# MATING SPEED AND DURATION OF COPULATION IN *DROSOPHILA PSEUDOOBSCURA*

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## 1. INTRODUCTION

Spiess and Langer (1964*a*) found mating speed differences for different homokaryotypes in *D. pseudoobscura* such that AR karyotypes mated the most rapidly, followed in order by ST, CH, TL and PP. Kaul and Parsons (1965) found the karyotype of the male to be of critical importance in determining both mating speed and duration of copulation for all possible combinations between the three karyotypes ST/ST, ST/CH and CH/CH. For mating speeds in the males ST/ST was fastest, followed by ST/CH and CH/CH, while durations of copulation were in the reverse order. Because of the large difference found by Spiess and Langer (1964*a*) between AR and PP homokaryotypes, it was decided to study mating speeds and durations of copulation for all possible combinations between AR/AR, AR/PP and PP/PP karyotypes at two temperatures, 20° C. and 25° C.

## 2. METHOD

The technique was essentially the same as that used by Kaul and Parsons (1965). After separation at eclosion, flies were stored singly in vials. At 4 days a single male was shaken into a vial with a single female and observed until copulation began. The time in minutes for this to occur is the mating speed. The recording of the duration of copulation followed immediately after. Any pairs not mating in $\leqslant 30$ minutes were recorded as unmated.

The larvae used for the mating experiments were cultured at a density of 40 larvae per vial at 20° C. and 25° C. respectively.

Fifty trials split into two replicates of 25 were carried out for each of the 9 possible karyotypic combinations for flies at both temperatures.

## 3. RESULTS

In table 1 the mean numbers mating out of 25 for the two temperatures in 5 minutes are given. The following observations can be made directly on the table:

(1) Fewer matings occurred at 25° C. than at 20° C. This temperature difference is significant (table 2) and is maintained at 30 minutes.

(2) The marginal means show that the karyotype of the females is more important than that of the males in determining the mating frequency especially at 25° C. Analyses of variance for the two temperatures (tables 2 and 3) confirm this, and although there is a significant effect due to the karyotype of

the males at 20° C. (table 3), it is far smaller than that due to the karyotype of the females.

(3) The marginal means for the karyotype of the females show that the main difference between the two temperatures is a low PP/PP mating frequency at 25° C. compared with 20° C., and

TABLE 1

*Mean number of matings out of 25 in 5 minutes for AR/AR, AR/PP and PP/PP karyotypes at 20° C. and 25° C.*

| 20° C. Male karyotypes | | AR/AR | AR/PP | PP/PP | |
|---|---|---|---|---|---|
| Female karyotypes | AR/AR | 11 | 12 | 7·5 | 10·17 |
| | AR/PP | 13·5 | 17·5 | 17·5 | 16·17 |
| | PP/PP | 12·5 | 21 | 21·5 | 18·33 |
| | | 12·33 | 16·83 | 15·5 | 14·89 |

| 25° C. Male karyotypes | | AR/AR | AR/PP | PP/PP | |
|---|---|---|---|---|---|
| Female karyotypes | AR/AR | 7 | 10·5 | 7·5 | 8·33 |
| | AR/PP | 17 | 16 | 18·5 | 17·17 |
| | PP/PP | 5·5 | 10·5 | 8 | 8 |
| | | 9·83 | 12·33 | 11·33 | 11·17 |

TABLE 2

*Analysis of variance of the number mating out of 25 in 5 minutes (after applying the angular transformation)*

| Source of variation | d.f. | m.s. | F | Probability |
|---|---|---|---|---|
| Female karyotypes | 2 | 929·55 | 28·08 | $<0·001$ |
| Male karotypes | 2 | 241·43 | 7·29 | $<0·05$ |
| Temperatures | 1 | 782·51 | 23·64 | $<0·001$ |
| Females × males | 4 | 122·36 | 3·70 | $<0·05$ |
| Females × temperatures | 2 | 658·48 | 19·89 | $<0·001$ |
| Males × temperatures | 2 | 20·36 | 0·61 | |
| Females × males × temperatures | 4 | 52·25 | 1·58 | |
| Error | 18 | 33·11 | | |

a somewhat lower AR/AR frequency at 25° C. than at 20° C. Hence there is strong heterokaryotype advantage for females at 25° C. but not at 20° C. It is not therefore surprising that there is a significant female × temperature interaction (table 2).

These various trends still occur for the numbers mating out of 25 in 30 minutes.

