DIRECTIONAL SELECTION FOR DURATION OF COPULATION IN *DROSOPHILA MELANOGASTER*[1]

IAN T. MACBEAN[2] AND P. A. PARSONS

School of Biological Sciences, La Trobe University, Victoria, Australia

Received December 12, 1966

A quantitative trait having some genetic basis should respond to selection, since by selecting extreme phenotypes, extreme genotypes will be selected. Initially, the magnitude of the response to selection will depend on the heritability of the trait and its selection differential, which is itself dependent on the proportion of the population selected and the standard deviation of the trait. In time, the rate of response to selection may be expected to diminish as genetic variability is lost (FALCONER 1960). However, rapid or "accelerated" responses to selection may occur after some generations at a plateau, and these may be due to recombination producing extreme gametes, and hence genotypes leading to extreme phenotypic expression of the trait under selection (BODMER and PARSONS 1962).

Artificial directional selection has been successful for several behavioral traits, for example defecation scores in rats (BROADHURST 1960), geotaxis (HIRSCH 1962; DOBZHANSKY and SPASSKY 1962) and mating speed in Drosophila (MANNING 1961, 1963). Further discussions of these and other traits appear in PARSONS (1967).

In this paper the results of a directional selection experiment for duration of copulation in *D. melanogaster* will be discussed. Evidence for the genetic control of this trait in *D. melanogaster* has been given by MERRELL (1949) and HILDRETH (1962) who found differences between strains, and by HOSGOOD and PARSONS (1965) who found differences between strains derived from single inseminated females taken from natural populations. A recent experiment based on inbred strains and their hybrids gave a heritability in the region of 0.15 to 0.20 (MACBEAN and PARSONS 1966). In *D. pseudoobscura* differences for duration of copulation, mainly controlled by the male, have been found between various karyotypes (KAUL and PARSONS 1965). Thus there is quite an amount of evidence for its genetic control, even though the heritability quoted is quite low. It was therefore decided to carry out directional selection for the trait in an attempt to assess its genetic basis more fully.

MATERIALS AND METHODS

The experimental testing procedure followed PARSONS (1964). Pairs of virgin flies aged about seven days were put together in vials without etherization and observed for 50 minutes. Pairs

[1] Supported in part by a grant from the Australian Research Grants Committee.

[2] Supported by a Commonwealth Post-Graduate Award held at the University of Melbourne.

not mating within 50 minutes were recorded as unmated. Mating speed, which is the time until mating commences, and duration of copulation, which is the period between the time when the genitalia are first observed to lock until they disengage, were recorded in minutes. All flies were grown and tested at 25°C.

The base population was the sixth laboratory generation derived from a single inseminated female captured in the wild at Rushworth, Victoria. Two control (C), high (H) and low (L) lines were set up in the following way using five pairs of flies in ½-pint milk bottles for each line. In the first generation the two control lines were formed by selecting five pairs of flies per line at random from 81 copulations, and the high and low lines were then set up by selecting pairs with high and low durations of copulation .The extreme five pairs selected in either direction were the series 1 lines, and the next most extreme five pairs the series 2 lines. The series 2 lines were set up for emergency purposes. In subsequent generations 50 pairs of flies per line were tested and the selection of the C, H and L lines was carried out as above. Whenever possible, all flies for a given generation were taken from the series 1 lines, but occasionally some series 2 flies had to be used. In any case, variation in the number of successful copulations, which determines the population size in a generation, prevents the maintenance of an absolutely constant selection differential.

