

# THE ATTRACTIVENESS TO MALES OF FEMALE *DROSOPHILA MELANOGASTER*: EFFECTS OF MATING, AGE AND DIET

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**Abstract.** Decapitated females were used to examine the attractiveness to males (duration of courtship) of female *Drosophila melanogaster*. Decapitated females show but few behavioural responses. The attractiveness of virgin females varied with age, being at a maximum on the day of eclosion, and declining thereafter. Even though decapitated inseminated females do not extrude their genitalia they received less courtship than decapitated virgin females. In protein-fed inseminated females attractiveness to males returned in parallel with sexual receptivity. A sucrose diet rendered both virgin and inseminated decapitated females more attractive to males, but did not prevent the change in attractiveness due to mating. The ejaculate and ovarian activation are suggested as causal factors in these processes.

Female *Drosophila melanogaster* are sometimes receptive to males, not permitting copulation even after extended periods of courtship. This may occur in four main circumstances: she may be too young (Manning 1967; Cook 1973), too old (Manning 1967), or she may be under the influence of an ejaculate, two main components of which appear to be active: male accessory gland secretion (Manning 1967; Merle 1968; Bennett et al. 1973), and the presence of sperm in the spermathecae (Manning 1962). Finally, the courtship received from the male may be inadequate, either originating from a male of a different species having different courtship parameters (Ewing & Bennett-Clark 1968), or from a conspecific with impaired courtship. Although often described as being 'passive', the female possesses a repertoire of rejection responses, some of which have deterrent effects on the courtship of the male. A recent study (Connolly & Cook 1973) has confirmed earlier suggestions that the most significant of these rejection responses is 'extrusion', described by Smith (1952). This response is found almost exclusively in inseminated females, usually as an immediate response to courtship, but sometimes when no courtship is apparently occurring. The genitalia are extruded towards the male in a directed nature of the response is one of its most remarkable features), forming a small tube. It has been suggested that this movement deters the male from courtship in some strains (Bastock & Manning 1955;

Connolly et al. 1974), or prevents him copulating without significant deterrence in another (Connolly & Cook 1973).

Before we can understand the causes of this response other problems must be elucidated. These include: the behavioural triggers for the occurrence of extrusion and its effects upon the male, and the more fundamental processes controlling the attraction of the male to the female, in particular the control of the onset and termination of courtship. Currently it is not possible to state whether the effects of extrusion on male behaviour are in any way independent of more fundamental changes in female attractiveness.

The technique of female decapitation, already used by Spieth (1966) in the analysis of male behaviour, reduces the complexity of the courtship situation by eliminating behavioural signals from the female. This technique has already been used in a first set of experiments reported elsewhere (Cook 1975). Here further results are presented which analyse age- and state-dependent phenomena which underly the sexual behaviour of the intact female.

Males will court decapitated females intensively, although possibly after a slightly longer latency than for the intact females; nevertheless, the decapitated female, which will survive for many hours, provides a relatively constant stimulus to the male, since she remains stationary, and makes but a few behavioural responses, none specific to courtship. Above all, such females do not extrude their genitalia in response to courtship (but see Cook 1974).

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Thus the behavioural response of extrusion may be excluded as an influence on male behaviour in these experiments.

### Methods

The behavioural assays of the attractiveness of females were made in the following manner: freshly decapitated females (at least  $\frac{1}{2}$  hr after the operation) were introduced into a round cell (18 mm diameter  $\times$  7 mm deep), four females per cell.

A total of twelve such cells was observed at any one time, six by each of the two observers. Cells containing different female types were alternated across the row of cells. After one male had been entered into each cell a sampling observation procedure was started which lasted a total of 10 min. Each cell was observed in turn for 5 s, totalling twenty 5-s periods per cell in the whole 10-min sampling period. Each 5-s sample was scored 1 if any courtship took place, 0 if not. The males were replaced after each 10-min sampling period until four males had been

measured. Each set of four females was discarded and measuring restarted with new animals in clean cells.

The experiments reported here assayed females of the Novosibirsk strain with males of the Pacific strain (see also Cook 1975). Insectary Novosibirsk females were obtained by removing copulating pairs from a mass mating chamber again using Pacific males.

