

Species-specific effects of single sensillum ablation on mating position in *Drosophila*

Angel Acebes¹, Matthew Cobb² and Jean-François Ferveur^{1,*}

¹UMR-CNRS 5548, Faculté des Sciences, Université de Bourgogne, 6 Boulevard Gabriel, F-21000 Dijon, France and

²School of Biological Sciences, University of Manchester, Oxford Road, Manchester, M13 9PL, UK

*Author for correspondence: (e-mail: jean-francois.ferveur@u-bourgogne.fr)

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Summary

Dipteran insects show a wide range of species-specific mating positions. Interspecific transitions from one position to another may reflect sexual or natural selection, or be pleiotropic consequences of other genetic changes. Like many cyclorrhaphan flies, *Drosophila* species mate with the male on the back of the female, positioned centrally. Mechanosensory sensilla on the male genitalia of three species of the *melanogaster* species sub-group of *Drosophila* have species-specific effects on mating position and on courtship success: ablation of a single pair of bristles on the genital claspers of *D. melanogaster* males halved homotypic mating success, and unilateral ablation produced a contralateral asymmetry in the male's mating posture. Ablation of mechanoreceptors on the male genital lateral plate affected mating posture less radically and had

no effect on mating frequency. Surprisingly, ablation of sensilla on the claspers of *D. simulans* and *D. sechellia* males showed no effect on homotypic mating. A similar result was found for *D. melanogaster* × *D. simulans* hybrid males. The existence of major differences in the sensory bases of mating position and copulation success in closely related species shows how differing mating positions may have evolved and underlines the need for detailed functional studies in studying the evolution of insect genitalia: homologous structures may serve different functions in different species.

Key words: lock and key hypothesis, sexual selection, mechanoreceptor, copulation, fruit fly, *Drosophila melanogaster*.

Introduction

Insect genitalia are some of the most gloriously elaborate structures in the natural world. The function – if any – of the variety of forms that can be observed remains a matter of debate. In 1844 the French zoologist Dufour argued that the variety of structures present on insect genitalia acted ‘as a guarantee of the conservation of types, and a safeguard for the legitimacy of species’ (Dufour, 1844). This ‘lock and key hypothesis’ is widely held, but there is little evidence to support it (Shapiro and Porter, 1989). It has also been argued that interspecific variation in genitalia is pleiotropic and effectively neutral (Mayr, 1963) and, more recently, that it is produced by sexual selection by females on male genitalia (Eberhard, 1985; Arnqvist, 1997, 1998), or as a result of conflicts of interest between the sexes (Alexander et al., 1997). The sensory roles of these structures have been largely ignored, and yet it seems likely that these may provide an insight into the diversity that can be observed.

This is particularly apparent in the case of the Diptera, which show the widest range of mating positions of any pterygote insect, with at least ten recorded positions (Alexander, 1964; McAlpine, 1981). Species-specific mating positions, which are frequently constant within a given genus, are thought to be driven by two factors: ecological selective pressures relating to

the evolution of courtship and mating on a substrate rather than in the air (McAlpine, 1981) and, in some species, the twisting of the final segments (‘hypopygium’) of the Dipteran male abdomen by 180° or even, bizarrely, by 360° in the Cyclorrhapha, which makes flexion of the final segments easier to effect, like a twist in a long balloon (Bickel, 1990). In virtually all Dipteran species, the initial coupling position involves both male and female facing in the same direction; the pair may then make synchronised movements to arrive at the species’ typical final mating position. These movements, and indeed mating itself, require both partners to be able to detect both their partner’s position and their own (proprioception). The most likely sources of this positional information are the genital sensilla. In crickets, for example, mechanoreceptors on the male cerci detect the female’s position and form part of the neural network underlying spermatophore transfer (Sakai et al., 1991; Snell and Killian, 2000).