RESULTS

Response to selection: The mean durations of copulation for the low and high lines over 20 generations of selection and the control line are shown in Figure 1. Initially, there was a general reduction in mean duration for all lines (Figure 1). Two causes come to mind. First, there is the possibility of an inbreeding effect imposed by the experimental design. Secondly, it could be due to adaptation to laboratory conditions. The second interpretation finds support from data of S. M. W. Hosgood (personal communication) which show a 5 to 8 minute reduction between the fourth and 14th laboratory generations for four *D. melanogaster*

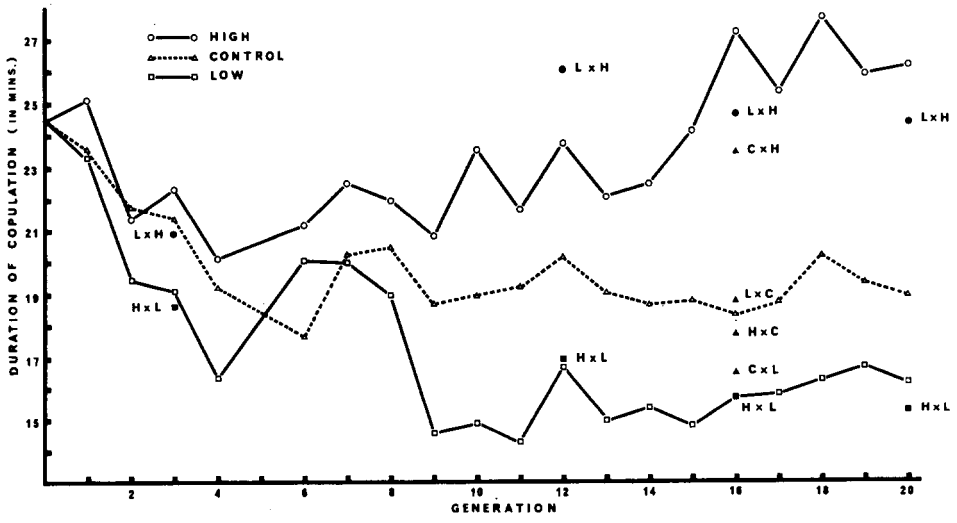

FIGURE 1.—Response to selection for high and low duration of copulation (minutes). No points appear for generation 5, which was untested (see text). At generations 3, 12, 16, and 20 various crosses between the selection lines (H and L) and controls (C) were made. In each cross the female is written first.

strains collected in the wild. Some fluctuations in the early generations may be due to a relaxation of selection at generation 5, which unfortunately could not be tested, and consequently parents of generation 6 were randomly selected from the nonvirgin generation-5 population.

In the low line, a response was obtained by generation 9, after which there was little change. The greatest variance within the low line was observed at generation 8, immediately before the large drop in mean duration at generation 9. In the high line there was a gradual response to generation 13, an accelerated response from generation 13 to 16, followed by some minor fluctuations. Again, the greatest variance within the high line was observed at generation 13, which was just before the accelerated response to generation 16. Recombination, as discussed in the introduction, is a possible explanation for the greater variance preceding these rapid responses. The divergence between the high and low lines increased from over 6 minutes at generation 9 when it first appeared obvious, to over 10 minutes at generation 20. Differences between the selected line means and those of the control line were significant from generation 6 onwards in the high line and from generation 9 onwards in the low line.

The percentage mating within the 50 minute observation period (Figure 2) is high in all lines. This is no doubt maintained by a weak secondary selection pressure imposed by the use of only those flies that mate within the limited observation period to give parents of the next generation. No associations between selection progress for duration of copulation and the percentage mating are apparent. The lines all behave in a similar manner from generation to generation for percentage mating, showing the importance of environmental variations between generations. Further work on environmental factors is currently in progress. In addition, further work on other traits which may be associated with duration of copulation is under way, and will be reported at a later stage. Even so, it is already clear that the reproductive success of both of the selection lines has fallen.

