
12	13.3 ± 0.5	5.6 ± 0.4	11.0 ± 0.9	3.3 ± 0.4	11.7 ± 0.6	6.0 ± 0.8
13	11.2 ± 0.9	4.3 ± 1.0	13.3 ± 0.3	2.7 ± 0.5		4.0 ± 0.2
14	12.1 ± 0.7	4.6 ± 0.5	13.1 ± 0.6	4.0 ± 0.3		
15	14.0 ± 0.4	2.5 ± 0.5	12.3 ± 0.5	4.3 ± 0.8		
16	14.5 ± 0.2	2.6 ± 0.5	13.3 ± 0.3	4.0 ± 0.4		
17	10.8 ± 0.7	1.5 ± 0.2	14.0 ± 0.2	4.0 ± 0.4		
18	13.0 ± 0.6	4.0 ± 0.5				
19	13.8 ± 0.3	3.1 ± 0.3				
20	14.3 ± 0.3	3.5 ± 0.4				

15 cups per cage) in the L and H selected lines in each generation studied. It can be seen that in the L lines the numbers increase, and in the H lines decrease (meaning, of course, that in the L lines the aggregation decreases and in the H lines it increases). In the monomorphic CH and AR populations the L lines have, in the 20th and 17th generations respectively, eggs in almost every cup ( $14.3 \pm 0.3$  and  $14.0 \pm 0.2$  out of the possible 15). In the H lines, on the contrary, most of the cups are unoccupied, and the eggs are concentrated in only  $3.5 \pm 0.4$  and  $4.0 \pm 0.4$  cups. The polymorphic AR + CH populations had to be discarded after only 12 and 13 generations of selection, and yet in the last selected generations the divergence was quite evident:  $11.7 \pm 0.6$  in L and  $6.0 \pm 0.8$  in H lines.

Table 2 gives the mean percentages of the eggs deposited in the cages which were found in the one cup containing the largest number of eggs. Since each cage had 15 cups equally suitable as oviposition sites, a random distribution would give 6.7 percent of the eggs per cup. Yet even at the start, between 40 and 57 percent of all the eggs were found in just one preferred cup. In the L lines the percentages declined to  $16 \pm 2$ ,  $16 \pm 1$ , and  $27 \pm 3$ . In the H lines, the increases were not nearly as striking; in fact, in the monomorphic AR population the last reading,  $45 \pm 2$  is below the first,  $57 \pm 9$  percent.

Table 3 reports the changes in the aggregation indices. These indices are calculated according to the formula  $(100\sqrt{S^2 - \bar{X}})/\bar{X}$ . This, the so-called Charlier coefficient of disturbance (COLE 1946), measures the deviation of a distribution from a Poisson distribution. With random distribution, the variance,

TABLE 2

*Mean percentages of the eggs in the cup containing the greatest number of eggs*

Generations	CH/CH		AR/AR		CH/AR	
	Low	High	Low	High	Low	High
1	49 ± 10	47 ± 6	49 ± 3	57 ± 9	47 ± 4	40 ± 9
2	56 ± 8	65 ± 11	45 ± 7	40 ± 7	41 ± 6	50 ± 4
3	28 ± 2	38 ± 6	30 ± 3	32 ± 3	54 ± 11	29 ± 4
4	31 ± 3	28 ± 2	32 ± 4	31 ± 5	28 ± 1	38 ± 8
5	40 ± 7	31 ± 3	23 ± 2	30 ± 4	23 ± 2	28 ± 4
6	37 ± 4	62 ± 12	38 ± 7	57 ± 11	18 ± 1	53 ± 10
7	64 ± 11	38 ± 4	34 ± 6	66 ± 7	48 ± 7	48 ± 5
8	37 ± 5	61 ± 9	42 ± 9	61 ± 10	45 ± 9	59 ± 7
9	40 ± 4	55 ± 10	36 ± 5	53 ± 5	29 ± 3	48 ± 11
10	41 ± 5	62 ± 8	21 ± 2	61 ± 10	26 ± 2	58 ± 7
11	38 ± 5	77 ± 6	36 ± 5	70 ± 6	31 ± 2	73 ± 6
12	22 ± 2	42 ± 3	33 ± 6	67 ± 6	27 ± 3	53 ± 5
13	33 ± 2	55 ± 7	19 ± 1	71 ± 10		63 ± 7
14	26 ± 2	50 ± 4	15 ± 1	57 ± 3		
15	16 ± 2	73 ± 10	22 ± 3	52 ± 3		
16	18 ± 2	68 ± 8	25 ± 3	51 ± 2		
17	30 ± 1	92 ± 6	16 ± 1	45 ± 2		
18	23 ± 1	48 ± 7				
19	23 ± 1	58 ± 5				
20	16 ± 2	55 ± 5				