### Results

Figure 1 shows the percentage of the 5-s periods spent in courtship as a function of the age, in days, from eclosion, in days, of decapitated virgin females. Each solid dot represents a mean of twelve cells of females, measured with a total of forty-eight males. The males were 2 days from eclosion in all cases. These data yield a correlation coefficient of  $-0.61$ , with a regression equation of  $y = 65.38 - 1.96x$ . The regression is significant at  $P < 0.001$  ( $t = 4.4$ ,  $df$  31). Excluding the data for the day of eclosion (day 0), or that for days 17, 18 and 19 leaves

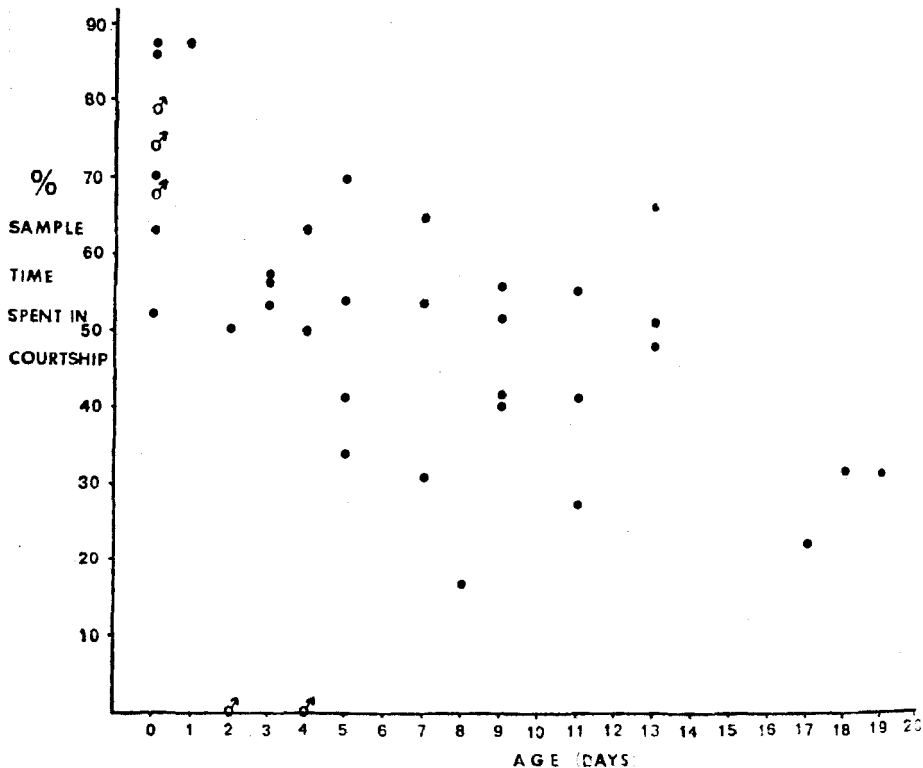


Fig. 1. Percentage of total sample time spent in courtship for samples of decapitated virgin Novosibirsk females (●) of different ages, with Pacific strain males aged 2 days from eclosion. Days 0, 2 and 4 include samples of Novosibirsk males (♂) treated in the same way as the females.

relationship significant ( $P < 0.01$  in both cases). Including both extremes simultaneously leaves the data for days 1 to 12 below significance ( $P < 0.10 > 0.05$ ). This result may suggest that a decline with age is not an entirely linear process.

The measures for day 0 were made within 2 days of eclosion; females of this age tend to attract much courtship from males. Samples of freshly eclosed males, treated in the same way as experimental females (i.e. decapitated) were also used, and they are marked on the figure with a square sign. The samples measured on day 0 showed a level of attractiveness comparable to that of females, whereas by days 2 and 4 males received virtually no courtship at all. It is possible that the high attractiveness of females on day 0 is due to an 'eclosion effect', and by days 2 and 4 therefore not necessarily on the continuum implied by the decline in attractiveness of females with age. But other hypotheses could apply equally well.

