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ORIGINAL RESEARCH

Sex Combs are Important for Male Mating Success in *Drosophila melanogaster*

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Abstract The sex comb is one of the most rapidly evolving male-specific traits in *Drosophila*, making it an attractive model to study sexual selection and developmental evolution. *Drosophila* males use their sex combs to grasp the females' abdomen and genitalia and to spread their wings prior to copulation. To test the role of this structure in male mating success in *Drosophila melanogaster*, we genetically ablated the sex comb by expressing the female-specific isoform of the sex determination gene *transformer* in the tarsal segments of male legs. This technique does not remove the sex comb entirely, but simply restores the morphology of its constituent bristles to the ancestral condition found in *Drosophila* species that lack sex combs. Direct observations and differences in long-term insemination rates show that the loss of the sex comb strongly reduces the ability of males to copulate with females. Detailed analysis of video recordings indicates that this effect is not due to changes in the males' courtship behavior. Rapid evolution of sex comb morphology may be driven either by changes in female preferences, or by co-evolution between sex combs and female external genitalia.

Keywords *Drosophila* · Sex comb · Sexual dimorphism · Sexual selection · Courtship · Female choice

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Introduction

Male-specific morphological structures play important roles in competition for mates by acting as display ornaments, weapons in male–male contests, and mediators of male–female tactile interactions during courtship and mating. These functions can place male sexual characters under intense sexual selection and, as a result, these characters tend to evolve more rapidly than other morphological traits (Andersson 1994). Often, male-specific structures show dramatic diversity among closely related species, and can be gained and lost on short evolutionary timescales. In many cases, the rapid evolution of male sexual characters may reflect equally rapid changes in female preferences for these traits (Wiens 2001; Wong and Rosenthal 2006).

In *Drosophila*, one of the most prominent male-specific structures is the sex comb—an array of modified mechanosensory bristles located on the males' first pair of legs. The sex comb is a recent evolutionary innovation; in fact, the vast majority of *Drosophila* species lack this structure. It is present only in the *melanogaster* and *obscura* species groups of the subgenus *Sophophora*, and (possibly independently) in the genus *Lordiphosa* (Lakovaara and Saura 1982; Lemeunier et al. 1986; Hu and Toda 2001). Sex comb morphology varies greatly among closely related species, especially in the *melanogaster* species group. Interspecific differences involve sex comb position (on the first and second tarsal segments in most species, but only on the first segment in some), orientation (along or perpendicular to the proximo-distal leg axis), the number of bristles ("teeth") of which the sex comb is composed, and the size, shape, and color of these teeth (Kopp and True 2002; Barmina and Kopp 2007). This variation makes the sex comb an attractive model for

understanding the genetic basis of morphological evolution and the interaction between developmental pathways and natural selection in shaping animal form. Quantitative-genetic analyses in several species have shown that interspecific and intraspecific differences in sex comb size are determined by multiple, as yet unidentified, loci (True et al. 1997; Macdonald and Goldstein 1999; Nuzhdin and Reiwitch 2000; Coyne et al. 2004; Tatsuta and Takano-Shimizu 2006; Graze et al. 2007). At the same time, developmental-genetic approaches are beginning to elucidate the molecular pathways involved in the specification and differentiation of sex combs (Barmina et al. 2005; Barmina and Kopp 2007).

To develop a comprehensive model of trait evolution, it is essential to understand both the genetic and cellular processes that generate natural variation in phenotypic traits, and the selective pressures acting on these traits. The function of sex combs in mating behavior has been described for a number of *Drosophila* species in the *melanogaster* and *obscura* species groups (Spieth 1952). Interestingly, this function varies considerably among species. For example, males of *D. melanogaster*, *D. simulans*, and *D. mauritiana* use sex combs for "precision grasping" of extruded female genitalia before mounting, whereas in *D. pseudoobscura* the sex combs are used by males to spread the females' wings (Spieth 1952; Cook 1977; Coyne 1985). In the former three species, there is only transient contact between the male's sex comb and the female's body. Once the male assumes the final copulatory position, his sex combs no longer perform any function. On the other hand, males of some species of the *montium* subgroup (*melanogaster* species group) use their sex combs to grasp the female's abdomen securely during copulation, sometimes with sufficient force to compress it visibly (Sturtevant 1942; Spieth 1952).

