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A *Drosophila* male pheromone affects female sexual receptivity

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Sex pheromones are chemical signals frequently required for mate choice, but their reciprocal role on mate preference has rarely been shown in both sexes. In *Drosophila melanogaster* flies, the predominant cuticular hydrocarbons (CHs) are sexually dimorphic: only females produce 7,11-dienes, whereas 7-tricosene (7-T) is the principal male CH. Males generally prefer females with 7,11-dienes, but the role of 7-T on female behaviour remains unclear. With perfumed males, control females mated faster and more often with males carrying increased levels of 7-T showing that this CH acts as a chemical stimulant for *D. melanogaster* females. Control females—but not antenna-less females—could detect small variation of 7-T. Finally, our finding that *desat1* mutant female showed altered response towards 7-T provides an additional role for this gene which affects the production and the perception of pheromones involved in mate choice, in both sexes.

Keywords: male pheromone; 7-tricosene; female receptivity; antenna; *desat1*; *Drosophila*

1. INTRODUCTION

The multiple sensory signals exchanged during courtship serve to precisely inform potential sex partners about their reciprocal reproductive status. Many studies involving both vertebrates and invertebrates have shown a clear relationship between mating success and the quality of various sex-specific characters, presumably subject to sexual selection (Andersson 1994). Among the sexually dimorphic signals exchanged during courtship, sex pheromones are frequently used for mate discrimination and choice (Wyatt 2003). However, in only a very few examples have the sex pheromones that reciprocally influence the mating response in both sexes been identified (Andersson 1994). In insects, and more specifically in moths, pheromones are multicomponent blends of chemicals, some of which tend to stimulate partner attraction while other components can induce repulsion (Linn & Roelofs 1989; Mustaparta 1996).

In *Drosophila* species, cuticular hydrocarbons (CHs) can differ between the sexes and be involved in mate recognition and preference (Ferveur 2005). In *D. melanogaster*, the predominant CHs of wild-type females, 7,11-dienes, tend (i) to increase male intraspecific courtship and mating and (ii) to inhibit interspecific male sexual activity (Antony & Jallon 1982; Coyne & Oyama 1995; Savarit *et al.* 1999). With conspecific males, 7,11-diene-rich females were preferred by males but produced less daughters (Marcillac & Ferveur 2004).

The male principal CH, 7-tricosene (7-T), is the most efficient pheromone able to prevent or reduce male homosexual courtship (Ferveur & Sureau 1996; Sureau & Ferveur 1999; Svetec & Ferveur 2005), but its role on female mating behaviour remains elusive. Studies, based on the comparison between *D. melanogaster* males that largely varied for their hydrocarbon profile but also for their genetic background (Averhoff & Richardson 1974; Jallon 1984; Scott 1994; Cobb & Ferveur 1996*a*; Coyne 1996), made it impossible to reveal the identity of the substance(s) involved. For example, to verify the hypothesis (Jallon 1984) that 7-T enhances female sexual receptivity, Scott (1994) compared the response of canton-S (Cs) and Tai females to each male (Cs = 7-T rich; Tai = 7-T-poor) with/out synthetic 7-T added on a piece of a filter paper placed in the mating chamber. However, the role that 7-T was playing in female receptivity was debated especially because (i) of the limited number of male types used (Cs and Tai) and (ii) male sexual activity was not measured (Cobb & Ferveur 1996*b*). The possibility of transferring specific CHs by rub-off from donors (Coyne *et al.* 1994) onto receiver flies partially or totally depleted for these CHs (Savarit *et al.* 1999; Marcillac & Ferveur 2004) is a powerful method which overcomes some of these limitations and allows to measure the behavioural effect of CHs carried by flies of a similar genotype.