In table 4 are the mean durations of copulation. The entries in the table are based on 22 observations, since there were 22 observations for the combination of PP/PP ♀×AR/AR ♂ at 25° C. in 30 minutes and more for all other contrasts. The excess over 22 for the other

TABLE 3

*Analysis of variance of the number mating out of 25 in 5 minutes at 20° C. and 25° C. separately (after applying the angular transformation)*

| Source of variation | d.f. | 20° C. | | 25° C. | |
|---|---|---|---|---|---|
| | | m.s. | F | m.s. | F |
| Female karyotypes . | 2 | 663·10 | 17·14*** | 924·93 | 33·61*** |
| Male karyotypes . | 2 | 199·43 | 5·15* | 62·35 | 2·27 |
| Males × females . | 4 | 132·35 | 3·42 | 42·26 | 1·54 |
| Error . . . | 9 | 38·7 | | 27·5 | |

*P<0.05 ***P<0.001

TABLE 4

*Mean durations of copulation (minutes) at 20° C. and 25° C.*

| 20° C. Karyotype of males | AR/AR | AR/PP | PP/PP | |
|---|---|---|---|---|
| Karyotype of females AR/AR | 4·05 | 4·27 | 4·00 | 4·11 |
| AR/PP | 4·07 | 4·30 | 3·98 | 4·11 |
| PP/PP | 4·34 | 4·61 | 3·75 | 4·23 |
| | 4·15 | 4·39 | 3·91 | 4·15 |

| 25° C. Karyotypes of males | AR/AR | AR/PP | PP/PP | |
|---|---|---|---|---|
| Karyotypes of females AR/AR | 6·00 | 4·34 | 4·05 | 4·80 |
| AR/PP | 4·93 | 4·50 | 5·57 | 5·00 |
| PP/PP | 4·80 | 4·70 | 4·59 | 4·70 |
| | 5·24 | 4·52 | 4·73 | 4·83 |

contrasts was removed by a randomisation procedure. The following observations can be made:

(1) The duration of copulation is greater at 25° C. than at 20° C. This is confirmed by an analysis of variance (table 5).

(2) At both temperatures the marginal means for the karyotype of the males are more variable than for females as is confirmed by the larger F values for male karyotypes in tables 5 and 6.

The male karyotype PP/PP has a shorter duration of copulation than AR/AR at both temperatures.

(3) The marginal means for the karyotype of the males show that the main difference between temperatures is that the durations of copulation of the homokaryotypes are increased relatively more than the heterokaryotype at 25° C., so that at 25° C. the heterokaryotype has the shortest duration of copulation. This explains the significant male × temperature interaction in table 5. A final distinction between temperatures is a significant female × male interaction at 25° C. but not at 20° C.

TABLE 5

*Analysis of variance of durations of copulation*

| Source of variation | d.f. | m.s. | F | Probability |
|---|---|---|---|---|
| Female karyotypes | 2 | 0·43 | 0·22 | |
| Male karyotypes | 2 | 4·77 | 2·41 | |
| Temperatures | 1 | 45·68 | 23·04 | <0·001 |
| Females × males | 4 | 5·77 | 2·91 | <0·05 |
| Females × temperatures | 2 | 1·49 | 0·75 | |
| Males × temperatures | 2 | 8·29 | 4·18 | <0·05 |
| Females × males × temperatures | 4 | 5·88 | 2·96 | <0·05 |
| Error | 378 | 1·98 | | |

TABLE 6

*Analysis of variance of durations of copulation at 20° C. and 25° C. separately*

| Source of variation | d.f. | 20° C. | | 25° C. | |
|---|---|---|---|---|---|
| | | m.s. | F | m.s. | F |
| Female karyotypes | 2 | 0·34 | 0·22 | 1·58 | 0·66 |
| Male karyotypes | 2 | 3·88 | 2·45 | 9·18 | 3·85* |
| Females × males | 4 | 0·73 | 0·46 | 10·91 | 4·58** |
| Error | 189 | 1·58 | | 2·38 | |

* P<0·05 ** P<0·01

## 4. DISCUSSION

In our experiments PP/PP did not mate slower than AR/AR as found by Spiess and Langer (1964*a*). This could be due to variations in genetic background or to differences in experimental technique, since Spiess and Langer used a mating chamber with 10 pairs of flies for their experiments, and aged their flies for 6 days prior to mating at 15° C., compared with the single pairs, aged apart for 4 days prior to mating in our experiments. Furthermore, their flies were all raised and tested at 25° C. It is clear from these and other experiments that environmental variations will lead to large differences for behavioural characteristics.

The importance of the karyotype of the female in determining mating frequency contrasts with the results of Kaul and Parsons (1965) who found the karyotype of the male to be of critical importance. However, Spiess and Langer (1964*b*) found that the karyotype of the female was more important in determining mating speed over a one hour period in *D. persimilis*. In *D. melanogaster* Parsons (1965) found the genotype of the male to be more important for various combinations between inbred lines over a one hour period, although several experiments have shown that the female becomes progressively more important with time (Parsons, unpublished). If mating is to be regarded as an interaction between the copulation tendency of males and avoidance tendency of females (Fuller and Thompson, 1960), one might expect according to the intensities of these opposing tendencies, that the male would be more important in certain genotypic combinations and the female in others.