Functional studies of insect male external genital structures are rare (Eberhard, 1993, 2001) and there have been no experimental studies of Dipteran mating position. To investigate the sensory mechanics of insect mating, we studied three closely related species of fruitfly, *Drosophila*

melanogaster, *D. simulans* and *D. sechellia*, all of which mate in the classic Cyclorrhaphan position with the male on the female's back and both partners facing the same way, following substrate-based courtship behaviours. Once mating has taken place, there is no subsequent change in position. Males of these species show specific differences in their genital structures, and in particular the shape of the claspers (CLs), the lateral plates (LPs) and the genital arch (Ashburner, 1989). In all three species the CLs and the LPs are covered with similar sex-specific mechanoreceptor sensilla, which in *D. melanogaster* show afferent projections to different levels of the abdominal ganglion (Taylor, 1989). To discover whether the information encoded by these sensilla is related to mating, they were ablated and the male's resultant behaviour was observed. The results were surprising.

Materials and methods

Fly strains and genetic procedures

All *Drosophila* stocks were maintained at 25°C on a standard cornmeal agar food under a 12 h:12 h dark:light cycle. We used *Drosophila melanogaster* (Canton-S=Cs strain), *D. simulans* (C-168.4 strain; a gift of Dr F. Lemeunier, Gif-sur-Yvette, France), and *D. sechellia* (Robertson strain; a gift from Dr J. A. Coyne, Chicago, USA). Hybrid males were generated by mass crosses between *D. melanogaster* males (3-days old) and *D. simulans* females (1-day old), following the procedure given in Manning (1959). To generate *D. melanogaster* females producing cuticular hydrocarbon that were attractive for *D. simulans* males, we crossed the Tp5670 PGal4 line (a gift from Dr T. Pr  at, Gif-sur-Yvette, France) with the UAS-tra2IR line (Fortier and Belote, 2000) (a gift from Dr J. M. Belote, Syracuse, Sicily). Such female flies produced high levels of 7-tricosene, which stimulates *D. simulans* males, and no 7,11 dienes, which normally strongly inhibit their courtship (Savarit et al., 1999; data not shown).

Sensilla ablation and scanning electron microscopy

Flies less than 2 h old were sexed under light CO₂ anaesthesia and subsequently kept in food vials until the behavioural test. Males were kept individually, and females were placed in groups of five. 1 day before the behavioural assay, 3- to 4-day-old males were CO₂ anesthetized and immobilized in a pipette tip with their abdomen protruding. Bristles were removed from the claspers or from the lateral plates with fine forceps (ref. 9980, Moria, France). Operated males were isolated in a fresh food vial until the next day when their behaviour was studied. Micrographs of male genitalia from control and ablated flies were taken using a scanning electron microscope (ESEM XL30; Philips, Eindhoven, The Netherlands).

Behavioural tests

Each male (4 or 5 days old) was tested once with a virgin intact female (3 days old) in a circular observation chamber (2.8 cm diameter, 0.5 cm height). Single pairs were observed

for 60 min (Savarit et al., 1999). In the case of hybrid males, however, which were difficult to obtain, behaviour was monitored with an alternative procedure. Instead of pairs of flies confined in a small mating chamber, 3–5 hybrid males (3- to 5-day-old control or operated flies) were placed with 10–20 females (2 days old) in a Petri dish (5 cm diameter), and their behaviour was monitored for 120 min. Moreover, each hybrid male was tested on two following days consecutively with *D. melanogaster* and with *D. simulans* females (or *vice versa*). All tests were performed at 25±0.5°C, at 65±5% humidity. In all cases, we recorded the time at which copulation occurred (copulation latency) and its duration. We noted the total number of mating pairs, and from that figure we calculated the frequency of abnormal and normal mating positions. Digital images of mating position were taken using a MZ8 binocular microscope (Leica, Munich, Germany) connected to a digital video cassette recorder (Panasonic AJ-D230E, Tokyo, Japan) coupled to a colour digital video camera (Sony SSC-DC38P, Tokyo, Japan). Flies were placed in specially designed plastic cells (internal dimensions: 16 mm × 12 mm × 4 mm).

Statistics

For each series of experiments, the significance level is indicated in the corresponding table. Mating frequency was tested by a χ^2 -test. The data for copulation latency, which were not normally distributed, were logarithmically (ln) transformed prior to being tested with an analysis of variance (ANOVA). Data obtained for copulation duration were normally distributed and were compared by means of an ANOVA.

