Effects of the *hypoactive* and *inactive* mutations on mating success in *Drosophila melanogaster*

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The effects on mating success of four X-linked mutations controlling locomotor activity are examined. The mutation *hypoE* has no effect on female mating success. The mutations *hypoC*, *iav* and *iav*² reduce female mating propensity and extend courtship durations. Unlike wild type females these mutant females fail to reach their maximum propensity for mating on the second day after eclosion. The *iav* females have normal wild type compositions of cuticular hydrocarbons and are highly attractive to courting males. Attenuated perception or aberrant processing of male courtship stimuli, and delayed sexual maturation are likely contributors to the poor mating propensity of *inactive* females. The *iav* mutation also causes decreased mating success and extended courtship duration in male mutants. This is largely a consequence of their inactivity. The duration of courtship latency, which is related to the number of phenotypically inactive flies present in the pairing, is positively correlated with age. Copulation duration, though primarily a male determined trait, is also affected by age. Some inferences regarding possible modes of action of the mutations are discussed.

INTRODUCTION

Since the description by Sturtevant (1915), sexual behaviour of *Drosophila* has been the subject of reviews and investigations (see Burnet and Connolly, 1974; Ewing, 1983; Siegel *et al.*, 1984). In *Drosophila melanogaster* the male orients himself towards the female and vibrates the wing nearest to her to produce a species specific song. He may then lick her genitalia, curl his abdomen and attempt to copulate.

Courtship behaviour involves visual, olfactory, tactile and auditory stimuli. Consequently there is a potentially large number of gene mutations likely to affect it. Spiess (1970) reviewed 20 investigations into the effects of specific single gene mutations on mating success. All the mutations were found to decrease mating success in males, whilst most mutant females showed reduced thresholds of receptivity. Two studies revealed that the mutations, *cut* (Merrell, 1949) and *aristapedia* (Tan, 1946), reduce female receptivity.

The effects of changes in locomotor activity on courtship behaviour have been examined using lines selected for high or low activity (Ewing, 1963; Kessler, 1968; Cook, 1973) and for fast or slow mating speed (Manning, 1961, 1963). Other than suggesting that the two systems are largely under independent genetic control, as Kyriacou (1981) noted, these studies show little agreement about the effects of activity on courtship behaviour. However, he concluded that there was some association between high activity and male mating success. More recently Cobb *et al.* (1987) found a correlation between high activity and short courtship latencies.

Gailey *et al.* (1986) report that the level of female locomotor activity decreases immediately prior to copulation. However, the precise role of locomotor activity throughout courtship remains unclear. This is surprising, given that during courtship transitions between male and female movement occur very frequently (Cobb *et al.*, 1986). Once courtship has started, male locomotor activity is largely dependent upon that of the female (Cook, 1979). The amount of locomotion by females also affects the intensity of licking stimuli that they receive (Burnet *et al.*, 1988).

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Single gene mutations controlling locomotor activity may also affect sexual behaviour. Homyk (1977) found that males with the X-linked mutations *hypoactive-B* (now *inactive*²) and *hypoactive-C* showed "normal" wing vibration during courtship and proposed that the mutations were neurological in origin. The *inactive* females show a deficit in octopamine titre in the brain and other parts of the nervous system (O'Dell *et al.*, 1987). More detailed information is required concerning the effect of these mutations on courtship behaviour and mating success. A description of the effects of four X-linked mutations *hypoactive-C*, *hypoactive-E*, *inactive*¹ and *inactive*² on locomotor activity has been given by O'Dell and Burnet (1988). The mutations cause changes in different specific aspects of locomotor activity such as amount or speed of movement and reactivity.

Any correlated change in mating success may direct attention to a relationship between particular aspects of locomotor activity and sexual behaviour. Such relationships may be revealed by the use of ethograms (Manning, 1959; Brown, 1964) and transition analyses of the sequential structure of specific courtship behaviours (Wood *et al.*, 1980; Markow and Hansen, 1981; Cobb *et al.*, 1986).