Copulations between lines: Copulations were observed reciprocally between the

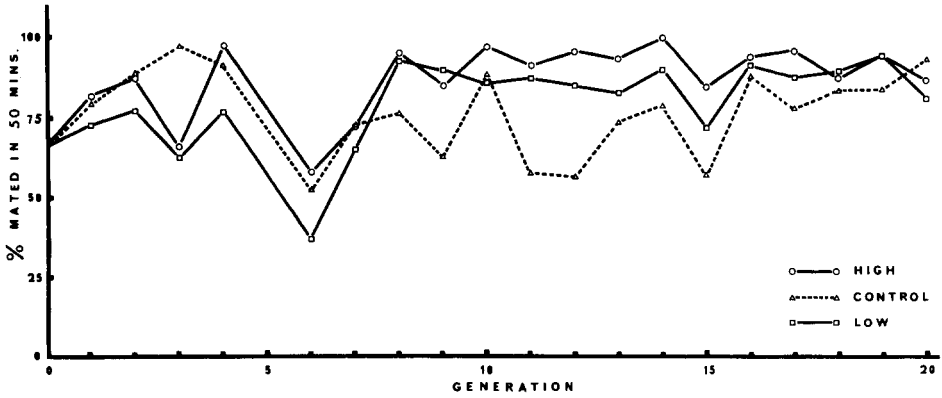

FIGURE 2.—Percentage mated within 50 minutes for the three lines given in Figure 1.

TABLE 1

Analysis of variance of the diallel cross between the three lines at generation 16

Source of variation	df	Mean square	Variance ratio
Males	2	1,647.49	184.37***
Females	2	14.97	1.68
Male × female interaction	4	47.31	5.29***
Error	216	8.94	

*** Significant at $P < 0.001$.

high and low lines at generations 3, 12, 16, and 20. The mean durations are plotted on Figure 1. Highly significant differences between reciprocal pairs of means were obtained, such that the duration followed the genotype of the male almost entirely. This agrees with data in *D. pseudoobscura* (KAUL and PARSONS 1965; PARSONS and KAUL 1966).

At generation 16, all possible combinations were observed between the high, low and control lines, making 9 combinations including the lines themselves. The data thus form a 3 × 3 diallel cross. An analysis of variance was carried out on these data based on 25 observations for each combination (Table 1). There is a highly significant male effect as expected, and the female effect is insignificant, although there is a significant male × female interaction which is very small compared with the male effect. The diallel cross therefore strikingly confirms the significance of the divergence between lines, and the importance of the genotype of the male for all three lines.

Variation in the pretransmission period: V. A. STRANGIO (personal communication) noticed fertility differences as assessed by the presence or absence of progeny, between mutant strains in *D. melanogaster* subjected to interrupted copulations, presumably because the males of some strains transfer sperm earlier than others. A preliminary test on the selection lines was carried out at generation 14 using his technique of interrupting copulation by sharply shaking the vials containing a copulating pair of flies after certain time intervals following the

TABLE 2

Percentage fertility, as assessed by the presence or absence of progeny, for interrupted copulations at generation 15

Duration of copulation prior to interruption in minutes	Line					
	Low		Control		High	
	n	Percent fertile	*n*	Percent fertile	*n*	Percent fertile
3	17	0	16	0	13	0
4½	15	40	16	0	16	0
6	16	69	14	0	14	0
7½	15	93	15	27	14	21
9	15	87	16	75	14	43

n is the number of copulations successfully interrupted.

commencement of copulation. Twice as many fertile matings, as assessed by the presence or absence of progeny, were observed for low line copulations interrupted at 6 minutes than for the control and high lines, while all lines showed high fertility percentages when interrupted at 9 minutes.

This was further tested at generation 15 where pairs of flies were interrupted after five different time intervals (Table 2). Fertility in the low line was observed for some of the copulations interrupted at 4½ and 6 minutes, but not for the control and high lines. Thus the time before sperm is transferred (the pretransmission period) has been changed as a correlated response to selection for reduced duration of copulation.

Some fertile matings occurred in the control and high lines for copulations interrupted at 7½ and 9 minutes, so that on these data there is little difference between the control and high lines, although the percentage fertility is somewhat higher in the control lines. It therefore seems that the response to selection in the high line involves either an addition to the transmission period itself, or to a post-transmission period, or both.