Interspecific divergence of sex comb morphology suggests that sexual selection on sex comb size and shape may be different in different species. Several observations in natural populations support this idea. For example, in *D. bipectinata*, males with larger sex combs have greater mating success (Polak et al. 2004), while the opposite is true in *D. simulans* (Markow et al. 1996), and in *D. pseudoobscura* sex comb size does not significantly affect mating success (Markow et al. 1996). These observations suggest that females of some *Drosophila* species may be able to perceive the size of the male's sex comb, and accept or reject potential mates based partly on this phenotype. Alternatively, it is possible that sex comb size has a purely mechanical effect on the efficiency of the males' grasping behavior, and females simply reject males whose initial mounting attempts are not successful. The large number of mechanosensory bristles covering the abdomen, genitalia, wings, and thorax of flies could provide anchors for grasping and, in principle, allow the female to detect the presence, size, and number of sex comb teeth. Unfortunately, *Drosophila* mating behavior has not been video-recorded with sufficient speed and resolution to identify specific female mechanoreceptors that come into contact with the male's sex comb.

Experimental removal of sex combs also suggests that they are important for male mating success. Precise manipulation of sex combs is difficult due to their small size, as the teeth are only $\sim$50 μm long (Hannah-Alavah 1958). Spieth (1952) and Coyne (1985) circumvented this difficulty by amputating the front legs of males above or below the sex comb-bearing segment. In *D. pseudoobscura* and *D. persimilis*, amputation of the leg above the sex comb strongly reduced the males' success in inseminating females, whereas removal of the front leg below the sex comb had little effect (Spieth 1952). Similarly, males of *D. simulans* and *D. mauritiana* had significantly reduced ability to inseminate females when their legs were amputated above, but not below, the sex comb-bearing segment; this effect was stronger in *D. mauritiana* than in *D. simulans* (Coyne 1985). Direct observations showed that males whose legs were amputated above the sex comb were less successful at grasping the females' genitalia than males whose legs were amputated below the sex comb (Coyne 1985). In a more refined experiment, Cook (1977) used fine forceps to remove sex combs while leaving the leg otherwise intact. This procedure also led to delayed copulation in *D. melanogaster* and *D. simulans*. Since other aspects of courtship were not visibly affected, Cook (1977) suggested that sex combs were important specifically for the effective grasping of the females' genitalia prior to intromission.

In this study, we revisit the effects of sex combs on male mating success in *D. melanogaster* using a genetic approach. Sex combs were genetically ablated by expressing the female-specific isoform of the sex determination gene *transformer* in the tarsal segments of male legs. Instead of physically removing the sex comb, this procedure results in the transformation of all sex comb teeth into normal, unmodified mechanosensory bristles such as those found in males of *Drosophila* species that lack sex combs. Leg morphology is otherwise unaffected in the genetically transformed males. We then compared the mating success of normal and transformed males, and used video recordings to analyze the progression of courtship in detail. Our results confirm that the sex comb contributes to male mating success by facilitating effective grasping. Interestingly, the impact of sex comb loss in males depends on the genotype of females.

Materials and methods

Drosophila strains and genetic crosses

All fly stocks were reared on standard yeast/cornmeal/glucose medium at 22°C under a 12 h light/12 h dark cycle. We used three different wild-type strains: Canton-S; WI89, a near-isogenic strain established from a single female collected in Winters, CA, and inbred by over 20 generations of single-pair, full-sib crosses; and WO, an outbred strain produced by combining 10 isofemale strains from the Winters population and allowing them to mix for at least four generations.

Sex combs were genetically ablated by expressing the female splicing isoform of the *transformer* (*tra*) gene (Ferveur et al. 1995) under the control of the *rn-Gal4* enhancer trap. This enhancer trap is expressed in all three pairs of legs from the distal first to the proximal fourth tarsal segments, as well as in restricted regions of the wing, haltere, antenna, proboscis, and genitalia (St. Pierre et al. 2002). *rn-Gal4/TM6, Tb Dr* and *UAS-traF* males were crossed separately to WI89 females. +/+; *rn-Gal4*/+ F_1 females from the first cross were then crossed to *UAS-traF*/+; +/+ males from the second cross. In the F_2, *UAS-traF*/+; *rn-Gal4*/+ males were identified by the loss of sex combs. No morphological changes were observed in any other structures, such as genitalia, wings, or probosci. However, these structures carry chemoreceptory neurons whose feminization may affect male behavior (see "Results and Discussion"). F_2 siblings with normal sex combs were used as controls in each experiment. These individuals were a mixture of three genotypes: (1) *UAS-traF*/+; +/+, (2) +/+; *rn-Gal4*/+, and (3) +/+; +/+. The effect of this crossing scheme is that the genetic background of the experimental and control males is effectively randomized, with the exception of the chromosomes that carry the *rn-Gal4* and *UAS-traF* transgenes. Since the expression of *traF* in the wing could affect the males' ability to produce correct mating song, we also examined the mating success of males with feminized wings. To obtain these males, *UAS-traF* was expressed under the control of the *vg-GAL4* driver, which is expressed throughout the wing blade but is not expressed in other appendages (Simmonds et al. 1995).