MATERIALS AND METHODS

The two wild type strains, Novosibirsk and Sierra Leone, and four mutants, *hypoactive-C*, *hypoactive-E*, *inactive*¹ and *inactive*² used here have been described by O'Dell and Burnet (1988). As Spiess (1970) noted, comparisons between the effects of a mutant allele and its corresponding wild type allele may be obscured by the effects of differences in the residual background and the effects of inbreeding depression. The investigations using the *inactive*¹ mutation described here have been conducted using the F₁ hybrids derived from reciprocal crosses between two pairs of isogenic strains that differ only by the allelic substitution *iav*⁺ or *iav*⁻ (O'Dell and Burnet, 1988). The problems of genetic background and inbreeding depression can therefore be circumvented by comparing the behaviours of the hybrid wild type to the hybrid *inactive* flies so that differences in behaviour can more confidently be attributed to the effect of the *iav* locus itself.

Flies were raised on a standard oatmeal and molasses medium, seeded with live yeast and maintained at 25 ± 0.5°C on a 12-hour light:dark cycle. The sexes were separated under ether on the

day of eclosion and maintained in groups of ten in vials.

Measurement of mating success

Scores of mating success, courtship latency (time elapsing between the male and female being placed together and courtship beginning), courtship duration (the time elapsing between the beginning of courtship, defined as the first male wing vibration, and copulation irrespective of breaks in courtship), and copulation duration, were made using single pair matings with virgin flies.

Observations of courtship behaviour were made in a circular mating cell milled from a block of polythene and fitted with a clear Perspex lid. The cells were 21.5 mm in diameter and 4 mm deep and could be divided in half by a retractable divider. On opposite sides of this arena were 3 mm entrance holes, stoppered with a Perspex plug. The male was aspirated into one side of the divided cell and the female into the other. The flies were left for 1–2 minutes to recover from the aspiration. The cell divider was then retracted. Each pair was observed for three hours (at three days old), one hour (at the other ages), or until copulation occurred, whichever was the shorter. Durations were measured to the nearest minute. The initiation of courtship was defined by the first orientation of the male to the female in which wing vibration occurred.

Analysis of courtship behaviour

Courtship behaviour was observed at 8× magnification using a Nikon double-headed binocular stereo microscope under the conditions described above. The flies were observed from the start of courtship (defined as above) until copulation occurred, or until ten minutes of unsuccessful courtship had elapsed. If no courtship commenced within ten minutes the pair of flies were discarded.

The pattern of courtship events was recorded as the frequency and duration of key presses using an Apple II microcomputer as a data logger. Two observers recorded the frequency and duration of ten courtship elements on ten separate channels. One observer recorded the male behaviours of orientation, vibration, wing scissoring, licking and genital contact, whilst the other scored the female behaviours of movement, flicking, kicking, raising and extrusion. These behaviours have been described by Cobb *et al.* (1985).

In addition the sequences of courtship events during the observation period were compiled as a

matrix. Wood *et al.* (1980), Markow and Hansen (1981) and Cobb *et al.* (1986) looked for significant transitions within matrices. However, this study is concerned with differences between transition matrices for courtship behaviour between wildtype and mutant. The emphasis is therefore on a comparison of the pattern and frequency of transitions between rather than within genotype groups and the results are presented in ethograms.

Extraction of cuticular hydrocarbons

Following the procedure of Jallon (1984), five individuals of each sex and genotype were bathed in 50 μ l hexane for five minutes and then vortexed. 5 μ l were then injected into a Girdel 300 gas chromatograph equipped with a Chrompack 25 m CpSil 5 capillary column. After integration with a Spectraphysics SP4270 integrator, chromatograms were compared within each group and with the reference chromatograms of *D. melanogaster* Canton-S males and females reported by Jallon (1984) and Antony *et al.* (1985).

RESULTS

Mating success

From the results in table 1 it is clear that three day old *inactive* males and females show reductions in mating success relative to the wild type control ($\chi^2 = 33.3$, $df = 1$, $P < 0.001$), the effect in the female being particularly marked. The *hypoC* and *iav*² females also show marked deficits in mating propensity but *hypoE* females are similar to wild type ($\chi^2 = 61.8$, $df = 3$, $P < 0.001$).

