Intraspecific variation in the effects of mating on emigration activity and fecundity in a natural population of Drosophila melanogaster

Kenji Mikasa
Biological Laboratory, School of Dentistry, Meikai University, Sakado, Saitama 350-0283, Japan
(Received 13 May 1998, accepted 3 September 1998)

Intraspecific variation in the effects of mating on the emigration response behavior and fecundity of Drosophila melanogaster was investigated using isofemale lines of the Himeji population in Japan. The emigration activities of the mated and unmated females were examined with Sakai’s population system. The isofemale lines were classified into two groups with respect to the effect of mating on emigration activity; 1) copulation decreased the emigration activity in 26 out of 28 isofemale lines, and 2) higher emigration activity was noted in the mated than in the unmated females in two lines. The percentage of expressed genotypic variance on emigration activity was higher in the unmated females than in the mated ones. Gregarious oviposition did not seem to be related to the decrease of emigration activity in the mated females.

INTRODUCTION

The movement of insects differs between mated and unmated females (Johnson, 1969). These differences are species specific. Drosophila is one of the organisms most extensively studied with respect to the movement of insects under natural conditions. Movement of Drosophila from one place to another is related to feeding, oviposition, search for a mate, and avoidance of deleterious conditions. Environmental factors such as temperature, light, wind, and different habitat conditions also influence the movement of Drosophila (Dobzhansky and Wright, 1943, 1947; Burla et al., 1950; Dyson-Hudson, 1956; Koch, 1967; Dobzhansky and Powell, 1974; Richardson and Johnston, 1975; Johnston and Heed, 1976; Powell et al., 1976; Dobzhansky et al., 1979). Because of the interactions among the above factors under natural conditions, it is very difficult to detect directly which biological and environmental factors influence the movement of flies in nature. Therefore, laboratory experiments are needed to examine the behavioral response of Drosophila (Dyson-Hudson, 1956). Sakai et al. (1958) devised a population system, termed Sakai’s population system, to investigate the movement of Drosophila under laboratory conditions. Such movement investigated by this system is defined as emigration response behavior, that is, overall movement from the original location to another location (Rockwell et al., 1978). The measured emigration activity is a polygenic character with a large additive effect and a small dominance effect (Mikasa, 1990). Natural populations of Drosophila have genetic variations for emigration activity (Narise, 1962; Rockwell et al., 1983; Mikasa and Narise, 1986; Rockwell and Levine, 1986; Mikasa, 1990). The emigration response behavior of Drosophila is influenced by various environmental factors, such as population density (Sakai et al., 1958; Takada, 1959; Narise, 1962; Tantawy et al., 1975), genetic constitution in a population (Narise, 1966, 1968, 1974; Narise and Mikasa, 1984), temperature (Tantawy et al., 1975; Mikasa and Narise, 1979, 1983a, b, 1986, 1990), light and height (Rockwell et al., 1978; Rockwell, 1979), and wind (Johnston, 1982). Narise and Narise (1991) found that palmitic acid and oleic acid influenced the emigration behavior of D. melanogaster. The flies responded to those acids in a strain-specific manner.

As mentioned above, there are many studies on emigration response behavior with respect to environmental factors. However, none of the studies was carried out with respect to the influence of mating conditions of the females on emigration response behavior. Thus, I examined the relationship between mating conditions and emigration activity. Mating conditions in female Drosophila seem to influence their movement from one place to another. Virgin females may exhibit a strong tendency to move to a new location in search of a mate, whereas mated females may remain at suitable places to oviposit. As the use of isofemale lines is a somewhat simpler approach for the assessment of the nature and range of phenotypic variation in natural populations (Parsons, 1980), 28 isofemale lines derived from the Himeji population were used. Additionally, to enable the comparison of results of previ-
ous studies on the variability of emigration activity in a natural population (Mikasa and Narise, 1986; Mikasa, 1992), females were mated with males of the same isofemale line. I will also report the results of examinations on the number of eggs oviposited by mated and unmated females. In this paper, I will discuss the effects of mating on emigration response behavior in a natural population of *D. melanogaster*. Furthermore, the relation between emigration response behavior and oviposition will be demonstrated.