Mating tests

Flies were sexed under light anesthesia with CO_2 within 8 h of their emergence. About 40 virgin males and females were maintained separately for 4–6 days in food vials until use. All experiments were conducted in the morning (8:30–11:00) under the same light and temperature regime (21–23°C).

Male mating success was measured in no-choice and multiple-choice experiments. For multiple-choice experiments, 55–60 males of each type and 120 WI89 females were simultaneously released into a population cage (18.75 cm × 27.5 cm ×35 cm) and kept under constant observation for 2 h. Each copulating pair was aspirated out of the cage and placed on a CO_2 stage to determine the sex comb phenotype of the male. The proportion of males without sex combs in the cage increased over time, since these males mated later and in smaller numbers (see "Results and Discussion"). For no-choice experiments, one male and one female were transferred to a food-containing vial with a confined space ($\sim$5 cm^3) by aspirator without anesthesia, and observed for 2 h. For each pair we recorded whether copulation occurred within this time period and, if so, its courtship latency (time between combining the male and female and the initiation of courtship), courtship time (time from courtship initiation to copulation), copulation latency (time between combining the male and female and the start of copulation), and copulation duration. After direct observation, the single pairs were left in their vials for 7 days, and the proportion of vials in which progeny were produced was recorded. Vials in which the females were dead were not counted. Genital tracts of females that did not produce any progeny were dissected, mounted in insect saline, and examined for the presence of sperm in the spermathecae under Nomarski optics at 400× magnification.

Behavioral observations

Virgin females of the WO strain were used for video recordings. Mating chambers were prepared by adding standard *Drosophila* medium to a well in a 24-well cell culture plate (well diameter 20 mm), which was then covered with a glass slide. One male and one female were introduced into the mating chamber by aspiration, and their behavior was videotaped using a Panasonic DV NV-GS500 recorder positioned directly above the chamber. All observations took place at 08:30–11:00 am. A total of 28 pairs were recorded, including 14 males without sex combs and 14 control males. Video recordings were then observed at a slower speed. We quantified the amount of time that males spent performing the following elements of courtship behavior:

1. Orienting/circling: male orients toward the female at any position or circles around the female.
2. Tracing: male follows after a running female.
3. Wing vibration: male extends his wing(s) and vibrates it/them.
4. Attempting: male bends his abdomen to attempt copulation.

Statistical analyses were performed using StatsDirect software (http://www.statsdirect.com).

Results and discussion

Our approach takes advantage of the fact that somatic sex determination in *Drosophila* is largely cell-autonomous (Baker and Ridge 1980). The *transformer* (*tra*) gene acts as a binary switch that directs each cell into an either male- or female-specific differentiation pathway (McKeown 1992). By expressing the female-specific isoform of *tra* in the males' tarsi, we can completely eliminate the sex comb by forcing the bristles from which it normally develops to assume a female identity (Fig. 1). We can then test the effects of this transformation on male mating success.

In no-choice experiments, 15.9% and 33.7% of males without sex combs mated with Canton-S and WO females, respectively, compared to 84.5% and 94.7% of control males with normal sex combs (Fig. 2a and Table 1). These proportions are significantly different for both female genotypes ($\chi^2 = 39.3$ with WO and $\chi^2 = 55.7$ with Canton-S, df $= 1$, $P < 0.001$). In the multiple-choice experiment, only 6.1% of males without sex combs mated successfully ($n = 3$), compared to 75.4% of control males ($n = 46$) ($\chi^2 = 63.7$, df $= 1$, $P < 0.001$).