Results

Bristle ablation affects mating posture...

Following ablation of both long mechanosensory clasper (CL) bristles (CLlbs; Fig. 1A), *D. melanogaster* males mated significantly less frequently than intact males (Table 1A; $\chi^2=21.7$; $P<0.001$), but showed the typical mating posture (Fig. 2A). Unilaterally ablated males mated as frequently as control males, but adopted a striking asymmetric mating posture, redolent of that shown by some Empidid flies, with the body bent to one side of the female's abdomen. This effect was contralateral to the ablation: left-side ablation yielded right-leaning males (Fig. 2B) whereas right-side ablation produced left-leaning males (Fig. 2C). The operation only affected mating position: no differences were observed in copulation latency or duration (data not shown) suggesting that the flies were not unduly affected by ablation, and that copulation proceeded according to its normal sequence. Unilateral or bilateral ablation of one or two of the four lateral plate bristles (LPlbs; Fig. 1D) increased copulation latency (Table 1), but did not affect mating frequency or copulation duration. Mating position was affected, but not as dramatically or in the same fashion as for the clasper sensilla. The data suggest that unilaterally and bilaterally ablated males had lost their ability to detect their position on the female: they randomly showed a central, a left-leaning or a right-leaning mating position.

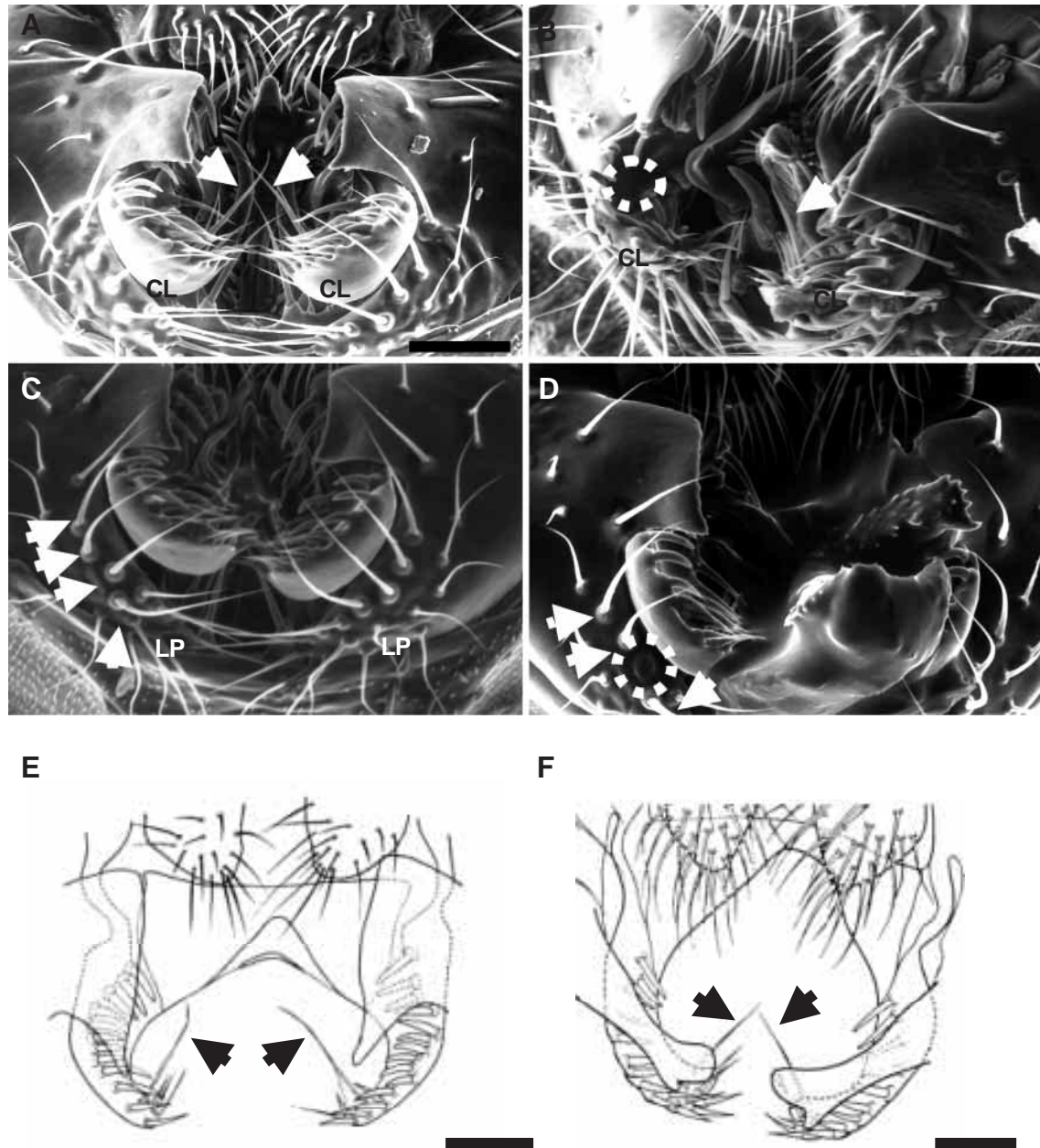


Fig. 1. Dorso-ventral views of male genitalia from *D. melanogaster* (A–D; scanning electron microscope images), from *D. simulans* (E) and *D. sechellia* (F) (reproduced with permission from Tsacas and Bächli, 1981; Tsacas et al., 1971). (A) The pair of long bristles (CLlbs; white arrows) on the claspers (CL) in a control male. (B) Ablation of the left CLlb (dotted circle). (C) The four long bristles (LPlbs; white arrows) on the male lateral plate (LP). (D) Ablation of a single left LPlb (dotted circle). (E,F) Arrows indicate the *D. simulans* and *D. sechellia* clasper bristles that are homologous to those in *D. melanogaster*. Scale bars, 50 μ m.

... But not in all species