The mating success of wild type and *iav* at different ages is shown in fig. 1. The isogenised *iav* females show marked deficits in mating success throughout their lives. The developmental pattern of mating propensity is significantly different from that of wild type females ($\chi^2 = 84.01$, $df = 8$, $P < 0.001$), being characterised by an apparent delay in the attainment of maximum propensity.

Figure 1 Relationship between mating success and age in isogenised wild type (●) and *iav* (○) females when paired to wild type males. $n = 100$ for each point.

The effect of age on the mating success in the other mutant females is shown in fig. 2. The *hypoE* females show a wild type pattern of receptivity with age, but the consistently low frequency of mating by *hypoC* and *iav*² females precludes any confident interpretation of temporal expression of sexual receptivity.

Table 1 Mating success and mean durations of latency, courtship and copulation in three day old flies in single pair mating trials over three hours. Mating success expressed as a percentage, durations of latency and courtship in log mins, duration of copulation in mins. For (a) $n = 200$, (b) $n = 100$, for durations of courtship and copulation n = number mating within each class. Wild type is Novosibirsk inbred. Figures in parentheses are standard errors

Female	Male	Success	Latency	Courtship	Copulation
a					
wild type	wild type	94	0.061 (0.010)	0.601 (0.030)	23.67 (0.34)
<i>iav</i>	wild type	35.5	0.332 (0.028)	1.274 (0.057)	23.83 (0.50)
wild type	<i>iav</i>	77.5	0.242 (0.024)	1.376 (0.033)	18.45 (0.44)
<i>iav</i>	<i>iav</i>	4	0.710 (0.050)	1.731 (0.118)	17.50 (1.94)
b					
wild type	wild type	92	0.089 (0.018)	0.878 (0.051)	25.29 (0.38)
<i>hypoC</i>	wild type	25	0.374 (0.036)	1.593 (0.086)	23.68 (0.87)
<i>hypoE</i>	wild type	90	0.261 (0.031)	0.911 (0.066)	26.41 (0.38)
<i>iav</i> ²	wild type	36	0.306 (0.037)	0.890 (0.102)	25.47 (0.64)

Figure 2 Relationship between mating success and age in *hypoC* (□), *hypoE* (△), *iav*² (○) and Novosibirsk inbred wild type (●) females when paired with wild type males. $n = 100$.

Latency

The effect of the mutations on courtship latency is shown in Table 1. Analysis of variance shows that the *iav* mutation extends the latency of both males ($df = 1$, $F = 79.38$, $P < 0.001$) and females ($df = 1$, $F = 138.59$, $P < 0.001$). The durations of latency for *hypoC* ($t = 2.85$, $P < 0.001$), *hypoE* ($t = 2.49$, $P < 0.05$) and *iav*² ($t = 2.61$, $P < 0.05$) females are all significantly longer than wild type.

Latency clearly varies according to the number of phenotypically inactive flies present in the pairing. The shortest latencies are shown by the wild type pairings, whereas the *iav* pairs show extended latencies. Those crosses involving one wild type and one phenotypically inactive individual are intermediate.

These results are confirmed and extended by the results presented in fig. 3. There is a clear relationship between latency duration and age. Both wild type ($df = 1$, $F = 29.34$, $P < 0.001$) and *iav* ($df = 1$, $F = 7.87$, $P < 0.001$) females showing significant increases in latency with age.

Courtship duration

The effects of the mutations on courtship duration are shown in table 1. Both *iav* males ($df = 1$, $F =$

Figure 3 Relationship between latency duration and age in isogenised wild type (●) and *iav* (○) females when paired with wild type males. $n = 100$.

286.14, $P < 0.001$) and females ($df = 1$, $F = 48.24$, $P < 0.001$) and also *hypoC* females ($t = 5.68$, $P < 0.001$) have significantly extended courtship durations, but neither *hypoE* ($t = 0.40$, ns) nor *iav*² ($t = 0.11$, ns) females show any significant difference from wild type females. However, when results are pooled over different age groups *iav*² females show significantly extended courtship durations ($t = 3.82$, $P < 0.001$).