DISCUSSION

The rate of response to selection is not rapid. However this is to be expected since the heritability of duration of copulation is not high, as discussed in the introduction. Even so, different species of Drosophila show characteristic durations of copulation, although different genotypes within species show variations, as already discussed. In *D. melanogaster*, the duration of copulation is commonly between 15 and 25 minutes (MACBEAN and PARSONS 1966), while in *D. pseudoobscura* it is rarely greater than 10 minutes (KAUL and PARSONS 1965; PARSONS and KAUL 1966). In a pair of South American sibling species, *D. gaucha* and *D. pavani*, which cross freely in the laboratory, the mean duration was found to be 31 minutes in *D. gaucha* and 50 minutes in *D. pavani* (LAMBOROT and KOREF-SANTIBAÑEZ 1964). Observations on many Drosophila species are given by SPIETH (1952), who regards duration of copulation as a characteristic that differentiates species.

In crosses between the three lines, duration of copulation is almost entirely male determined in agreement with other evidence cited. Even so, it should be pointed out that there are some movements of the female near the end of copulation in *D. paulistorum* (EHRMAN 1964) and *D. pavani* (KOREF-SANTIBAÑEZ 1963), which appears to indicate that the female may be of importance in initiating separation. Perhaps this is tied up in some way with the time of completion of sperm transfer, at which stage the female shows the first visible reaction. For mating speed, however, male determination does not always occur. In *D. melanogaster* PARSONS (1965) suggested that the genotype of the female increases in importance with time. If mating is considered as an interaction between the copulatory tendency of males and the avoidance tendency of females, it may be expected, according to the intensities of these opposing tendencies, that the male would be more important in certain genotypic combinations and the female in

others. (See KAUL and PARSONS [1965] for a discussion of mating speed in other species of Drosophila.) In the case of duration of copulation, however, because it is almost entirely male determined, the male is clearly active and the female passive. In any case, it is unlikely that copulation would cease before sperm transmission is completed. From this point of view, further work on the pretransmission period, the transmission period, and the possible posttransmission period will be of interest, and would permit a greater understanding of the duration of copulation. Finally, a recent experiment in *D. melanogaster* shows the very great importance of the genotype of males in determining the copulation frequency of a male placed with six females over a 12 hour period. The proportion of females inseminated represents total sexual activity and clearly incorporates mating speed and duration of copulation in successive matings of the same male (FULKER 1966). In this connection, the result of KAUL and PARSONS (1965) in *D. pseudoobscura*, who studied mating speed and duration of copulation for the karyotypes ST/ST, ST/CH and CH/CH (ST = Standard and CH = Chiricahua gene arrangements) is of interest, since they found heterokaryotype advantage controlled by the male for the sum of mating speed plus duration of copulation, but not for these two variables taken in isolation. Clearly the sum of the variables is more closely correlated with total sexual activity than the variables taken alone, as was pointed out by KAUL and PARSONS (1965). In any case far more work is needed to assess the biological significance of duration of copulation.

Duration of copulation seems to be less sensitive to environmental variation than mating speed in *D. melanogaster* (MACBEAN unpublished). This means that it could be a useful trait for further genetic analysis in attempts to locate genetic activity to chromosomes and even to regions of chromosomes (see THODAY 1961; BODMER and PARSONS 1962). Perhaps the only behavioral trait for which genetic activity has been localized to chromosomes is geotaxis in *D. melanogaster* (HIRSCH and ERLENMEYER-KIMLING 1962), if we exclude associations with karyotypes, as found for example in *D. pseudoobscura* for geotaxis (DOBZHANSKY and SPASSKY 1962) and for mating speed and duration of copulation (PARSONS and KAUL 1966; SPIESS and LANGER 1964). For duration of copulation there would be some complications because it is sex-limited to the male, however, it would be a far simpler trait to handle from the point of view of genetic analysis than mating speed, which is determined more by an interaction between the sexes.

The experimental work was carried out at the Department of Genetics, University of Melbourne. We wish to thank certain people of that Department for help in various ways, namely PROFESSOR M. J. D. WHITE for his support, DR. V. A. STRANGIO and MISS S. M .W. HOSGOOD for valuable discussions and permission to quote unpublished results, DR. B. T. O. LEE for helpful discussions, and MISS H. GIDLEY for competent technical assistance.

SUMMARY

Selection for high and low duration of copulation has been carried out with responses in both directions. In agreement with work on unselected Drosophila stocks, the duration of copulation was found to be male-determined. A correlated reduction of the period before sperm was transferred (the pretransmission period) was obtained in the line selected for low duration of copulation.