**MATERIALS AND METHODS**

*D. melanogaster* strains. Collection of *Drosophila* was conducted near human habitation at Himeji City (N34°50', W134°40') in October 1988. The collection sites were the same as those in the previous study of Mikasa (1991). The isofemale lines of *D. melanogaster* were established from single inseminated females. They were maintained in vials containing yeast-glucose-cornmeal-agar medium at a temperature range of 19–21°C. Twenty-eight isofemale lines were used for the experiments which were performed two years from the time of capture of the flies.

Emigration activity. Batches of nine males and nine females from each isofemale line were cultured separately in a half-pint milk bottle containing yeast-glucose-cornmeal-agar medium. The bottles were kept in a 25°C room with a LD 12:12 photocycle (lights turned on at 08:30). The flies were transferred into new bottles with fresh medium every day or every other day. Two replicate bottles were prepared for each isofemale line. Then, the young adult flies eclosed within eight hours were collected from the culture bottles and sexed under light anesthesia. These flies were randomly assigned to two groups; (1) mated group (20 females and 5 males), and (2) unmated group (20 females). The individuals in each group were maintained in a vial with the medium as mentioned above and transferred into new vials with fresh medium every day or every other day. At the fourth day after eclosion, the males in the mated group were removed by aspiration, and the 20 mated females were introduced into a Sakai tube with fresh medium. The 20 virgin females in the unmated group were placed in another Sakai tube with fresh medium. Density in an experimental population is known to influence the emigration activity scored with Sakai’s population system, and low density results in density-independent emigration response behavior (Sakai et al., 1958; Narise, 1962). Therefore, a density of 20 individuals was chosen to decrease the effect of density on emigration activity. The procedures for introduction of flies into a Sakai tube were always conducted at 16:00–17:30. The next morning, 9:00–9:30, three new Sakai tubes with fresh medium were radially connected to the original one (Sakai et al., 1958; Mikasa and Narise, 1979; Rockwell, 1979). In Sakai’s system, the flies that move into the newly connected tubes can move back to the original tube. After six hours, the number of flies observed in the outer three tubes was counted, and the total was defined as emigration activity. Five replications were done for each group of each isofemale line.

Mating conditions. In order to verify the virginity in the unmated group and to confirm whether or not the mated females laid eggs, the following procedures were done for each group. First, in the unmated group, the appearance of F1 larvae was checked in the vials in which the virgin females had been maintained for one or two days. Second, after the measure of emigration activity in each group, all the females used were individually introduced without anesthesia into small vials containing food and the appearance of F1 larvae was examined to check whether or not females were mated.

Fecundity. After the series of experiments to examine emigration activity, the number of eggs laid by mated and unmated females was examined in newly cultured flies. The rearing conditions of the flies and the experimental treatment of emerged females were the same as those for the examination of emigration response behavior. A batch of 20 mated and 20 unmated females were introduced, respectively, into vials with the same diameter as Sakai’s tube at 16:00–17:30 and allowed to oviposit on the fresh medium at 25°C for about 24 h. All the eggs oviposited by a batch of 20 unmated females were counted. However, as the group of 20 mated females oviposited so many eggs on the medium that it was too laborious to count all of them, a count was made of those on one fifth of the surface of the medium. This number was then multiplied by five for the estimated total. Five replications were carried out to examine the fecundity in each line. Mating conditions of each female were also checked by the procedure mentioned previously. The fecundity of 16 of 28 isofemale lines was examined. The other lines were lost in an accident.