Wild type females have long courtship durations on the first day after eclosion, but consistently shorter durations thereafter (fig. 4). This first day level is comparable with the durations of isogenised *iav* females which are consistent from day 2 to day 32.

Figure 4 Relationship between courtship duration and age in isogenised wild type (●) and *iav* (○) females when paired with wild type males. $n =$ number of pairs mating, (see fig 1).

The effects of age on courtship durations for the other mutants is shown in fig. 5. The *hypoE* females clearly show a wild type pattern of courtship duration with age. There may be some decrease in courtship duration as *iav*² females age, but the low frequency of matings by one day old *hypoC*, *iav* and *iav*² flies preclude any meaningful comparisons at this age.

Figure 5 Relationship between courtship duration and age in *hypoC* (□), *hypoE* (△), *iav*² (○) and Novosibirsk inbred wild type (●) females when paired with wild type males. *n* = number of pairs mating, (see fig. 2).

Copulation duration

The effects of the mutations on copulation duration are shown in table 1. The *iav* males have a significantly shorter copulation duration than wild type males ($df = 1$, $F = 109.11$, $P < 0.001$). Fig. 6 shows the effect of age on copulation duration. Both wild type and *iav* females show very similar patterns of expression.

Courtship behaviour

Ethograms describing courtship are shown in fig. 7(a)–(c). The male maintains a position close to the female with his body axis oriented towards her. If she moves, he follows, so that during courtship his locomotor activity is largely dependent on that of the female. For wild type flies (fig. 7(a)) the

Figure 6 Relationship between copulation duration and age in isogenised wild type (●) and *iav* (○) females when paired with wild type males. *n* = number of pairs mating, (see fig. 1).

most frequent transitions are those between moving and standing by the female (12.2 and 11.2 per cent) indicating that much of the courtship time is spent in motion. Other important transitions include vibration-locking (9.4 per cent) and moving-vibration (7.8 per cent), whereas the reverse transitions occur relatively infrequently (1.9 and 1.9 per cent). The licking-genital contact transition (2.2 per cent) indicates that wild type courtship is characterised by the sequence: vibration-licking-genital contact.

The most frequent transition for the *inactive* female and wild type male pairings (fig. 7(b)) is from vibration to licking, suggesting that much of courtship time is spent in close contact. Analyses of variance show that, relative to the wild type females, *iav* females elicit significantly more vibration from the courting male over the ten minute observation period ($F = 23.45$, $df = 1$, $P < 0.001$), and respond with significantly more kicking and fending ($F = 14.52$, $df = 1$, $P < 0.001$). There are fewer movement transitions than in wild type courtships (5.8 and 4.1 per cent), and a low frequency of orientation-vibration transitions (0.6 per cent), suggesting that there is little locomotor activity and few breaks in courtship. Licking-genital contact transitions are infrequent (0.3 per cent), whereas those from scissoring to vibration are relatively more frequent (3.3 per cent).

The most frequent transitions for the wild type female and *inactive* male pairings (fig. 7(c)) are those involving movement (9.3 and 7.8 per cent). The *inactive* males show a low frequency of transitions from moving to vibration (4.8 per cent) and

Figure 7A-C Ethogram (10 × 10 matrix) for courtship in *Drosophila melanogaster*. Frequency of transitions (per cent) denoted by thickness of arrow. A, female wild type × male wild type. B, female *iaav* × male wild type. C, female wild type × male *iaav*.

vibration to licking (5.8 per cent). No transitions associated with genital contact occur at a frequency of greater than 1.5 per cent over the ten minute observation period. In addition the *inactive* males show transitions between scissoring and vibration (3.3 and 1.5 per cent), and following female movement (2.0 per cent). They also show recurrence of scissoring (2.0 per cent).