LITERATURE CITED

BODMER, W. F., and P. A. PARSONS, 1962 Linkage and recombination in evolution. Advan. Genet. **11**: 1–100.

BROADHURST, P. L., 1960 Experiments in psychogenetics. Applications of biometrical genetics to the inheritance of behaviour. pp. 1–102. *Experiments in Personality*. Vol. I. Edited by H. J. EYSENCK. Routledge and Kegan Paul, London.

DOBZHANSKY, TH., and B. SPASSKY, 1962 Selection for geotaxis in monomorphic and polymorphic populations of *Drosophila pseudoobscura*. Proc. Natl. Acad. Sci. U.S. **48**: 1704–1712.

EHRMAN, L., 1964 Courtship and mating behavior as a reproductive isolating mechanism in Drosophila. Am. Zoologist **4**: 147–153.

FALCONER, D. S., 1960 *Introduction to Quantitative Genetics*. Oliver and Boyd, Edinburgh.

FULKER, D. W., 1966 Mating speed in male *Drosophila melanogaster*: a psychogenetic analysis. Science **153**: 203–205.

HILDRETH, P. E., 1962 Quantitative aspects of mating behavior in Drosophila. Behaviour **19**: 57–73.

HIRSCH, J., 1962 Individual differences in behavior and their genetic basis. pp. 3–23. *Roots of Behavior*. Edited by E. L. BLISS. Harper, New York.

HIRSCH, J., and L. ERLENMEYER-KIMLING, 1962 Studies in experimental behavior genetics: IV. Chromosome analyses for geotaxis. J. Comp. Physiol. Psychol. **55**: 732–739.

HOSGOOD, S. M. W., and P. A. PARSONS, 1965 Mating speed differences between Australian populations of *Drosophila melanogaster*. Genetica **36**: 260–266.

KAUL, D., and P. A. PARSONS, 1965 The genotypic control of mating speed and duration of copulation in *Drosophila pseudoobscura*. Heredity **20**: 381–392.

KOREF-SANTIBAÑEZ, S., 1963 Courtship and sexual isolation in five species of the Mesophragmatica group of the genus Drosophila. Evolution **17**: 99–106.

LAMBOROT, M., and S. KOREF-SANTIBAÑEZ, 1964 Actividad sexual de *Drosophila pavani* y *Drosophila gaucha*. Biológica (Chile) **36**: 17–26.

MACBEAN, I. T., and P. A. PARSONS, 1966 The genotypic control of the duration of copulation in *Drosophila melanogaster*. Experientia **22**: 101–102.

MANNING, A., 1961 The effects of artificial selection for mating speed in *Drosophila melanogaster*. Anim. Behaviour **9**: 82–92. —— 1963. Selection for mating speed in *Drosophila melanogaster* based on the behaviour of one sex. Anim. Behaviour **11**: 116–120.

MERRELL, D. J., 1949 Mating between two strains of *Drosophila melanogaster*. Evolution 3: 266–268.

PARSONS, P. A., 1964 A diallel cross for mating speeds in *Drosophila melanogaster*. Genetica **35**: 141–151. —— 1965 The determination of mating speeds in *Drosophila melanogaster* for various combinations of inbred lines. Experientia **21**: 478. —— 1967 *The Genetic Analysis of Behaviour*. Methuen, London.

PARSONS, P. A., and D. KAUL, 1966 Mating speed and duration of copulation in *Drosophila pseudoobscura*. Heredity **21**: 219–225.

SPIESS, E. B., and B. LANGER, 1964 Mating speed control by gene arrangements in *Drosophila pseudoobscura* homokaryotypes. Proc. Natl. Acad. Sci. U.S. **51**: 1015–1019.

SPIETH, H. T., 1952 Mating behavior within the genus Drosophila (Diptera). Bull. Am. Museum Natural History **99**: 399–474.

THODAY, J. M., 1961 Location of polygenes. Nature **191**: 368–370.