**RESULTS**

Emigration Activity. The emigration activities of the mated females were generally lower than those of the unmated ones (Fig. 1). As presented in Table 1, the two main factors, line and treatment, and the interaction between these two factors had statistical significance (*P* < 0.01 or *P* < 0.001). The variance components in each source were estimated by equating observed and expected mean squares (Table 1). The component due to the isofemale line was defined as “expressed” genotypic variance by Rockwell (1980). The order of the amount for the variance components is *L* × *T* (σ2L × T = 1.52) < *Line* (σ2L = 3.54) < Treatment (σ2T = 7.06). To examine to what extent mating influenced emigration activity in each line, the
Intraspecific variation in the effect of mating

ratios, \( R_m = (M - U) / U \), were calculated; \( M \) is the mean emigration activity of the mated females and \( U \), the unmated. The ratio shows the effect of mating with respect to emigration response behavior. These values ranged from –0.72 to 0.38 and the medium value was –0.50. The univariate procedure of SAS was conducted in order to check the distribution pattern for these values. The statistical results indicated that these values significantly deviated from normal distribution (\( P < 0.0003, W = 0.830 \)). The distribution pattern is bimodal, with one large peak and one small peak. The former consisted of twenty-six lines out of twenty-eight used ones. The range of the ratios (\( R_m \)) was –0.72 to –0.21. The latter comprised two lines (lines 18 and 19 as shown in Fig. 1). The ratios of the two lines were 0.38 and 0.26 respectively.

As shown in Figure 1, the highest emigration activities of the mated and unmated females were 8.4 in line 24 and 13.4 in line 8, respectively; the lowest emigration activities of the mated and unmated females were 1.4 in line 18 and 1.6 in line 14, respectively. The range of emigration activity for the unmated females (1.6–13.4) was wider than that for the mated ones (1.4–8.4). To estimate the amount of variability due to the isofemale line for emigration activity, I conducted one-way ANOVA on emigration activity in each treatment (Table 2). The components of variance due to isofemale lines were 7.05 ± 2.26 in the unmated females and 2.13 ± 0.90 in the mated ones. The standard error was estimated following the method as presented in Mukai (1978). The percentages of the variance component to total phenotypic variance were 53.0 (= 7.05 × 100 / (7.05 + 6.25)) % in the unmated females and 27.4 (= 2.13 × 100 / (2.13 + 5.64)) % in the mated ones. There was a statistically significant difference between the two percentages (\( P < 0.01, \chi^2 = 8.11, df = 1 \)).

Correlation between emigration activity and the degree of the effect of mating. As mentioned above, the emigration activities and the effects of mating on emigration activity (\( R_m \)) varied among the lines. Therefore, I examined whether the degree of the effects of mating on emigration activity was related to the emigration activities of the unmated females within the group in which emigra-
tion activity decreased in the mated females (Fig. 2). As shown in Figure 2, there was no significant correlation ($r = -0.002$, df = 24, $P > 0.05$).

**Fecundity.** The mean number of eggs oviposited in the 16 isofemale lines of the mated and unmated females is shown in Table 3. The unmated females of lines 17 and 24 did not oviposit. The coefficient of variation for fecundity in the unmated females was remarkably high (0.46–2.24). Then, further statistical analysis was not carried out.

**Relationship between emigration behavior and fecundity.** Negative correlation was found between emigration activity and fecundity in the mated group, but the correlation coefficient was not statistically significant ($P > 0.05$, $r = -0.228$, df = 14).

### DISCUSSION

Most of the mated females produced progeny. However, one or two females out of the 20 in one or two replicates in several lines did not produce offspring, as observed after the measurement of emigration activity and fecundity in the mated females. In the present study, 20 females were inseminated by five males of the same isofemale lines. A batch of 20 newly emerged female and 5 male flies was kept for four days. These males were allowed to mate multiply. Male flies become rather sterile after mating to four or five females within a short period of time (Lefevre and Johnson, 1962; Strømøe and Kvelland, 1962; Petit et al., 1980). But, if the males were young, they would recover within 24 h (Kaufmann and Demerec, 1942). Thus, I could speculate that there might be little influence due to bias of the sex ratio for reproduction in the mated populations.