Pheromones and other cuticular hydrocarbons

Gas chromatographic analyses on a capillary column using hexane extracts from wild type and *inactive* individuals of both sexes were compared with those for Canton-S wild type flies described previously (Jallon, 1984). All major peaks co-migrated with those of the reference strain. The

Table 2 Cuticular hydrocarbon compositions of wild type and *inactive* males and females from the Novosibirsk background. Expressed as percentage of all characterised hydrocarbons with standard error

Hydrocarbon percentage	Niav + female	Niav - female	Niav + male	Niav - male
7-tricosene	2.8 ± 0.6	2.8 ± 0.7	46.2 ± 1.8	44.1 ± 2.8
<i>n</i> -tricosane	11.6 ± 1.4	9.6 ± 0.9	16.5 ± 1.4	14.2 ± 1.2
7-pentacosene	3.9 ± 0.5	4.7 ± 0.6	5.9 ± 2.0	10.0 ± 0.2
<i>n</i> -pentacosane	6.3 ± 0.9	5.7 ± 0.8	4.6 ± 1.8	3.0 ± 0.3
7, 11-heptacosadiene	24.1 ± 2.0	23.8 ± 1.2		
2-methyl-hexacosane	15.7 ± 0.5	15.3 ± 0.4	7.7 ± 0.9	7.5 ± 0.9
7, 11-nonacosadiene	10.9 ± 2.8	13.4 ± 1.5		
2-methyl-octacosane	7.4 ± 1.0	7.0 ± 0.9	6.4 ± 0.4	6.9 ± 1.1

mean percentages of the major components are listed in table 2. In the Novosibirsk genetic background, no marked quantitative differences were found between wild type and *inactive*. Most importantly no differences were found in the relative abundance of 7,11-heptacosadiene, the major pheromone of *D. melanogaster* (Antony *et al.*, 1985). In addition no clear differences were found in any cuticular component between wild type and *inactive* males, nor females, within the Sierra Leone genetic background (data not shown).

DISCUSSION

According to Manning (1967), female receptivity is affected by two independent processes, "switch-on" and summation. Switch-on is associated with female sexual maturity and determines whether the female is in the right physiological state to mate and sexually attractive to the courting male. Summation is connected with the amount of courtship stimulation required by the female before she copulates. Low female mating propensity could be determined by any of these processes.

Wild type females show a pattern of receptivity similar to that described by Manning (1967). However, their extended courtship durations on day 1 support the conclusions of Cook (1973). Mating propensity and courtship duration in young adult females are negatively correlated, suggesting that switch-on and summation are not entirely independent processes.

With the exception of the duration of latency, the courtship behaviour of *hypoE* females appears to be indistinguishable from wild type. This mutation is known to cause a deficit in speed but not amount of movement (O'Dell and Burnet, 1988). The *hypoC*, *iav* and *iav*² females show deficits in mating propensity and extended courtship dur-

ations. They also show reduced speed and amount of movement (O'Dell and Burnet, 1988) which suggests that there is an association between amount of locomotor activity and mating success.

Contrary to the criticisms of Robertson (1982), Manning's (1967) assertion that *Drosophila* courtship has a stimulatory function is supported by evidence presented by Cobb *et al.* (1986, 1987). Low mating propensity and extended courtship durations characteristic of *hypoC*, *iav* and *iav*² mutants may be the consequence of aberrant female summation of male courtship stimuli. This could result either from attenuated perception or abnormal processing of the male courtship stimuli.

Through mosaic analysis and fate mapping Homyk (1977) proposed that the foci for aberrant locomotor activity in both *hypoC* and *iav*² are neurological, the former in the suboesophageal ganglion and the latter in the thoracic ganglion. Such observations are consistent with poor summation through aberrant processing of courtship stimuli associated with some generalised neural defect. O'Dell *et al.* (1987) found that, relative to wild type females, *iav* mutants have significantly lower levels of octopamine in the brain as well as other parts of the nervous system, which is consistent with a neural effect of this mutation.

Another possibility is that the mutant females may be relatively unattractive. The two major components of female attractiveness are chemical and locomotor. The chemical component has been analysed in detail by Antony and Jallon (1982) and Antony *et al.* (1985). The most abundant epicuticular hydrocarbon found in mature wild type *D. melanogaster* females has a chain 27 carbons long with double bonds in positions 7 and 11. This is absent in mature conspecific males and in females of the sibling species *D. simulans*. The abundance of this hydrocarbon in mature (4 day old) *inactive* females seems normal.