Drosophila female discrimination and receptivity to male courtship is genetically based (Pineiro *et al.* 1993; Doi *et al.* 2001), and partially depends on the antenna and on a dorso-anterior brain region (Mayr 1950; Petit 1958; Manning 1967; Tompkins & Hall 1983). The distal part of the female antenna (consisting of the arista attached to the third antennal segment) is clearly involved in the perception of male acoustic signals ('love song') and changes in female receptivity (Bennet-Clark & Ewing 1967; Kyriacou & Hall 1982; Göpfert & Robert 2002; Tauber & Eberl 2003). The few studies suggesting a relationship between female receptivity and chemical signal(s), have been either based (i) on mutant females with defective sensory perception (Tompkins *et al.* 1982; Gailey *et al.* 1986), or (ii) on males with abnormal CH profiles (Ferveur & Jallon 1993*b*; Rybak *et al.* 2002).

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But in all these cases, the multiple effects caused by mutant genes and/or by the experimental treatment equally came to no conclusion in this respect.

Here, we tested the effect of male pheromones, and especially that of 7-T which is the principal male-specific component, on female mating activity. First, we used three genetically related males producing radically different levels of 7-T and compared female receptivity to these males. Then, sibling males genetically depleted for 7-T and other unsaturated CHs were perfumed with a slightly variable quantity of 7-T to evaluate whether females could detect smaller variations in male pheromones. The comparison of male courtship towards decapitated and intact females was carried out to assess the respective activities of the male and the female during courtship. We also measured the response of females homozygous for the *desat1* mutation previously shown to alter male perception of sex pheromones (Marcillac *et al.* 2005*a*). Finally, different segments of female antenna were surgically removed to determine their role in male pheromone perception.

2. MATERIAL AND METHODS

(a) Drosophila melanogaster *strains and crosses*

All *D. melanogaster* strains were raised on yeast/cornmeal/agar medium and kept at 24±0.5 °C with 65±5% humidity on a 12 : 12 h light/dark cycle.

We chose homozygous mutant $desat1^{1573\text{-}1}$ males (*desat1*) as tester males because they were drastically depleted for 7-T, while they still show a strong courtship (Marcillac *et al.* 2005*a*). Mutant *desat1* females were also used. These flies contain a transposable PGal4 element inserted in the *desat1* gene.

Homozygous $desat1^{1573\text{-}N2}$ (*N2*) male and female flies were also used. *N2* is an allele resulting from the complete and precise excision of the transposable PGal4 element, and is the best available control genotype to assess the effect of the $desat1^{1573\text{-}1}$ mutation. Indeed, *N2* males showed a fully rescued production and male perception of sex pheromones (Marcillac *et al.* 2005*a*, in press). In preliminary experiments, *N2* females paired with control males showed a mating pattern that resembled those shown by control females.

EP males result from the cross between *desat1* virgin females and *E(p)-10164* males. The *E(p)-10164* strain (a gift of Toshiro Aigaki, Tokyo Metropolitan University) contains a transposable element inserted in *desat1* that can deregulate the expression of this gene under the conditional activation of *Gal4* (Rörth 1996). We used *EP* males because they produced a very high amount of 7-tricosene (7-T). Therefore, all tester males are genetically related: *desat1* and *N2* males are similar, except at the *desat1* locus; *EP* and *desat1* males share the same X chromosome and half of their autosomal genes.

(b) *Characterization of cuticular hydrocarbons*

CHs were analysed from 5 day old intact individual flies by gas chromatography following hexane extraction according to standard procedures (Antony & Jallon 1982; Ferveur 1991). Analyses were performed with a Varian CP3380 chromatograph, equipped with a Cp-sil 25 m capillary column with hydrogen as the carrier gas. All the *D. melanogaster* predominant CHs have already been identified and characterized (Pechine *et al.* 1985). Twenty-four CHs were systematically detected in female flies, and 14 in male flies, both with a chain length ranging from 23 to 29 carbons (see Marcillac *et al.* in press for the complete list of compounds analysed in male and female flies). For the sake of clarity, the only amounts shown here are for 7-tricosene (7-T, 23C), 7-pentacosene (7-P, 25C), *n*-tricosane (23Lin), *n*-pentacosane (25Lin), and the sum of all CHs (SumCHs).