TABLE 3

*Means of the aggregation indices*

Generations	CH/CH		AR/AR		CH/AR	
	Low	High	Low	High	Low	High
P	183 ± 21	183 ± 21	176 ± 10	176 ± 10	150 ± 11	150 ± 11
1	207 ± 35	198 ± 21	224 ± 10	239 ± 31	203 ± 13	180 ± 32
2	230 ± 23	271 ± 38	190 ± 23	178 ± 24	176 ± 17	215 ± 15
3	133 ± 12	168 ± 20	132 ± 9	149 ± 13	234 ± 38	144 ± 14
4	138 ± 13	136 ± 5	147 ± 13	146 ± 22	117 ± 6	175 ± 28
5	177 ± 21	143 ± 9	113 ± 11	147 ± 12	114 ± 13	131 ± 13
6	168 ± 18	242 ± 43	167 ± 24	239 ± 37	117 ± 8	226 ± 34
7	254 ± 40	175 ± 15	155 ± 19	263 ± 24	205 ± 20	207 ± 13
8	167 ± 20	254 ± 31	191 ± 31	260 ± 32	191 ± 29	243 ± 24
9	172 ± 13	231 ± 34	157 ± 15	223 ± 16	133 ± 11	217 ± 40
10	170 ± 15	252 ± 29	107 ± 9	253 ± 33	124 ± 13	245 ± 24
11	157 ± 20	311 ± 16	160 ± 18	279 ± 21	138 ± 11	291 ± 19
12	110 ± 5	185 ± 9	148 ± 19	271 ± 19	121 ± 11	220 ± 16
13	149 ± 9	230 ± 26	85 ± 4	292 ± 32		254 ± 25
14	116 ± 8	216 ± 12	96 ± 4	235 ± 11		
15	76 ± 9	292 ± 33	111 ± 11	223 ± 11		
16	84 ± 7	282 ± 25	113 ± 11	218 ± 8		
17	139 ± 5	354 ± 18	78 ± 7	206 ± 29		
18	112 ± 11	214 ± 18				
19	107 ± 9	245 ± 12				
20	66 ± 6	233 ± 18				

$S^2$ , should be as large as the mean,  $\bar{X}$ , of the numbers of eggs per cup, which would make the index equal to zero. Positive values of the coefficient indicate aggregation, while the coefficient would become an imaginary number (a negative value under the square root) with overdispersion, that is, if the eggs are more evenly distributed than would occur in the Poisson case. Table 3 shows that in reality all indices are much higher than zero. Before the selection started (generation P) the average values in the three populations ranged from  $150 \pm 11$  to  $183 \pm 21$ . In the L lines the values declined to  $66 \pm 6$ ,  $78 \pm 7$ , and  $121 \pm 11$ ; in the H lines they rose to  $233 \pm 18$ ,  $206 \pm 29$ , and  $254 \pm 25$ . An analysis of the regression of the aggregation indices on time is presented in Table 4. The regression coefficients are always positive in the H lines and always negative in the L lines, and they

TABLE 4

*Regression of the aggregation indices on time*

Population	Low		High		D.F.
	<i>b</i>	<i>t</i>	<i>b</i>	<i>t</i>	
CH/CH	-5.75 ± 0.9	6.44	5.07 ± 1.2	4.07	112
AR/AR	-5.72 ± 0.9	6.24	3.96 ± 1.4	2.92	100
CH/AR	-5.64 ± 1.9	3.01	8.23 ± 2.5	3.77	70

*b*, coefficient of regression; *t*, for the significance of the regression.