Emigration activities differed very much among isofemale lines of the Himeji population, as Rockwell et al. (1983) previously reported in natural populations of *D. busckii*. In addition to the effect of different lines on emigration activity, effects of mating conditions were found. The degree of the differences between the unmated and mated females ($\sigma^2_T = 7.06$) was twice as great as that between isofemale lines ($\sigma^2_L = 3.54$). Although there was a significant line × treatment interaction, the effect of the interaction ($\sigma^2_L \times r = 1.52$) was smaller than that of the two main factors. Mating is a very important factor for emigration response behavior.

The females of *D. melanogaster* could be classified into two groups with respect to the effect of mating on emigration activity. In the first group, comprised of 26 of 28 isofemale lines, the mated females had lower emigration activity than the unmated ones. Three reasons may be considered for this decrease: 1) mating makes female flies change from a mobile to a sedentary phase. As mated females must expend energy for reproduction, such females may not attempt to move from a place suitable for oviposition to another place. 2) The mated females used in the

### Table 2. One-way ANOVAs on emigration activities of the unmated and mated flies in three groups

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>Expected M.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>139</td>
<td>1820.14</td>
<td></td>
<td>1070.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Line</td>
<td>27</td>
<td>1120.14</td>
<td>41.49***</td>
<td>439.74</td>
<td>16.29***</td>
<td>$\sigma^2_T + 5\sigma^2_L$</td>
</tr>
<tr>
<td>Error</td>
<td>112</td>
<td>700.00</td>
<td>6.25</td>
<td>631.20</td>
<td>5.64</td>
<td>$\sigma^2_L$</td>
</tr>
</tbody>
</table>

### Table 3. Mean and standard deviation of fecundity in 20 unmated and 20 mated females

<table>
<thead>
<tr>
<th>Line</th>
<th>Unmated</th>
<th>Mated</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.2 ± 0.4</td>
<td>531.0 ± 68.8</td>
</tr>
<tr>
<td>4</td>
<td>31.2 ± 34.3</td>
<td>854.0 ± 137.0</td>
</tr>
<tr>
<td>5</td>
<td>5.0 ± 6.4</td>
<td>655.0 ± 90.1</td>
</tr>
<tr>
<td>6</td>
<td>44.6 ± 22.9</td>
<td>582.0 ± 198.0</td>
</tr>
<tr>
<td>9</td>
<td>73.4 ± 70.4</td>
<td>679.0 ± 195.7</td>
</tr>
<tr>
<td>10</td>
<td>38.4 ± 37.6</td>
<td>814.0 ± 183.9</td>
</tr>
<tr>
<td>11</td>
<td>46.2 ± 24.8</td>
<td>702.0 ± 223.7</td>
</tr>
<tr>
<td>13</td>
<td>408.0 ± 282.3</td>
<td>1264.0 ± 100.3</td>
</tr>
<tr>
<td>15</td>
<td>1.6 ± 3.6</td>
<td>505.0 ± 200.7</td>
</tr>
<tr>
<td>17</td>
<td>0.0 ± 0.0</td>
<td>558.0 ± 118.8</td>
</tr>
<tr>
<td>19</td>
<td>4.2 ± 4.2</td>
<td>853.0 ± 139.7</td>
</tr>
<tr>
<td>21</td>
<td>7.8 ± 3.6</td>
<td>538.0 ± 109.4</td>
</tr>
<tr>
<td>22</td>
<td>0.6 ± 1.3</td>
<td>536.0 ± 45.5</td>
</tr>
<tr>
<td>24</td>
<td>0.0 ± 0.0</td>
<td>588.0 ± 127.8</td>
</tr>
<tr>
<td>25</td>
<td>10.0 ± 8.5</td>
<td>624.0 ± 158.8</td>
</tr>
<tr>
<td>27</td>
<td>85.6 ± 73.3</td>
<td>736.0 ± 80.9</td>
</tr>
</tbody>
</table>
present experiments were 4 days old and matured to oviposit (Table 3). D. melanogaster females exhibit gregarious oviposition behavior (del Solar and Palomino, 1966; del Solar and Ruiz, 1992). As there seemed to be sufficient food for ovipositing females under our experimental conditions, many of the mated females may have stayed at a place suitable for oviposition. However, as there was no significant negative correlation between emigration activity and number of eggs oviposited by mated females, gregarious oviposition behavior may not be related to the decrease of emigration activity in the mated females. 3) The presence of an aggregation pheromone is known in D. melanogaster (Bartelt et al., 1985). It is located in the male reproductive tract, is transferred to females during copulation, and females would probably emit it on the surface of food. The material emitted on the food would act as a co-tractant of food odors. The flies would aggregate preferentially at sites where the pheromone was emitted. Therefore, the influence of the pheromone may not be negligible in emigration response behavior. We need further experiments to examine the effect of the aggregation pheromone with respect to emigration response behavior. In the second group, comprised of lines 18 and 19, the reverse was observed; i.e., emigration activities were greater in the mated females than in the unmated ones, although there were no significant differences in the mean emigration activity between the mated and unmated females. The unmated females of the former group had a stronger tendency to move from their original place to a new one, probably to search for a mate. Those of the latter seemed to be sedentary rather than vagile. These results show that different adaptive features may operate among the unmated females in a natural population of D. melanogaster.