(c) *Hydrocarbon transfer*

We adapted the procedure described by Coyne *et al.* (1994). Briefly, one hundred and fifty 4–7 day old donor males were instantly killed by immersion in liquid nitrogen and placed, after thawing, in a fresh food vial to be confined overnight with fifteen 4 day old tester *desat1* males. The plug of the vial was pushed down to reduce the volume to 2 cm^3, and the vial was held upside down to increase the contact between donors and testers for a period of 14–16 h. Immediately before the test, the plug was pulled up, and each tester male was aspirated without anaesthesia. Tester *desat1* males to be perfumed received the CHs of one of three donor genotypes: *desat1* (shown as *desat1* in the text), *N2* (*N2*) and *EP* (*EP*). The comparison between three perfumed males allowed thus to eliminate any stressful effect caused by the transfer procedure. We do not know whether or not the transferred CHs were deposited homogeneously on the body.

(d) *Female antennal surgery*

One day old *N2* females were anaesthetized with carbon dioxide (CO_2) and their antennae removed under a binocular microscope: both aristae or both funiculi were removed with a pair of dissecting forceps (MC32, Moria, Paris, France). As the arista is attached to the funiculus, the removal of funiculi implies that aristae were also eliminated. Control females were treated with the same experimental procedure, but their antennae were not removed.

(e) *Behavioural experiments*

Two kinds of experiments were carried out: (i) with decapitated females to measure male sexual activity and (ii) with intact females to estimate female willingness to mate. Decapitated females were alive but did not respond to male courtship: this allowed us to assess male heterosexual activity independently of female response. Our results are mostly based on the observation that the frequency and the latency to mate did not follow the propensity to court decapitated females, between male genotypes, and this may reflect the active involvement of the female and her preference to mate.

All flies were isolated under light CO_2 anaesthesia 0–4 h after eclosion. Tester male flies (i.e. those whose sexual responses to target flies were measured) were held individually in fresh glass food vials for 4 days (in case of hydrocarbon transfer) or for 5 days, before testing. Donor males (i.e. those whose hydrocarbons were transferred) were kept in vials in groups of 20 until 4 days old, and females were kept for 5 days in groups of five.

All experiments, carried out with a heterosexual pair of 5 day old flies, were performed in a room at 24±0.5 °C with 65±5% humidity. Tests were performed simultaneously over several days for the tester males of each type and always took place 1–4 h after lights on. Tester males were individually aspirated (without anaesthesia) under a watch glass used as an observation chamber (1.6 cm^3). After 10 min, a virgin female was introduced.

Male courtship index (CI) was measured during a 10 minute period with a single decapitated virgin. CI is the

Table 1. Production of cuticular hydrocarbons in various male flies. (Data shown are mean absolute amounts (±s.e.m.; in ng) for 7-tricosene (7-T), 7-pentacosene (7-P), *n*-tricosane (23Lin), *n*-pentacosane (25Lin) and for the sum of all detected cuticular hydrocarbons (SumCHs). Five day old males had either (*a*) different genotypes: homozygous for the *desat1*$^{1573\text{-}1}$ mutant allele (*desat1*), homozygous for the rescued *desat1*$^{1573\text{-}N2}$ allele (*N2*), F1 sons of the cross between *desat1* virgin females and *10164-EP* males (*EP*), or (*b*) they shared the same *desat1* genotype, but were perfumed by rub-off with the CHs of *desat1* males (*desat1*), of *N2* males (*N2*), or of *EP* males (*EP*). The value and significance of the ANOVA carried out for each type of male is shown below each CH parameter; the letters in brackets indicate the significant differences. (*a*) $n=9$–13; (*b*) 52–83.)

male genotype	perfume	cuticular hydrocarbons 7-T	7-P	23Lin	25Lin	SumCHs
(*a*) *plain genotypes*						
desat1	—	63 (7) (a)	39 (2) (a)	936 (172) (a)	346 (45) (a)	2432 (245) (a)
N2	—	450 (26) (b)	176 (11) (b)	144 (10) (b)	43 (8) (b)	1284 (63) (b)
Ep	—	1294 (59) (c)	156 (8) (b)	57 (8) (b)	11 (1) (b)	2054 (57) (a)
ANOVA *F*		263,50	57,76	30,40	61,71	21,12
(d.f.=29, 2) *p*		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
(*b*) *perfumed genotype*						
desat1	*desat1*	52 (3) (a)	35 (2) (a)	1223 (45) (a)	361(12) (a)	2580 (73) (a)
desat1	*N2*	68 (4) (b)	50 (2) (b)	935 (42) (b)	280 (11) (b)	2147 (72) (b)
desat1	*Ep*	90 (3) (c)	31 (1) (c)	1004 (41) (b)	300 (11) (b)	2281 (71) (b)
ANOVA *F*		39,37	36,91	11,66	12,02	8,65
(d.f.=206, 2) *p*		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