TABLE 5

*Average total numbers of eggs deposited per cage per generation*

Generations	CH/CH		AR/AR		AR/CH	
	Low	High	Low	High	Low	High
1	235 ± 67	120 ± 16	198 ± 30	209 ± 49	155 ± 15	262 ± 34
2	256 ± 81	137 ± 30	534 ± 47	364 ± 77	204 ± 27	148 ± 44
3	483 ± 78	246 ± 82	565 ± 55	613 ± 43	168 ± 53	350 ± 56
4	496 ± 88	491 ± 33	448 ± 87	496 ± 64	452 ± 40	431 ± 71
5	347 ± 76	420 ± 36	644 ± 43	447 ± 23	408 ± 66	443 ± 83
6	256 ± 68	160 ± 38	408 ± 74	301 ± 53	475 ± 82	247 ± 70
7	148 ± 23	282 ± 71	364 ± 68	254 ± 34	152 ± 47	165 ± 36
8	252 ± 66	283 ± 80	271 ± 59	296 ± 83	230 ± 58	137 ± 35
9	206 ± 29	258 ± 70	345 ± 39	264 ± 28	312 ± 49	263 ± 45
10	253 ± 40	304 ± 37	559 ± 47	287 ± 39	379 ± 42	323 ± 57
11	162 ± 34	138 ± 31	279 ± 68	247 ± 33	221 ± 31	136 ± 37
12	415 ± 53	588 ± 59	497 ± 101	173 ± 57	301 ± 42	361 ± 51
13	206 ± 26	282 ± 77	670 ± 55	181 ± 24		267 ± 23
14	430 ± 14	348 ± 40	591 ± 32	270 ± 40		
15	640 ± 88	126 ± 25	444 ± 60	318 ± 28		
16	817 ± 40	166 ± 38	336 ± 26	373 ± 39		
17	310 ± 62	61 ± 14		205 ± 28		
18	428 ± 75	225 ± 19				
19	536 ± 104	245 ± 25				
20	575 ± 76	203 ± 20				

are in all cases statistically significant at probability levels of one per cent or better.

The number of the eggs deposited per cage varied greatly, and since the averages, shown in Table 5, were computed from only six replications per line per generation, the sampling errors are sometimes very large. Nevertheless, the data suggest that the fecundity, i.e., the total number of eggs deposited per cage, increased in the L lines. To test this suggestion further, in the 17th generation of selection three samples of 15 females each were taken from the H line, and an equal number from the L line, of the monomorphic CH populations. The flies were placed in population cages for a 10-day period. Each day the cups with the culture medium were changed, and the numbers of eggs deposited were recorded. Table 6 shows the results obtained with the three samples, denoted A, B and C. The mean numbers of eggs per day of the 10-day period are greater in the low aggregation line than in the high aggregation line. The aggregation indices and the mean numbers of the cups utilized differ in the expected direction with the 10-day periods, as they do in the main experiment in which 1-day periods are used.

The question arises whether the increasing fecundity in the L lines is creating spurious changes in the index of aggregation. Thirty-four samples were found in generations 8 to 15 in which the average numbers of eggs in the H and L lines of each population were approximately the same (differences in less than 24 eggs). In 33 of these samples the H line showed a higher index of aggregation

TABLE 6

Mean number of eggs, aggregation indices and numbers of cups with eggs in the High and Low lines of the CH population after 17 generations of selection