The genetic variability of emigration activity was significantly higher in unmated than in mated females. Different adaptive features would operate between unmated and mated females in the natural populations. Because molecules such as sex-peptides and others which are transmitted from male to female during copulation influence oviposition and remating of mated females (Chen and Bühlcr, 1970; Aigaki et al., 1991; Fuyama and Ueyama, 1997; Wolfner, 1997), I speculate that these molecules may mediate the decrease of emigration activity in mated females. Although it was suggested that the emigration activities of the sexes were under different genetic control (Mikasa, 1992), the factors derived from males from copulation may not be ignored with respect to the variability of emigration activity in the mated females. In this study, the females of every isofemale line in the mated group were mated with males of their own line to compare previous experimental results. The genetic variability in the mated female group seems to include variability of factors derived from males. However, the obtained genetic variability in the present study may be one of the indices reflecting that of natural populations of mated females.

The effect of mating on emigration activity (Rm) did not phenotypically correlate with the emigration activity of the virgin females (Fig. 2). These results may suggest that the effect of mating on emigration activity and the emigration activity of the unmated females would be under different genetic controls.

The experimental populations for the examination of emigration activities in previous studies consisted of equal numbers of sexes (50 males and 50 females). The percentages of genetic variance in emigration activity to the total variance were estimated in the female flies to be 33.0% (Mikasa, 1992) and 49.8% (calculated from the data of Mikasa and Narise [1986]). Those values and the values in the present study (27.4 and 53.0%) seemed to be within the same range, although different experimental conditions and different natural populations were used and the experimental data were too small for comparison.

The isofemale lines were maintained under conditions whereby the populations were small. These maintenance procedures would cause inbreeding and random genetic drift in each isofemale line (Hoffmann and Parsons, 1988). Inbreeding and random drift might cause an increase in divergence among isofemale lines. The amount of expressed genotypic variations for emigration activity in a natural population may thus be overestimated in the present study.

In the absence of mating, some virgin females of D. melanogaster can delay oviposition for several days under laboratory conditions (Merle and David, 1967). A great deal of variability in the duration of this retention period is revealed among the virgin females of temperate populations (Boulétreau-Merle, 1982). The delay ranges from 2 to 25 days.

Under natural conditions, most of the unmated females will move to another location, probably to search for a mate if conditions are such that males cannot be found nearby. A small portion of the unmated females will remain at a suitable place to wait for the arrival of male flies. On the other hand, the mated females may stay at a suitable place to oviposit.

I am grateful to T. Narise, Meikai University, and anonymous referees for their valuable suggestions and comments.

REFERENCES


Sakai, K. I., Narise, T., Hiraizumi, Y., and Iyama, S. (1958) Studies on competition in plants and animals. IX. Experimental studies on migration in Drosophila melanogaster. Evolution