Other experiments (Fig. 2) compared the amount of courtship elicited by virgin and inseminated decapitated females. The latter were assayed on day 2 from eclosion and assayed on days 3, 5, 7, 9 and 11. On the vertical axis is plotted the mean number of 5-s sample periods

containing courtship per set of four females, combining the data for the four males measured. The experiment was repeated with consistent results, and the data for both replicates are combined in the figure. Ninety-five per cent confidence limits for the means are shown. *t*-test comparisons between the two conditions are significant at  $P < 0.001$  for days 3 and 5,  $P < 0.01$  for day 7, and non-significant for days 9 and 11.

Thus insemination reduces the amount of courtship the female receives for approximately 5 days, in the absence of any of the observable behaviours of rejection. After this period the level characteristic of virgin females of this age is apparently restored. This period parallels closely the time for which receptivity is switched off by mating in this strain (Cook & Connolly, unpublished data).

The presence of the ejaculate in female *D. melanogaster* is thought to inhibit receptivity (Merle 1968; Burnet et al. 1973) and at the same time stimulate egg maturation (Garcia-Bellido 1964), inseminated females laying large quantities of eggs (David 1963). Keeping females on a protein-free diet of sucrose reduces greatly the production and output of eggs (Manning 1967; Merle 1968) and was thus used to assess further

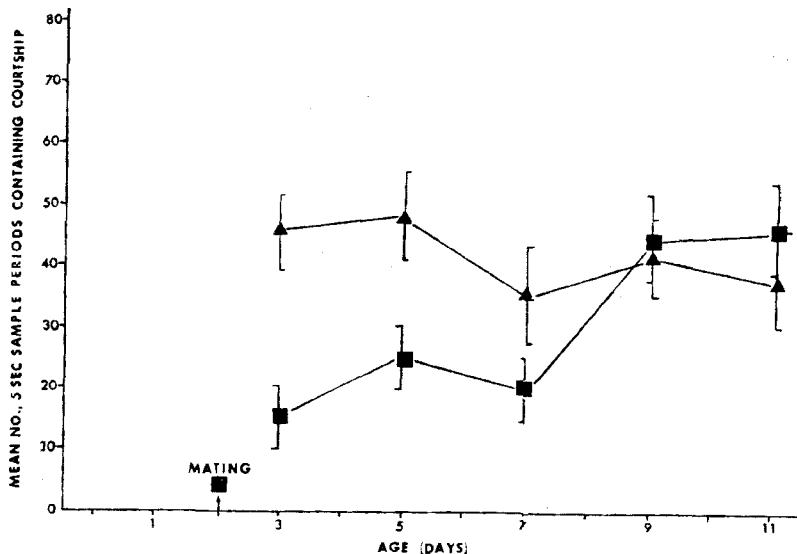


Fig. 2. Comparison of the mean number of 5-s sample periods which contained courtship per set of four decapitated females, pooling across four different males, obtained from virgin females ( $\blacktriangle$ ), with those from females inseminated on the second day from eclosion ( $\blacksquare$ ), showing the 95 per cent confidence limits for the means.