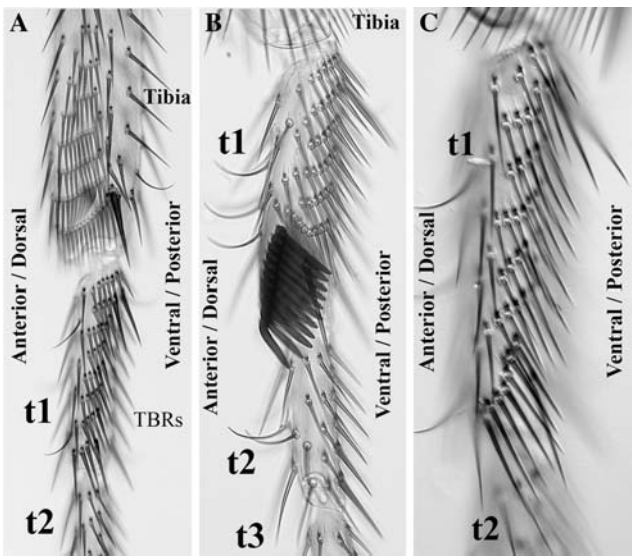

Fig. 1 The prothoracic (T1) leg of *D. melanogaster*. Distal is down and anterior is to the left in all panels. (**a**) Ventral view of the female T1 leg. The distal tibia and the first two tarsal segments (t1 and t2) are shown. Note the densely packed transverse bristle rows (TBRs) on the distal tibia and t1. (**b**) Anterior–ventral view of the male T1 leg. The most distal TBR on the first tarsal segment is transformed into a darkly pigmented sex comb oriented along the proximo-distal axis of the leg. (**c**) Effects of expressing female-specific transformer protein in the T1 leg of *UAS-traF/+*; *rn-GAL4/+* males. The sex comb fails to develop, and the distal-most TBR on the basitarsus assumes a female-like appearance

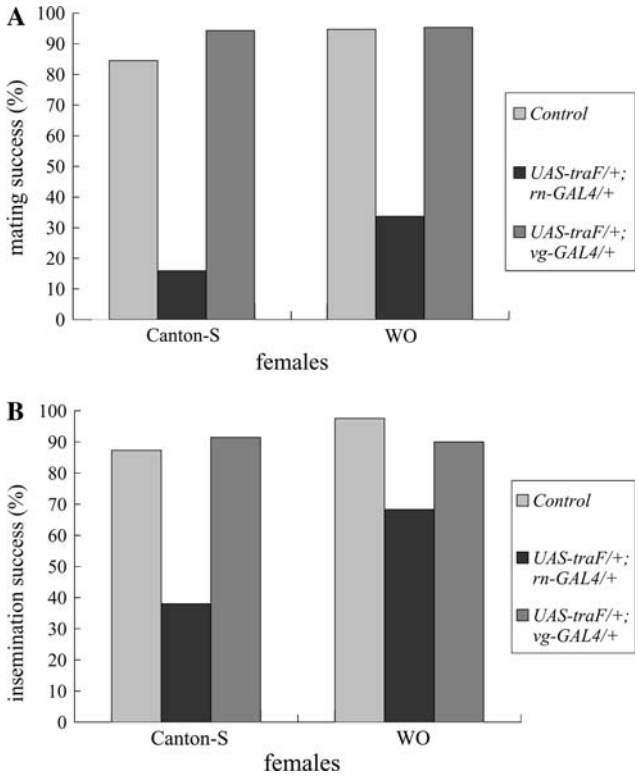

Fig. 2 Loss of sex comb reduces male mating success. (**a**) Percentage of wild-type, *UAS-traF/+*; *rn-GAL4/+*, and *UAS-traF/vg-GAL4* males that mated with Canton-S and WO females within a 2 h observation period. (**b**) Percentage of wild-type, *UAS-traF/+*; *rn-GAL4/+*, and *UAS-traF/vg-GAL4* males that inseminated Canton-S and WO females within 7 days. In both assays, female genotype has a strong effect on the mating success of males that lack sex combs

When males without sex combs were left with females for 7 days, offspring were produced by 38% of Canton-S females and 68.3% of WO females, compared to 87.3% and 97.6%, respectively, for control males (Fig. 2b). These proportions are also significantly different for both female genotypes ($\chi^2 = 27.8$, df $= 1$, $P < 0.001$ for Canton-S females; $\chi^2 = 8.8$, df $= 1$, $P < 0.01$ for WO females). The two female genotypes differ significantly both in the rate of acceptance of males without sex combs within 2 h ($\chi^2 = 9.4$, df $= 1$, $P < 0.01$), and in the rate of insemination over a 7-day period ($\chi^2 = 13.4$, df $= 1$, $P < 0.001$). No sperm were found in the reproductive tracts of females that produced no progeny, confirming that the males failed to copulate. Dissection of males' testes showed that the sperm of males without sex combs had normal motility, indicating that the failure of insemination was not due to male sterility.