The shapes of the claspers on *D. simulans* and *D. sechellia* males are different from those of *D. melanogaster*, but both species possess a pair of long sensilla located at a similar position to CLlbs in *D. melanogaster* males (Tsacas et al., 1971; Tsacas and Bachli, 1981) (Fig. 1E,F), suggesting that these structures are homologous in all three species. Surprisingly, unilateral and bilateral ablation of CLlbs in *D. simulans* and *D. sechellia* males had no effect on mating

posture, courtship success as measured by mating frequency or copulation latency in either species (Table 2). These results contrast with those obtained for *D. melanogaster*, which demonstrates interspecies differences in the sensory systems underlying mating and mating position and may indicate that these genital sensilla have different functions in the three species. The CLlbs are not involved in maintaining mating posture in *D. simulans* and *D. sechellia*; other structures presumably are involved.

Table 1. *Effects of male genital sensilla on mating in three Drosophila species*

Species	N	Mating frequency (%)	Mating posture			Mean copulation latency (min)
			Central (%)	Left side (%)	Right side (%)	
<i>D. melanogaster</i>						
Ablation of clasper long sensilla						
Control	52	88.5	82.6	6.5	10.9	8.8±0.7
Bilateral ablation	30	36.7	72.7	18.2	9.1	10±2
Right-side ablation	36	72.2	11.5	57.7	30.8	11.5±2.6
Left-side ablation	33	66.7	4.5	17.4	78.3	11.2±1.3
<i>D. melanogaster</i>						
Ablation of lateral plate long sensilla						
Control	55	85.4	74.5	12.8	12.8	6.7±0.6
Bilateral ablation	35	71.4	40	24	36	12.2±1.9
Right-side ablation	35	80	28.6	35.7	35.7	10.7±1.3
Left-side ablation	35	65.7	30.4	30.4	39.2	8.5±1.7
<i>D. simulans</i>						
Ablation of clasper long sensilla						
Control	56	32	94.2	5.8	0	18.7±3.4
Bilateral ablation	41	15	100	0	0	18.7±4.6
Right-side ablation	41	27	81.8	18.2	0	16.3±4.1
Left-side ablation	41	27	81.8	9.1	9.1	24.7±6.3
<i>D. sechellia</i>						
Ablation of clasper long sensilla						
Control	40	15	100	0	0	7.2±2.1
Bilateral ablation	38	13	80	20	0	14.6±5.2

Uni- and bilateral ablations were performed on the long bristle of the clasper in *D. melanogaster*, *D. simulans* or *D. sechellia* and on 1 or 2 sensilla of the lateral plate in *D. melanogaster*.

