

PHYSIOLOGICAL FACTORS IN THE COURTSHIP PROCESSING OF *DROSOPHILA MELANOGASTER*

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Abstract—The sexual receptivity of young virgin *Drosophila melanogaster* females develops between 24 and 48 hr from eclosion. Measurement of this process suggests that percentage receptivity (reflecting 'switch-on') and courtship duration (reflecting 'courtship processing') may not be controlled by entirely different processes. A decline in courtship duration was found to accompany the switch-on of receptivity, suggesting that the settings in the courtship processing system are under the control of the same developmental process.

Once a female has become sexually mature her courtship processing system is set to require certain quantities of courtship before acceptance occurs. This setting is stable, at least while the female is still virgin, and is correlated with a fundamental variable in the reproductive biology of the female, her fecundity.

INTRODUCTION

MANNING (1967) has argued that the sexual behaviour of *Drosophila* females is composed of two distinct processes. The first is the switch-on and switch-off of receptivity which determines whether or not the female is 'accessible' to stimulation by courtship from a male. The second process is called 'courtship summation' and reflects the 'heterogeneous summation' of courtship which occurs given that receptivity is switched on.

The existence of a second process such as this, which has quantitative as well as qualitative requirements for courtship, has also been suggested by other work (EWING, 1964; BENNET-CLARK and EWING, 1967; BURNET *et al.*, 1971).

The present paper reports experiments designed to examine the validity of the idea that a female has an intrinsic manner of processing courtship which is reflected in the lag between the onset of courtship and the commencement of copulation (courtship duration).

DEVELOPMENT OF RECEPTIVITY

The ascription of distinctness to the processes of switch-on and courtship summation by MANNING (1967) was largely based on inference from data on the development of receptivity. It was found, in *Drosophila simulans*, that percentage receptivity, reflecting switch-on, increased rather gradually over the period from 20 to 52 hr after eclosion. However, no evidence was found of a shift in the distribution of courtship times of those receptive which could be attributed to a

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corresponding gradual change in courtship duration. It was consequently concluded that the process of switch-on was not brought about by gradual reduction of the amount of courtship a female required, but was, rather, a process independent of courtship.

Since this is a crucial point in the understanding of receptivity, I determined to ascertain whether this was also true in the Pacific strain of *Drosophila melanogaster* in our laboratory.

Materials and Methods

The animals examined were from the populations, selected and wild, of the Pacific strain of *D. melanogaster* used by CONNOLLY (1966, 1968) in a selection experiment for high and low locomotor activity.

This experiment was conducted after approximately 135 generations of maintenance in Connolly's experiment, and selection on activity had been relaxed for several generations prior to this.

The three strains, *active*, *inactive*, and *control*, were tested on separate occasions but all within a period of 2 months. Each strain was reared and tested under similar conditions.

Newly emerged animals were collected and sexed throughout the day of eclosion. They were maintained in 4×1 in. vials containing culture medium seeded with live yeast, and in densities of not more than 20 animals per vial.

Proportional receptivity and courtship duration of the females were measured at various intervals after eclosion. Since different groups had hatched at different times of day it was possible to conduct the measurements in a manner which minimized the effects of any diurnal rhythm in receptivity—several groups, of different ages, being measured at the same time of day.

Males of 3 to 4 days of age were used in these measurements, and single pairs of flies were observed in the small mating cells described by COOK (1972a). Observations were terminated after 30 min if no copulation occurred, as suggested by MANNING (1967).

Results

The changes in percentage receptivity found with age are shown in Figure 1(a) for the *control* lines, and Fig. 1(b) for the two selected populations, *active* and *inactive*. All lines demonstrated a marked increase in percentage receptivity over the period of measurement, although there is variation in the rate at which the effect occurs.

Figure 2 shows the corresponding mean courtship times for the animals which were receptive. There is a marked decrease in mean courtship time with age for all strains. This decrease is an extremely rapid one, with the greatest portion of the decline occurring between 24 and 48 hr for all three strains.

There are two other outstanding features of Fig. 2. First, the points do not start at the ceiling level set by the 30 min observation period, but rather are first

seen at the 10 to 11 min level. Second, for the wild-type strain in particular, mean courtship duration is still decreasing after there is 100 per cent receptivity.