Line	Replication	Eggs	Index	Cups with eggs
High	A	297 ± 35	200 ± 17	5.2 ± 0.6
	B	242 ± 46	156 ± 19	7.4 ± 0.8
	C	234 ± 47	246 ± 28	4.2 ± 0.8
Low	A	625 ± 42	93 ± 7	13.3 ± 0.4
	B	544 ± 78	103 ± 9	13.0 ± 0.5
	C	465 ± 51	126 ± 11	11.3 ± 0.5

than the L line. Another test was made by dividing the number of eggs in the six replications per generation of the L line into two groups, three with more and three with fewer eggs. The mean numbers of eggs were then compared with the mean index of aggregation. Out of 20 comparisons, 13 showed a higher index of aggregation associated with the smaller mean number of eggs. The remaining seven cases show the opposite situation. A correlation analysis was made in both groups. There is no significant association between the aggregation index and the number of eggs. The correlation coefficients were 0.014 and  $-0.044$ .

The frequencies of the heterokaryotype AR/CH in the polymorphic population were determined in generations 1, 3, 5 and 12, after the start of the selection, by cytological examination of a sample of 100 larvae. No significant differences were found among generations nor between the H and L lines.

#### DISCUSSION

The way *Drosophila* flies distribute their eggs among the available oviposition sites is evidently important for the survival of their progenies. *Drosophila flavopilosa* studied by BRNCIC (1962, 1966), develops in the flowers of a single plant species, *Cestrum parqui*. Young larvae feed on flower sap, and older ones on pollen. An ovipositing female makes a hole in the base of the flower, large enough to deposit a single egg. Only very rarely flowers are found with more than a single egg. Species of *Drosophila* which feed in fermenting fruits or on tree sap deposit often several eggs close together. As shown particularly by SOKOLOFF (1955) in *D. pseudoobscura*, and in the related species *D. persimilis* and *D. miranda*, the effects of crowding in the culture medium may be quite unfavorable. The rate of survival, the weight of the emerging adults, and the speed of the development decrease at high population densities. The optimal conditions are, however, found not at the lowest density (one larva per culture) but at certain intermediate ones. *Drosophila* larvae have a "conditioning" effect on the medium in which they live. The presence of larvae of similar or of different genotypes in the culture medium influence the environment of the developing larvae in various ways (WEISBROT 1966).

It is evidently disadvantageous for a population of *Drosophila* to have all the

eggs which the females deposit concentrated in a single oviposition site, leaving all other potential food sources unexploited. It is apparently also disadvantageous to have so few eggs deposited that the food is not properly conditioned by the larvae. As is usual in evolution, natural selection strikes a compromise. The aggregation behavior leads to some oviposition sites receiving larger numbers of eggs than others, and some sites (at least in the experimental environments) remaining unoccupied. The point which the data reported in the present article demonstrate is that the degree of aggregation is under genetic control, and can be changed within a small number of generations by selection. Thus, the competition among the developing larvae may be held at levels at which it is not too detrimental, and at the same time the available food sources are exploited as fully as possible.

The unequal distribution of eggs among the oviposition sites has been described as "aggregation." It is evident that the aggregation is an outcome of a complex of behavior patterns. A single female may deposit several eggs close together, or it may place them in several sites. In addition, a mechanism of social interaction may be involved, making different females stay together in groups, or spread as widely as possible in the available space. Experiments to be reported elsewhere seem to indicate that the fecundity of the females selected for high and low aggregation is greatly modified by the space available to them. As shown in Tables 5 and 6, the L flies deposit more eggs than do the H flies in the population cages. The same L and H lines were tested in  $15 \times 2.9$  cm vials, into which paper spoons with OHBA's medium were inserted and changed daily. Under these conditions, the fecundity of H females is statistically significantly higher than that of the L females. These behavioral components may be genetically conditioned separately. Experiments of this kind are being planned.

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#### SUMMARY

When offered a number of equivalent oviposition sites, the females of *Drosophila pseudoobscura*, like those of other species previously studied, do not distribute their eggs at random. Some oviposition sites are not utilized at all, while many eggs are deposited on other sites. The degree of aggregation is under genetic control, and can be changed by selection. Three populations, two of them monomorphic for AR or for CH gene arrangements in their third chromosomes, and one population polymorphic for these gene arrangements, were selected for high and for low aggregation. A response to selection was obtained in all the populations.

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