Courtship latency was somewhat increased in males without sex combs compared to control males (Table 2). This increase was significant when males were paired with

Table 1 Number of mated and unmated pairs as a function of male and female genotypes

♀	♂	Replicate	Mated	Unmated	Total
Canton-S	Control	1	18	5	23
		2	18	1	19
		3[a]	13	3	16
	rn-GAL4/+; UAS-*traF*/+	1	3	11	14
		2	1	16	17
		3	2	19	21
		4	5	16	21
		5[a]	3	12	15
	vg-GAL4/UAS-*traF*	1	20	0	20
		2	13	2	15
WO	Control	1	18	1	19
		2	21	0	21
		3[a]	14	2	16
		4[a]	19	1	20
	rn-GAL4/+; UAS-*traF*/+	1	12	8	20
		2	11	11	22
		3	7	16	23
		4[a]	1	15	16
		5[a]	3	17	20
	vg-GAL4/UAS-*traF*	1	22	1	23
		2[a]	19	1	20

[a] Excluded from the analysis in Table 2 due to insufficient data on courtship latency and courtship time

WO females (one-way ANOVA: $F = 4.77$, df $= 2$, $P = 0.010$; Tukey–Kramer test: $P = 0.022$), but not significant when they were paired with Canton-S females (one-way ANOVA: $F = 2.96$, df $= 2$, $P = 0.055$; Tukey–Kramer test: $P = 0.148$). Males without sex combs showed significantly longer courtship time and copulation latency than control males (Table 2), indicating that they had to expend more effort to achieve a successful copulation, when they could achieve it at all. Copulation latency of males without sex combs is in fact underestimated, since most of them did not mate within the 2 h observation period. In contrast, most control males were accepted by females in the first 30 min. No significant differences of copulation duration were found between males without sex combs and normal males (Table 2). These results suggest that sex combs are required for courtship, but not for secure copulation.

The *rn-Gal4* enhancer trap used to drive the expression of the female Tra protein is expressed not only in the tarsi but also in the wings, antennae, and parts of the genitalia. Thus, the observation that *UAS-traF*; *rn-Gal4* males have reduced mating success is open to alternative interpretations. For example, male courtship songs produced by wing vibration play an important role in *Drosophila* mating behavior (Ritchie et al. 1999), and it is possible that feminization of the wings contributes to the lack of mating success. To examine this possibility, we feminized the

Table 2 Parameters of male courtship behavior (mean ± standard error) as a function of male and female genotypes

♀	♂	Courtship latency	Courtship time	Copulation latency	Copulation duration
Canton-S	Control [a]	6.71 ± 0.85	25.24 ± 4.33	25.63 ± 2.74	24.44 ± 0.67
	rn-GAL4/+; UAS-*traF*/+ [b]	9.67 ± 1.19	29.04 ± 19.79	34.58 ± 5.19	22.69 ± 2.00
	vg-GAL4/UAS-traF [c]	6.18 ± 0.88	14.71 ± 1.89	20.02 ± 1.84	17.82 ± 0.75
WO	Control [d]	7.03 ± 0.98	17.45 ± 3.29	24.49 ± 3.39	24.23 ± 0.68
	rn-GAL4/+; UAS-*traF*/+ [e]	14.13 ± 2.37	49.48 ± 6.98	66.96 ± 6.83	22.40 ± 1.09
	vg-GAL4/UAS-traF [f]	6.46 ± 1.60	14.22 ± 3.16	19.29 ± 3.05	20.32 ± 0.96

Canton-S: ** between *rn*-GAL4/+; UAS-*traF*/+ and *vg-GAL4/UAS-traF*; *** between Control and *vg-GAL4/UAS-traF*