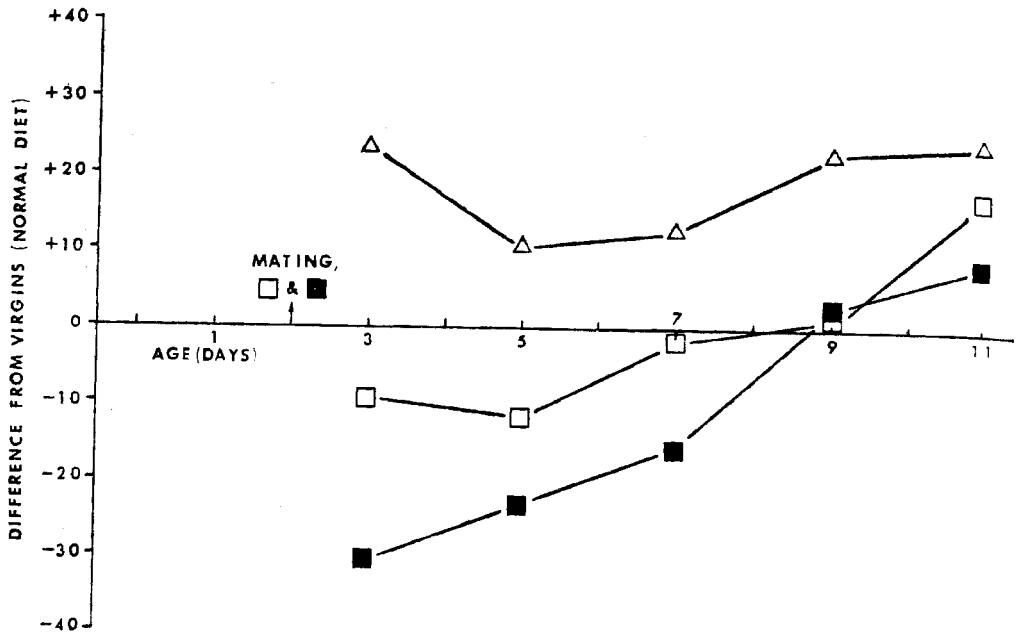


Fig. 3. Mean number of 5-s sample periods containing courtship for decapitated sugar-fed virgins ( $\Delta$  = SV), decapitated sugar-fed females mated on day 2 ( $\square$  = SF) and decapitated normal-diet females mated on day 2 ( $\blacksquare$  = NF), expressed as differences from decapitated normal-diet virgin females (NV) of the same age.

the control of the attractiveness to males of females. Figure 3, again based on two independent experiments, shows the results for females in three conditions, each expressed as a deviation from the values obtained for control virgins females on normal diet (NV). In the figure open triangles represent virgin females kept from eclosion on 10 per cent sucrose (SV), open squares, females kept from eclosion on sucrose and mated on day 2 (SF) and closed squares, females kept on normal diet and mated

on day 2 (NF). Multiple *t*-tests were performed on this data, and are shown in Table I. SV are significantly higher than NV females on days 3, 9 and 11, and significantly higher than SF females on all days but the last. The latter group was not significantly lower than NV, although on day 11 it is significantly higher and effectively back to the SV level. The NF females are significantly lower than the SF females for days 3, 5 and 7. Maintaining virgin females on a sucrose diet therefore makes them more attractive

Table I. Statistical Analysis

	Day 3		Day 5		Day 7		Day 9		Day 11	
	<i>t</i>	<i>df</i>	<i>t</i>	<i>df</i>	<i>t</i>	<i>df</i>	<i>t</i>	<i>df</i>	<i>t</i>	<i>df</i>
SV $\times$ NV	4.67***	22	1.70	22	1.90	22	4.05***	30	4.32***	30
SF $\times$ NV	1.30	22	1.63	22	0.25	22	0.09	30	3.06**	30
NF $\times$ NV	6.90***	38	4.93***	38	3.13 **	38	0.57	50	1.56	38
SV $\times$ SF	4.93***	22	4.04***	22	2.38*	22	3.98***	30	1.56	30
SF $\times$ NF	3.33**	30	2.46*	30	2.47*	30	1.00	40	0.79	34

\**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001.