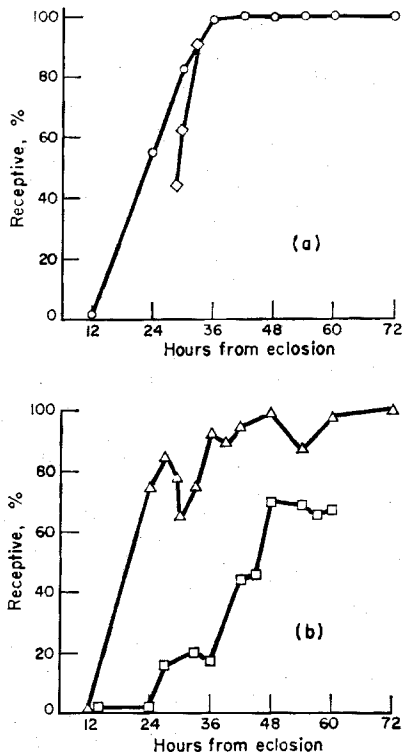


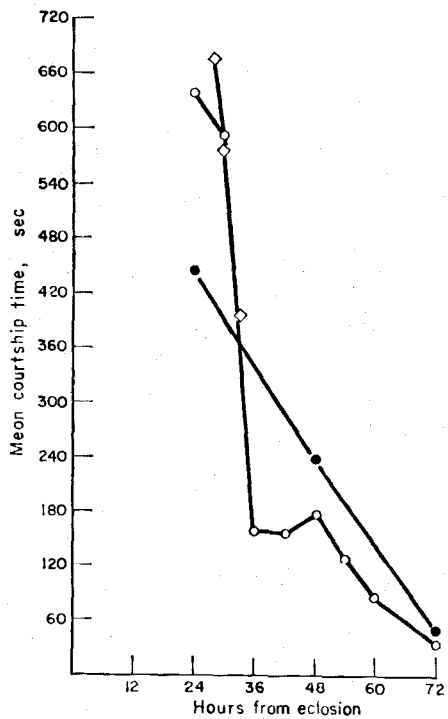
FIG. 1. Percentage receptivity of females at 12-hourly intervals from eclosion. (a) Control strain (mean n per group = 34). (b) Active (Δ) (mean n per group = 33.4) and inactive (\square) (mean n per group = 37.6).

Discussion

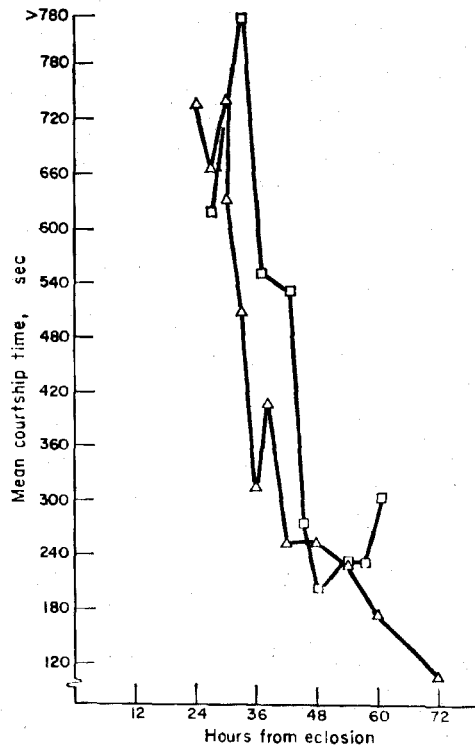
These data strongly suggest that in *D. melanogaster* the control of courtship duration may be closely connected with the systems controlling the onset of receptivity or switch-on. The changes in the two variables are clearly negatively correlated.

However, the observation that the courtship time of the 24-hr-old flies does not exceed 15 min does suggest that the range of the process underlying courtship duration is limited. In this respect the data are clearly consistent with those of MANNING (1967, Fig. 3) for *D. simulans*. The data reported here show that it is within this 15 min period that changes correlated with switch-on may occur.

Furthermore, the finding that mean courtship time continues to decrease after the achievement of total switch-on shows that the observed association of these two processes is not merely an inevitable statistical occurrence. Rather, they appear to be two manifestations of the same underlying continuous process.



(a)



(b)

FIG. 2. Mean courtship duration in receptive females at 12-hourly intervals from eclosion. (a) Control strain (●, unpublished data collected by Professor K. CONNOLLY). (b) Active (Δ) and inactive (□) strains.

TEMPORAL STABILITY OF COURTSHIP DURATION

A further derivative of the idea of courtship duration as a reflection of properties of the courtship processing system of the female is that there should be consistency in the amount of courtship required when measurements are made successively. Effectively, courtship duration, measured repeatedly in a mature female, should be a reliable phenomenon.

Materials and Methods

A technique was developed which enabled the separation of copulating pairs before insemination and consequent loss of receptivity could occur. This procedure allowed the repeated measurement of courtship duration for the same group of females, with different males on each occasion, on successive days.

The animals used in these experiments were from the Pacific strain of *D. melanogaster* reared and collected under conditions similar to those in the last experiment.