The frequency of mating posture is relative to the total number of successful matings. *D. melanogaster* males with bilaterally ablated clasper sensilla mated significantly less frequently than intact males: $\chi^2=21.7$; $P<0.001$.

Copulation duration showed clear interspecific differences: 18.3±0.3 min in *D. melanogaster*, 24.0±0.8 min in *D. simulans*, 25.8±2.2 min in *D. sechellia* ($F_{2,67}=40.03$, $P=0.0001$). No differences were found between control and experimental males (data not shown).

For copulation latency, a significant difference was found only for *D. melanogaster* LPIb ablation ($F_{3,118}=5.63$, $P=0.001$), but not in the other cases: *D. melanogaster* CL1b ablation ($F_{3,101}=0.97$, NS), *D. simulans* CL1b ablation ($F_{3,42}=0.392$, NS), *D. sechellia* CL1b ablation ($F_{1,9}=1.81$, NS).

NS, not significant.

Table 2. *Effect of genital sensilla of hybrid males (D. melanogaster female × D. simulans male) with females of the two parental species*

	N	Mating events	Mating posture			Copulation latency (min)
			Central (%)	Left side (%)	Right side (%)	
<i>(D. melanogaster × D. simulans) hybrid males with D. simulans females</i>						
Control	30	26	92.4	3.8	3.8	47.8±6.8
Left-side ablation	18	10	90	0	10	37.4±9.2
<i>(D. melanogaster × D. simulans) hybrid males with D. melanogaster females</i>						
Control	30	2	100	0	0	24.5±4.5
Left-side ablation	18	2	100	0	0	49±18

To increase the probability of mating, flies were observed *en masse* for 2 h. No significant effects were found on copulation latency or duration (data not shown).

To investigate the underlying genetic control of this effect, interspecific hybrid males were produced by crossing *D. melanogaster* males with *D. simulans* females. Hybrids showed a single pair of long clasper bristles, as in both parental species. Hybrid males were observed with virgin females of both parental species. Unilateral ablation of CL1b sensilla in hybrid males produced unambiguous results, although only a few pairs copulated (Table 2): like their *D. simulans* parents, operated hybrid males adopted a normal mating posture with females of both species, showing that the control of mating posture does not depend upon female type. No systematic differences were found for other mating or courtship features.

Discussion

The present study indicates that in *D. melanogaster*, information associated with the presence of mechanoreceptors on the male's claspers is involved in determining the mating position adopted by the pair of flies and the outcome of courtship, as measured by mating frequency. Information associated with the lateral plate bristles also plays a role in determining the position adopted by the mating pair, but does not affect courtship outcome.

The mechanisms by which these effects occur was further investigated, with the most striking result for mating position. Given the mechanosensory nature of the bristles, one possibility is that the male uses proprioceptive information to determine his position on the female, perhaps coded *via* the sexually dimorphic neurons and axonal arborisation that have been observed in the central nervous system (Taylor, 1989). In the absence of such information, he may incline his position as far as is possible, or until he obtains similar information from other sensory sources. It is also possible that the female reacts to the absence of information normally provided by these bristles, and changes her behaviour accordingly, leading to the pair adopting a lateral mating position.

The effects on mating frequency are more complex. Ablation may have affected the quantity or the quality of male courtship. In particular, during the final stages of courtship, males of all three species sometimes make what appear to be attempts at copulation. Failure to detect the female, or to provide her with appropriate stimuli, or both, may lead to a decrease in the frequency with which these attempts at copulation were transformed into successful matings, as shown by lower mating frequencies observed in bilaterally ablated flies. Similar effects may produce the increased mating latency observed in unilaterally ablated flies. These sensilla may therefore play a role in both mating and the final, decisive stages of courtship.