WO: * between Control and *rn*-GAL4/+; UAS-*traF*/+; *** between Control and *vg-GAL4/UAS-traF* (Courtship time); *** between *rn*-GAL4/+; UAS-*traF*/+ and *vg-GAL4/UAS-traF* (Courtship time); *** between Control and *rn*-GAL4/+; UAS-*traF*/+ (Copulation latency); *** between Control and *vg-GAL4/UAS-traF* (Copulation latency); ** between Control and *vg-GAL4/UAS-traF* (Copulation duration)

Virgin females of stains Canton-S and WO were used. Not all the data from Table 1 are subjected to the analysis in this table (see Table 1)

[a] 36 out of 42 control males mated

[b] 11 out of 59 males without sex combs mated

[c] 33 out of 35 males with feminized wings mated

[d] 39 out of 40 control males mated

[e] 30 out 65 males without sex combs mated

[f] 22 out of 23 males with feminized wings mated

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (ANOVA Tukey–Kramer test)

males' wings using the wing-specific *vg-Gal4* driver. These males (*vg*-GAL4/UAS-*traF*) had mating and insemination success of 94.3% and 91.4%, respectively, with Canton-S females, and 95.3% and 90% with WO females (Fig. 2). These proportions are not significantly different from those observed for control males ($\chi^2 = 0.193–1.45$, df = 1, $P > 0.1$), indicating that wing feminization does not affect male mating success. Wing feminization also had no significant effect on courtship latency, courtship time, and or copulation latency (Table 2).

Another potential caveat is that feminization of tarsi and antennae may have affected male courtship behavior. The front legs of males carry several chemosensory bristles that express a male-specific gustatory receptor protein, Gr68a. Inactivation of these sensory organs increases courtship latency and reduces mating success (Bray and Amrein 2003). Antennal sensory organs also express olfactory receptor proteins that are involved in the detection of sex-specific pheromones (Kurtovic et al. 2007; van der Goes van Naters and Carlson 2007). Thus, the low mating success of *UAS-traF/+; rn-GAL4/+* males could in principle be due to their reduced responsiveness to female pheromones, which would lead them to delay courtship or to court females less vigorously. To address these possibilities, we compared the courtship behavior of mutant and control males using video recordings. The courtship latency of *UAS-traF/+*; *rn-GAL4/+* males was indeed increased by 44–100% (Table 2), consistent with the report that loss of male-specific gustatory organs delays courtship (Bray and Amrein 2003). However, once courtship commenced, *UAS-traF/+*; *rn-GAL4/+* males exhibited normal courtship behavior with undiminished intensity and persistence. These males spent significantly more time than control males circling the females, vibrating their wings, and attempting to copulate (Fig. 3). The fact that control males spent less time attempting to copulate is clearly due to their high success rate in the first attempts. Feminized males were observed to attempt copulation up to 270 times within 2 h, whereas control males usually succeeded after 5 or fewer attempts. One possibility is that, as suggested by Bastock and Manning (1955), males may be seeking to overcome rejection by intensifying their courtship. Although feminization of olfactory or gustatory organs is probably responsible for increased courtship latency, it is unlikely to account for the reduced mating success of *UAS-traF/+*; *rn-GAL4/+* males under our experimental conditions. We suggest that the quantitative differences in courtship parameters between males without sex combs and normal males are due to the females' receptiveness to their efforts, rather than to any intrinsic differences in the males' behavior.

Our results confirm the importance of sex combs in *Drosophila* mating behavior. Consistent with previous