For duration of copulation, the karyotype of the male is clearly more important than that of the female in agreement with Kaul and Parsons (1965). It is unlikely that copulation would cease before the sperm is transferred. This is presumably mainly male determined, so it is not unreasonable for the duration of copulation to be in general determined by the karyotype of the male.

The temperature 25° C. is generally less favourable for mating than 20° C. since the total mating frequency over 30 minutes is lower at 25° C. This is reasonable as 25° C. is close to the upper limit at which *D. pseudoobscura* can be maintained. In females, which is the sex important in determining mating frequency in these experiments, the fall in frequency affects the homokaryotypes only leading to the development of extreme heterokaryotype advantage.

If a short duration of copulation is selectively advantageous, which seems reasonable as those pairs completing copulation the most rapidly will leave genes in subsequent generations, then 25° C. is less favourable than 20° C. for this trait. The significant female × male interaction at 25° C. is perhaps indicative of a breakdown of strict male determination expected for the duration of copulation, and may indicate a reduction in the efficiency of copulation. Furthermore, in males, which is the sex important in determining duration of copulation, the increase in duration affects the homokaryotypes much more than the heterokaryotypes, leading to slight heterokaryotype advantage at 25° C.

Thus, considering the sex important in controlling mating frequency and duration of copulation, the heterokaryotypes vary less between temperatures than the homokaryotypes, *i.e.* the heterokaryotypes show a higher degree of homeostasis than the homokaryotypes. The differential effect of temperatures on the heterokaryotypes and homokaryotypes helps to explain the significant genotype × environmental interactions found in tables 2 and 5.

Somewhat analogous results were found by Parsons (1959) in a

study of larval survival of some inbred lines and their $F_1$'s in *D. melanogaster* under 6 different temperature regimes. The $F_1$'s were found to be substantially less variable between temperatures than the inbred lines, showing therefore more homeostasis in their adaptation to temperature and, as expected, the genotype × temperature interactions were smaller for the former. Among several other papers indicating the same general type of result is that of Dobzhansky and Levene (1955) on *D. pseudoobscura*, who showed that the viabilities of homozygotes of 19 different second chromosomes are more variable under a series of environments than the corresponding heterozygotes.

For mating frequency this heterokaryotypic stability between temperatures leads to extreme heterosis. Heterosis at extreme temperatures has been demonstrated several times for various fitness factors, *e.g.* larval survival in *D. melanogaster* (Parsons, 1959), relative viability in *D. pseudoobscura* (Dobzhansky, *et al.*, 1955) and growth rates in *Arabidopsis thaliana* (Langridge, 1962). This and other evidence is discussed by Langridge (1962) who maintains that heterosis under these circumstances is due to recessive, temperature-sensitive alleles occurring in natural populations which therefore lead to temperature sensitivity in homozygotes rather than in heterozygotes, although this seems to be an oversimplification.

This paper and Kaul and Parsons (1965) show that the two behavioural traits under investigation have properties similar to the various components of fitness more classically studied in *D. pseudoobscura* (*e.g.* Dobzhansky, 1957). Furthermore in *D. melanogaster* both these traits show reasonably high heritabilities (Parsons, 1964; MacBean and Parsons, unpublished), as do other behavioural traits in other organisms (Broadhurst, 1960; Broadhurst and Jinks, 1963). The difficulty in studying behaviour often revolves around finding an objective measure of the trait under investigation. This is perhaps one reason why the study of behavioural traits has lagged until recently. Another reason is that such traits are generally even more remote from the primary gene products than the quantitative morphological traits usually studied with the methods of biometrical genetics, which means that there may be frequent opportunities for large genotype environmental interactions.

## 5. SUMMARY

1. The karyotype of the female is of predominant importance in the determination of mating frequency for all combinations between the karyotypes AR/AR, AR/PP and PP/PP at 20° C. and 25° C., although the karyotype of the male has some effect at 20° C.

2. Duration of copulation is determined mainly by the karyotype of the male.

3. The temperature 25° C., which is extreme for *D. pseudoobscura*, reduces the mating frequency and lengthens the duration of copulation for the homokaryotypes with little effect on the heterokaryotypes so

that, in agreement with work on other fitness factors, the heterokaryotypes show more homeostasis between environments than the homokaryotypes.

4. There is extreme heterokaryotype advantage for mating frequency at 25° C. in agreement with other fitness factors at extreme temperatures.

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