Surprisingly, ablation of the apparently homologous sensilla in *D. simulans* and *D. sechellia* did not produce the same effect: ablation of the CL1bs had no effect on mating position in these species. There are several possible explanations for this interspecific difference: the sensilla may have different functions, the neural networks to which they are connected may have different functions, female criteria may show interspecific

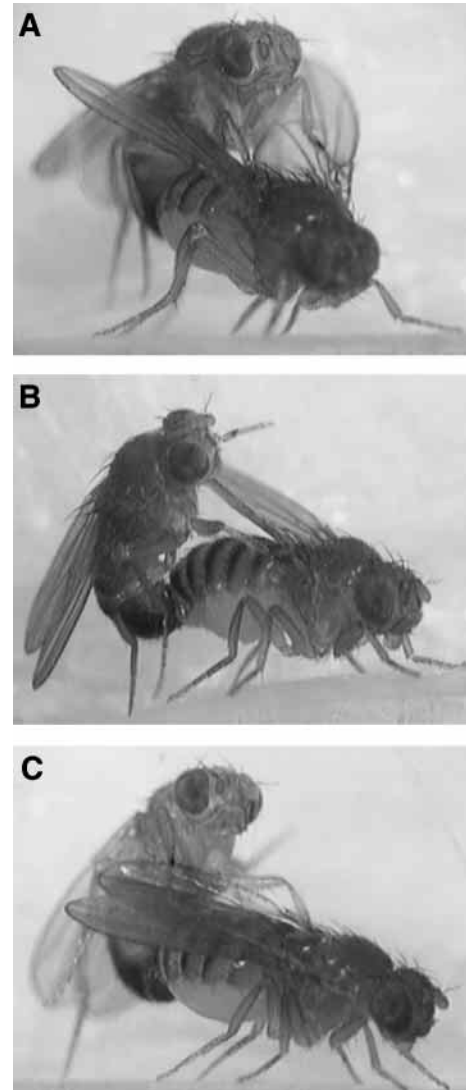


Fig. 2. Mating position in *D. melanogaster*. (A) A control pair showing a stereotypic position, (B) a left-CL1b ablated male leaning to the right of the female, and (C) a right-CL1b ablated male leaning to the left of the female.

differences or males may be differentially able to compensate for the absence of specific stimuli from their genitalia. Whatever the precise interaction that is taking place here, this result is of fundamental importance for studies that attempt to interpret the evolution of insect genitalia in terms of either the 'lock and key' hypothesis or sexual selection (Arnqvist, 1998; Alexander et al., 1997). It suggests that from a functional point of view rather than from a simple anatomical standpoint, the diversity of insect genitalia may be greater than hitherto suspected. Furthermore, the mating pair must be considered as an interacting pair, and not an active male with a passive female. Stimulation, response and interactions are taking place during courtship and mating. Functional studies will be required to make real progress in this field, as apparently identical structures in closely related species can form sensory systems with radically different functional characteristics.

It is striking that many other species of Diptera show similar long bristles on the male's claspers (e.g. Diopsidae, Mycetophilidae, Sciardiidae and Tanypezidae (McAlpine et al., 1981, 1987). This suggests that the effects observed here might apply to all Diptera and that changes in the detection or expression of sensory information in either or both sexual partners may have contributed to the changes in mating position that have taken place in Dipteran species. For example, some Empid flies couple with the male on the female's back, then during the course of mating the male bends his genitalia down and to one side of the female (McAlpine, 1981). This clearly requires proprioceptive information of the type that is apparently coded by the male clasper long bristles in *D. melanogaster*. The decrease in mating frequency shown by unilaterally ablated males suggests, however, that any mutant lacking that particular mechanoreceptor would be at a clear selective disadvantage. Coadaptive change by both the sender and receiver of the information associated with these mechanoreceptors would clearly be necessary to ensure the adoption of a new mating position by such a mechanism.

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