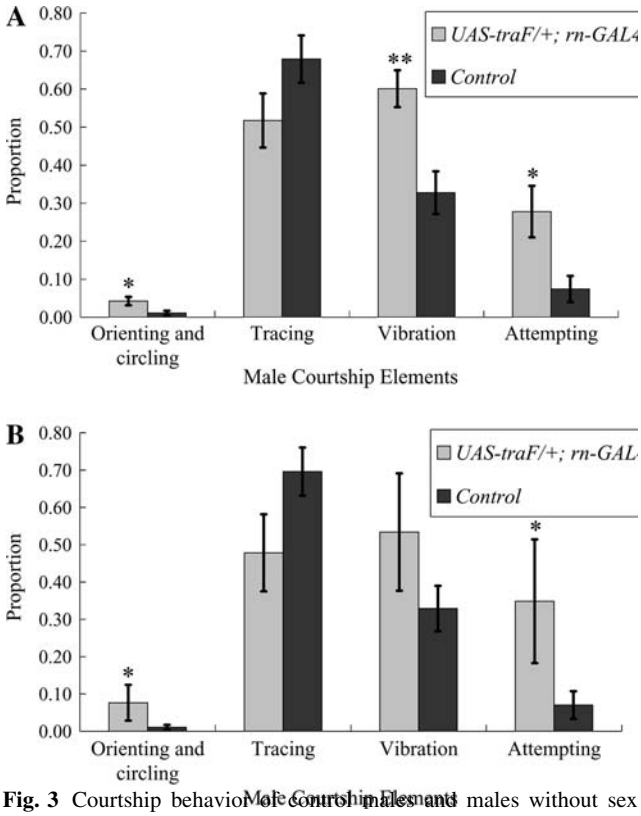

Fig. 3 Courtship behavior of control males and males without sex combs. Bars indicate the proportion of time expended by males on each courtship element. These proportions add up to more than 100% since some courtship elements can be performed simultaneously. Error bars represent standard errors. (**a**) All observed males ($n = 15$ for males without sex combs and control males, respectively). (**b**) Males that mated successfully during the observation period ($n = 3$ and $n = 14$ for males without sex combs and control males, respectively). Significant differences are indicated by asterisks: $*P < 0.05$; $**P < 0.01$ (Student's *t*-test)

findings (Cook 1977; Coyne 1985), the loss of sex combs strongly reduces, but does not fully eliminate, the ability of males to mate with females. In contrast to these earlier experiments, our genetic ablation technique does not remove the sex comb entirely, but merely restores the morphology of its constituent bristles to the ancestral, sexually monomorphic condition found in *Drosophila* species that lack sex combs. Thus, our results demonstrate that the effectiveness of sex combs is due to their modified male-specific morphology and not simply to the presence of bristles on the distal basitarsus. There are two possible explanations for this effect. First, females may be able to detect the size and shape of the sex comb through their mechanosensory organs, and reject males that lack a proper sex comb. Alternatively, males whose sex comb teeth are not curved and oriented properly may be unable to grasp the females' genitalia, preventing them from achieving intromission. In accordance with these different models, the origin and diversification of sex combs could be driven either by changes in female behavioral preferences, or by

co-evolution between sex comb morphology and the shape of female genitalia. Interestingly, the mating success of males without sex combs is strongly affected by the genotype of females they are paired with (Fig. 2). Coyne (1985) has suggested that the function of the sex comb may be to grasp the row of stout "thorn" bristles located on the female ovipositor. We find that the mean number of these bristles differs only slightly between the two genotypes [13.9 ± 0.4 bristles in Canton-S ($n = 15$) versus 15.3 ± 0.5 in WO ($n = 15$); Kolmogorov–Smirnov test, $P > 0.1$]. This mechanical difference could potentially account for the effect of female genotype on the mating success of males without sex combs. However, an equally likely explanation is that the females are able to perceive the shape of the male sex combs or other tactile stimuli through their sensory organs, and to react to this information in a genotype-specific manner. In the future, it would be interesting to use the recently developed phylogenetic framework (Barmina and Kopp 2007) to test for correlated evolution of male mating behavior, sex comb morphology, and female genitalia across species.

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References

Andersson MB (1994) Sexual selection. Princeton University Press, Princeton, NJ

Baker BS, Ridge KA (1980) Sex and the single cell. I. On the action of major loci affecting sex determination in *Drosophila melanogaster*. Genetics 94(2):383–423

Barmina O, Gonzalo M, McIntyre L, Kopp A (2005) Sex- and segment-specific modulation of gene expression profiles in Drosophila. Dev Biol 288:528–544

Barmina O, Kopp A (2007) Sex-specific expression of a HOX gene associated with rapid morphological evolution. Dev Biol 311:277–286

Bastock M, Manning A (1955) The courtship of *Drosophila melanogaster*. Behaviour 8:85–111

Bray S, Amrein H (2003) A putative Drosophila pheromone receptor expressed in male-specific taste neurons is required for efficient courtship. Neuron 39(6):1019–1029

Cook RM (1977) Behavioral role of the sexcombs in *Drosophila melanogaster* and *Drosophila simulans*. Behav Genet 7(5):349–357

Coyne JA (1985) Genetic studies of three sibling species of Drosophila with relationship to theories of speciation. Genet Res 46(2):169–192