to males than those on normal diet, keeping  
 closer to the level characteristic of eclosion.  
 The two sucrose diet conditions appear simply  
 demonstrate an upward displacement en  
 of the results from the two normal diet  
 conditions. On day 3 the difference SV minus SF  
 34-33, and NV (the baseline) minus NF is  
 40. Such a displacement of the two sucrose  
 conditions could be produced either by direct  
 effects of diet on their attractiveness  
 for example contamination of the experimental  
 with sugar, or the slightly thinner appearance  
 (their abdomen) or by the effects of the sucrose  
 on the systems normally controlling female  
 attractiveness; the activity of the ovaries is the  
 most likely hypothesis here. Other data collected  
 show that females maintained on sucrose from  
 eclosion until day 6 show little decline in attrac-  
 tiveness to males on the first day after they have  
 been transferred to a normal protein medium.  
 Moreover, protein uptake is only reflected in in-  
 creased egg output on the second day after that  
 change of diet, regardless of whether the female  
 was mated on the second day from eclosion or  
 the day of reversal of the diet. Therefore direct  
 effects on attractiveness through contamination  
 of females with sucrose seems, at least with  
 virgin females, to be a less plausible  
 hypothesis than the effects of the completion of  
 egg maturation. Furthermore, since the abdomen  
 increases in volume within minutes of giving a  
 protein diet it also is unlikely that the appearance  
 of the deprived female is especially important.  
 Since mating has an equivalent effect on the  
 amount of courtship directed to females regard-  
 less of their diet, a component of the effect due to  
 insemination must be controlled by a mechanism  
 which is independent of the dietary conditions.  
 The causal factor must therefore be at least one  
 stage further back towards the ejaculate.  
 The SF condition returns towards the SV  
 level towards the end of the experiment in a  
 manner parallel to the return of the NF con-  
 dition, the influence of copulation gradually  
 waning in both cases. The current hypotheses  
 necessitate that in the former case it is largely  
 the effects of the ejaculate that are waning where-  
 as in the second this change is also accompanied  
 by waning of the high degree of ovarian activa-  
 tion occasioned by the mating.  
 The decline in the activation of the ovaries  
 with time after mating is correlated with declining  
 fecundity, and therefore declining sperm  
 quantity (David 1963) and receptivity returns  
 when almost no sperm remains in the spermatha-

cae (Manning 1967). In females kept on sucrose,  
 however, the sperm is not used up sufficiently and  
 receptivity remains switched off (Manning 1967)  
 or returns only very gradually. We found that  
 only 25 per cent of females kept on sucrose were  
 receptive at day 16 after mating on day 2, 14  
 days after mating, and that such females do still  
 contain some live sperm. Thus the temporal  
 effect on attractiveness shown here by SF, and  
 possibly also NF, could be due rather to a waning  
 of the influence of the accessory gland com-  
 ponent of the ejaculate, already well known to  
 have a relatively transient effect on egg  
 production, as well as being implicated in the  
 switching off of receptivity, and not to the effects  
 of sperm depletion. A better idea of the action  
 of the switching system involved in the insemina-  
 tion and dietary effects on female attractiveness  
 will be gained by studies currently being con-  
 ducted with the female sterile mutant Fs(2)B,  
 which lacks functional ovaries.

Discussion

These results indicate the importance of taking  
 into account changes in more fundamental  
 systems controlling female attractiveness before  
 complex behaviours such as extrusion can be  
 understood; also, in assessing the 'receptivity'  
 of a female, her attractiveness to males should  
 be taken into account as a factor influencing the  
 amount of courtship she receives. The experi-  
 ments of Shorey & Bartell (1970) deal directly  
 with the attractiveness of *Drosophila* females  
 to males, demonstrating the existence of an  
 airborne factor emanating from females (and  
 to a lesser extent from males also) which in-  
 creases the tendency of males to start courting.  
 The chemical nature of this pheromone remains  
 unknown. It has not yet been possible for us to  
 demonstrate the nature of the communication  
 implicit in our results, but the system is probably  
 operating either through contact or by air-  
 borne chemoreception. Attempts to reduce the  
 amount of courtship directed to virgins by the  
 proximity through gauze of inseminated females,  
 and to increase that to inseminated females by  
 proximity through gauze of virgin females have  
 failed. This implies the probability of discrimina-  
 tion of type from extremely close range. Shorey  
 & Bartell (1970) apparently did not assay any  
 inseminated females.

But from the standpoint of the evolution of  
 behaviour and its co-ordination with reproduc-  
 tive physiology some interesting questions are  
 raised. Why, for example, should the time when

the female is maximally attractive to males also be the time when she is unreceptive? Receptivity only switches on during the second 24 hr from eclosion, when female attractiveness has already begun to decline somewhat, but we do not know what effects prolonged courtship might have on a female's rate of development. Also, the unreceptivity produced in a female by mating would appear to feed back upon the male's courtship through two systems: genital extrusion, and the underlying change in attractiveness shown by these experiments. In intact animals these may not be distinct systems, for genital extrusion may be a means of concentrating or directing the factor demonstrated here.

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