The experimental females were kept in groups until the day before the start of the experiment (day 2 from eclosion), when they were etherized and transferred to the individual maintenance assemblies described by COOK and CONNOLLY (1968).

There were slight variations in the procedure in the three replications which were conducted. The paradigm required repeated measurements of courtship duration on successive days. In the first replicate ($n = 17$) measures were made on 4 consecutive days, whereas in the further two replicates measures were made on alternate days, largely for convenience in measuring the larger numbers of females ($n = 25$ and 46 respectively). In the latter two replicates egg production was also measured.

The means of separating newly copulating pairs was adapted from MANNING (1967). As soon as a pair had copulated, the courtship cell was flooded with carbon dioxide, rendering the animals immobile immediately. A pair of fine forceps was then used to grasp the male's abdomen in such a way that the animals usually parted readily. The female was then returned to her assembly and the male discarded. Since NONIDÉZ (1920) showed that ejaculation does not occur until approximately 10 min after the start of copulation, it is unlikely that any of these females was inseminated. Subsequent tests showed no evidence of any effect of this treatment on egg production and so they may be safely regarded as 'virgins'.

Since wing vibration is known to be of great importance in *Drosophila* courtship, measures of the number of bouts and its total duration were obtained. Courtship durations were also noted.

The measurements were made in the small courtship cells used previously, the male flies being 4 to 6 days post-emergence.

Results

An overall estimate of the degree of similarity in the repeated measures in the experiment may be gained by employing Kendall's coefficient of concordance,

W (SIEGEL, 1956). Table 1 shows the results of this analysis. Variability exists between the replicates in the magnitude of the values of W and in the extent of their significance. Each of the variables measured is significant in two of the three replicates.

TABLE 1—CONCORDANCES IN COURTSHIP MEASURES OVER FOUR MEASUREMENTS OF RECEPTIVITY

	n		No. of bouts of vibration	Vibration duration	Courtship duration
Replicate 1	17	$W =$	0.40	0.42	0.34
		$X^2 =$	25.55	26.55	21.70
		$P =$	<0.05	<0.05	NS
Replicate 2	25	$W =$	0.42	0.50	0.40
		$X^2 =$	46.69	46.50	38.79
		$P =$	<0.05	<0.01	<0.05
Replicate 3	46	$W =$	0.32	0.31	0.35
		$X^2 =$	57.10	55.73	62.38
		$P =$	NS	NS	<0.05

Discussion

These experiments support the notion that courtship time and other more detailed courtship parameters are fundamentally female-controlled variables, at least in this strain of *D. melanogaster*. The repeated measures of courtship were made with a different male on each occasion, and this would be expected to introduce considerable variability into the data, which would work to reduce the concordances. Thus this is probably an underestimate of the extent to which the courtship needs of a female are reflected in her courtship time.

It should, however, be noted that the three variables measured do intercorrelate very highly (all significant positive correlations); it is impossible to assess whether these correlations are created by significant biological factors or by similarity in the measures used. Nevertheless, the fact that in two out of the three experiments the highest concordances were for vibration duration suggests that this may be the best measure of the requirements of the courtship processing system.

RECEPTIVITY AND EGG PRODUCTION

A causal association was demonstrated by MANNING (1967) of juvenile hormone activity and the switch-on of receptivity. If 'courtship summation' is controlled by the same processes as switch-on, as suggested by the first experiment reported here, then this also should be correlated with juvenile hormone activity. Evidence has been collected which is consistent with this hypothesis.

The evidence derives from an analysis of the relationship between the fecundity of a female and her subsequent receptivity in the Pacific strain of *D. melanogaster*.

The analysis has been performed twice, with similar results. The correlation of egg output with subsequent receptivity is shown in Fig. 3. In the mated animals a strong positive correlation develops as the time of measurement of courtship duration is approached. This is significantly different to the negative correlation observed in the virgins ($P < 0.01$).

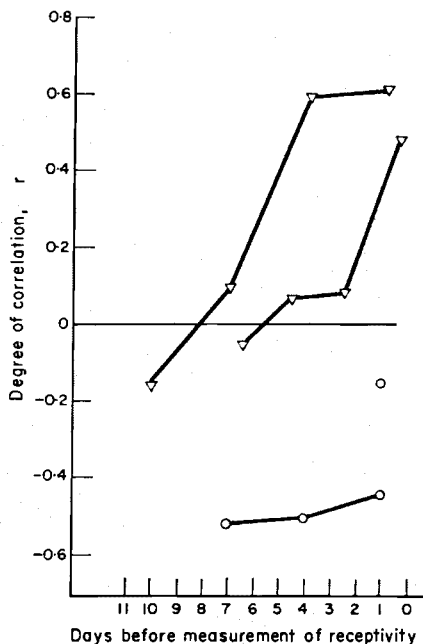


FIG. 3. Correlation of egg output on days before the measurement of receptivity with subsequent courtship duration. Δ , Inseminated females; \circ , virgin females.