Coyne JA, Elwyn S, Kim SY, Llopart A (2004) Genetic studies of two sister species in the *Drosophila melanogaster* subgroup, *D. yakuba* and *D. santomea*. Genet Res 84(1):11–26

Ferveur JF, Stortkuhl KF, Stocker RF, Greenspan RJ (1995) Genetic feminization of brain structures and changed sexual orientation in male Drosophila. Science 267(5199):902–905

Graze RM, Barmina O, Tufts D, Naderi E, Harmon KL, Persianinova M, Nuzhdin SV (2007) New candidate genes for sex comb divergence between *Drosophila mauritiana* and *Drosophila simulans*. Genetics 176:2561–2576

Hannah-Alavah A (1958) Morphology and chaetotaxy of the legs of *Drosophila melanogaster*. J Morph 103:281–310

Hu Y-G, Toda MJ (2001) Polyphyly of Lordiphosa and its relationships in Drosophilinae (Diptera: Drosophilidae). System Entomol 25:1–17

Kopp A, True JR (2002) Evolution of male sexual characters in the oriental *Drosophila melanogaster* species group. Evol Dev 4(4):278–291

Kurtovic A, Widmer A, Dickson BJ (2007) A single class of olfactory neurons mediates behavioural responses to a Drosophila sex pheromone. Nature 446(7135):542–546

Lakovaara S, Saura A (1982) Evolution and speciation in the *Drosophila obscura* group. In: Ashburner, Carson, Thompson (eds) The genetics and biology of *Drosophila*, vol 3b, pp 1–59

Lemeunier F, David J, Tsacas L, Ashburner M. (1986) The *melanogaster* species group. In: Ashburner, Carson, Thompson (eds) The genetics and biology of *Drosophila*, vol 3e, pp 147–256

Macdonald SJ, Goldstein DB (1999) A quantitative genetic analysis of male sexual traits distinguishing the sibling species *Drosophila simulans* and *D. sechellia*. Genetics 153(4):1683–1699

Markow TA, Bustoz D, Pitnick S (1996) Sexual selection and a secondary sexual character in two Drosophila species. Anim Behav 52(4):759–766

McKeown M (1992) Sex differentiation: the role of alternative splicing. Curr Opin Genet Dev 2(2):299–303

Nuzhdin SV, Reiwitch SG (2000) Are the same genes responsible for intra- and interspecific variability for sex comb tooth number in Drosophila? Heredity 84:97–102

Polak M, Starmer WT, Wolf LL (2004) Sexual selection for size and symmetry in a diversifying secondary sexual character in Drosophila bipectinata Duda (Diptera: Drosophilidae). Evolution 58(3):597–607

Ritchie MG, Halsey EJ, Gleason JM (1999) Drosophila song as a species-specific mating signal and the behavioural importance of Kyriacou & Hall cycles in *D. melanogaster* song. Anim Behav 58(3):649–657

Simmonds AJ, Brook WJ, Cohen SM, Bell JB (1995) Distinguishable functions for engrailed and invected in anterior–posterior patterning in the Drosophila wing. Nature 376(6539):424–427

Spieth HT (1952) Mating behavior within the genus Drosophila (Diptera). Bull Am Museum Nat Hist 99(7):395–474

St Pierre SE, Galindo MI, Couso JP, Thor S (2002) Control of Drosophila imaginal disc development by rotund and roughened eye: differentially expressed transcripts of the same gene encoding functionally distinct zinc finger proteins. Development 129(5):1273–1281

Sturtevant AH (1942) The classification of the genus Drosophila, with descriptions of nine new species. Univ Texas Publ 4231:6–51

Tatsuta H, Takano-Shimizu T (2006) Genetic architecture of variation in sex-comb tooth number in *Drosophila simulans*. Genet Res 87(2):93–107

True JR, Liu J, Stam LF, Zeng ZB, Laurie CC (1997) Quantitative genetic analysis of divergence in male secondary sexual traits between *Drosophila simulans* and *Drosophila mauritiana*. Evolution 51:816–832

van der Goes van Naters W, Carlson JR (2007) Receptors and neurons for fly odors in Drosophila. Curr Biol 17(7):606–612

Wiens JJ (2001) Widespread loss of sexually selected traits: how the peacock lost its spots. Trends Ecol Evol 16(9):517–523

Wong BB, Rosenthal GG (2006) Female disdain for swords in a swordtail fish. Am Nat 167(1):136–140