According to Tompkins *et al.* (1982) and Jallon (1984) males need to perceive female movement to initiate courtship and court immobilised females with far less intensity than wild type females. This contrasts the findings of Bastock (1956), Ewing (1963) and Kyriacou *et al.* (1978) who noted that males direct a greater intensity of courtship to stationary females. Males have even been known to copulate at low frequencies with etherised (Streisinger, 1948) and decapitated (Spieth, 1966) females.

Female movement during courtship is important to the male, initially as a stimulator to initiate courtship. Subsequently, reduction or cessation of female movement may serve as a signal of acceptance (Tompkins *et al.*, 1982; Gailey *et al.*, 1986; Cobb *et al.*, 1986). The *inactive* females may be providing the males with an inappropriate response, indicating that she is about to mate when in fact she is not. This appears to change the male's behaviour to a frustration response characterised by high levels of scissoring.

Courtships involving *inactive* females are characterised by reduced locomotor activity, and high frequencies of male vibration and licking. The frequent rejection responses, and low rates of genital contact suggest that the *inactive* females are unreceptive rather than unattractive.

The developmental changes in *iav* female mating propensity indicate that there may be some delay in switch-on. A similar delay in the onset of maximum levels of activity in *iav* mutants has been reported (O'Dell and Burnet, 1988). The extended courtship durations of *iav* females are remarkably similar to those of immature (one day old) wild type females, suggesting a possible association between sexual immaturity and courtship behaviour in three day old *inactive* females.

Mature *inactive* females have a cuticular hydrocarbon profile that is very different from that in young flies which exhibit non-sex-specific compounds that disappear about 24 hrs after eclosion (Antony and Jallon, 1981; Pechine *et al.*, 1988), and this is also the case for cuticular hydrocarbons of *inactive* males. Retention of an immature hydrocarbon pattern has been found in *apterous-4* males (Jallon *et al.*, 1986). However, this does not preclude the possibility of a short delay in maturation of *inactive* females as it is not yet clear whether the switch-on of female receptivity is temporally linked to changes in the female's pheromone coat.

The relative contribution of poor neural processing and sexual immaturity to the poor mating success of these mutant females requires further examination. It is quite possible that a primary

neural focus could be a common factor underlying both effects. This is supported by Homyk (1977), who found a thoracic neural focus for aberrant locomotor activity, and by O'Dell *et al.* (1987) who found a deficit in the neurotransmitter octopamine in *inactive* females. The corpora cardiaca and corpora allata associated juvenile hormone release, and sexual maturation in several insect species, are known to be octopaminergic (Evans, 1980).

The *inactive* males are characterised by deficits in several aspects of locomotor activity (O'Dell and Burnet, 1988). Decreased mating success, and increases in the durations of latency and courtship may be a direct consequence of reduced activity. Mutant male courtship behaviour is characterised by frequent breaks from close contact with the female, low incidence of genital contact and an increased frequency of scissoring. This latter behaviour is rare in wild type males and is characteristic of prolonged unsuccessful courtship, indicating that it may be a frustration response. This strongly supports the notion that inactivity is the major contributory factor in the poor mating success of the *inactive* males. However, in the light of the observations on the *iav* females the actual situation is probably more complex.

The observation that latency is associated with the number of phenotypically *inactive* flies present in a pairing agrees with that of Cobb *et al.* (1987), who found an inverse relationship between activity and courtship latency in the *melanogaster* species subgroup. However, the positive correlation between latency and age is not in accord with that observed by Eastwood and Burnet (1977) and Cobb *et al.* (1987), although this may be a consequence of the different age ranges investigated.

MacBean and Parsons (1967) noted that copulation duration is primarily a male determined trait, which our results confirm, although there is some effect of age.

The reduced mating success of *iav* females, and to a lesser extent of *iav* males, appears to be a direct consequence of some neurological effect of the mutation rather than a secondary effect of reduced locomotor activity *per se*, but the precise mode of action of the mutation requires further investigation.

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