If one regards the virgins as representing the basal state of the system, then the negative correlation of their fecundity with courtship duration is consistent with the hypothesis, since high titre of juvenile hormone, possibly reflected in high egg production, should lead to short courtship duration.

Nevertheless insemination, which indirectly leads to elevation of the juvenile hormone titre (MANNING, 1967), results in a reversal of the sign of the relationship. Furthermore, since no differences were found between the courtship durations of the virgin and the fertilized groups it is difficult to argue that egg laying *per se* was in any way impeding the receptivity of the inseminated females. Also, care was taken to ensure that the measurements of receptivity of the fertilized females were made *after* receptivity had switched on once more. The explanation of this change of sign with fertile mating probably lies in as yet unknown changes in courtship processing following a period of egg laying.

The fundamental negativity of the correlation has been confirmed on a strains basis by examination of the fecundity of the strains selected by COOK (1972a, b)

for receptivity to wingless males. Taking strain means as opposed to individual correlations is not an ideal method of elucidating a possible relationship, since absolute levels of variables may alter across strains independently of the standard scores on which an individual correlation is based. The technique is only likely to be valid in closely related strains such as those studied here.

The mean egg output over 4 days for virgin and fertilized females of each of the six strains is shown in Fig. 4. The open symbols represent strains selected for receptivity to wingless males and their hybrid (Selx) and the black symbols are all unselected controls.

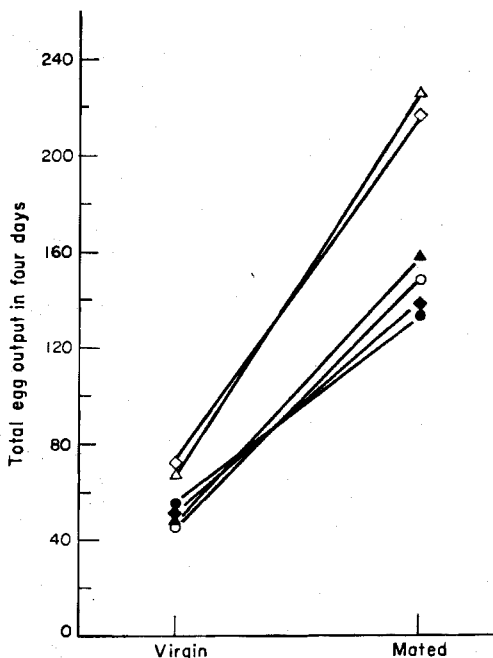


FIG. 4. Egg production over 4 days for samples of virgin and fertilized females from all strains. Δ , VBL 1; \blacktriangle , VBL 1 (control); \circ , VBL 2; \bullet , VBL 2 (control); \diamond , Selx; \blacklozenge , Contx.

Analysis of variance on these data has demonstrated the difference between the virgin and mated conditions ($P < 0.001$). Selx and VBL 1 do not differ significantly from one another, and the remaining four strains also do not differ amongst themselves. Both VBL 1 and Selx are significantly higher than the remaining four strains ($P < 0.01$). At the time when these measurements of egg production were made the receptivity of VBL 2 had declined from the peak achieved at the height of its response to selection (COOK, 1972a). The two strains most receptive to the wingless males, VBL 1 and Selx, have significantly higher egg production than their unselected controls.

DISCUSSION

The three experiments reported here support the view that the courtship duration of a female *D. melanogaster* is determined by internal factors which control the quantity and quality of the courtship necessary to enable the female to mate. These internal factors may be conveniently referred to as the courtship processing system.

It is tempting to assume from the results of the first experiment that the shift in courtship time with the onset of receptivity is undoubtedly brought about by changes in the 'setting' of the requirement for wing vibration by the courtship processing system. This is of course not necessarily the case, since other variables may influence the courtship duration. For example, CONNOLLY and COOK (1972) found that high activity was characteristic of virgin females in courtship on the day of eclosion. High activity could affect courtship duration, but there is no evidence that it did so in this experiment. Rather, CONNOLLY and COOK (1972) argued that high activity in the young females was a consequence of being courted, rather than spontaneously emitted behaviour. Hence it was regarded as a symptom, rather than a cause of unreceptivity. It is possible then that the 'setting' for the requirement for wing vibration alters gradually with the development of receptivity. This conclusion is supported by the third experiment which confirms that the courtship processing system is linked with factors in the reproductive system.

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