proportion of time that the male spends in active courtship (tapping, wing vibration, licking and attempting copulation). Prior to the test, anaesthetized females were decapitated with a razor blade cleaned with ethanol between each treated genotype. They were kept in groups of 10 in food vials and allowed at least 15 min to recover. Only standing headless females were used for the test.

For the mating experiment, each tester male was paired with an intact or antenna-less (and non-anaesthetized) virgin female, for 1 h. We measured the latency to copulate (time in min from the introduction of the female into the chamber until copulation), the duration of copulation (time in min from the copulation onset until disengagement), as well as the overall frequency of copulating pairs for each treatment. We measured the CH profile of males that were, or were not perfumed.

(f) *Statistics*

All tests compare flies bred and used during the same period of time. To compare the levels of CHs, of CI, of copulation latency and duration between male genotypes or between perfumed males (separately for each female genotype), and between different genotypes or females which had been operated on (with either control or perfumed males), we used an ANOVA with Bonferroni tests (or Fisher's PLSD *post hoc* test for CH levels). Data for copulation latency were log-transformed to normalize their distribution before the ANOVA test. A chi-square homogeneity test was used to compare copulation frequency between two or three samples.

3. RESULTS

(a) *Mating of control females is enhanced with 7-T-rich males*

N2, *desat1* and *EP* males largely differ for their level of 7-tricosene (7-T; table 1*a*). *EP* males produced much more 7-T (1294 ng) than *N2* males (450 ng), and both genotypes showed much higher levels of 7-T than *desat1* males (63 ng). *EP* and *N2* showed similar amounts for all other unsaturated CHs, including 7-pentacosene (7-P) that was much abundant than in *desat1* males ($p<0.0001$). Conversely, *desat1* males produced more saturated linear CHs (including *n*-tricosane=23Lin and *n*-pentacosane=25Lin) than *EP* and *N2* males which had similar levels. Finally, the global amount of CHs (SumCHs) was higher in *EP* and *desat1* males than in *N2* males.

The courtship activity of these three males (CI) as measured to decapitated control *N2* females, was similar (figure 1*a*, white bars). However, intact *N2* females induced a different mating pattern in the three male genotypes: *EP* males mated faster than *N2* males that mated faster than *desat1* males ($p<0.001$; figure 1*b*). Also, *EP* and *N2* males mated more frequently than *desat1* males ($p<0.01$). The comparison with decapitated *N2* females suggests that intact *N2* females detect differences between male genotypes. Since the mating performance of *N2* females was parallel to the quantitative variation of 7-T (*EP*>*N2*>*desat1*), but not that of other substantial male CHs, we tested the involvement of 7-T in female receptivity.

(b) *Female mating is enhanced by increased amounts of 7-tricosene*

To measure the influence of male CHs—and try to rule out the contribution of other characters—on female mating, we exclusively used *desat1* males and perfumed them by rub-off with the CHs provided by donor males of the three genotypes. As *desat1* males are largely depleted for 7-T, CH transfer with either *desat1*, *N2* or *EP* donors yielded perfumed males (respectively, noted *desat1*, *N2* and *EP*; see §2) with the same genotype but carrying different CHs. Rub-off induced significant differences between the three perfumed males for 7-T and 7-P (table 1*b*). Despite the fact that the variation of the absolute amounts of 7-T was moderate (20–40 ng), its relative increase was substantial: *EP* males carried 73% more 7-T than *desat1* and 32% more than *N2* males. The quantitative variation of 7-P was much smaller and

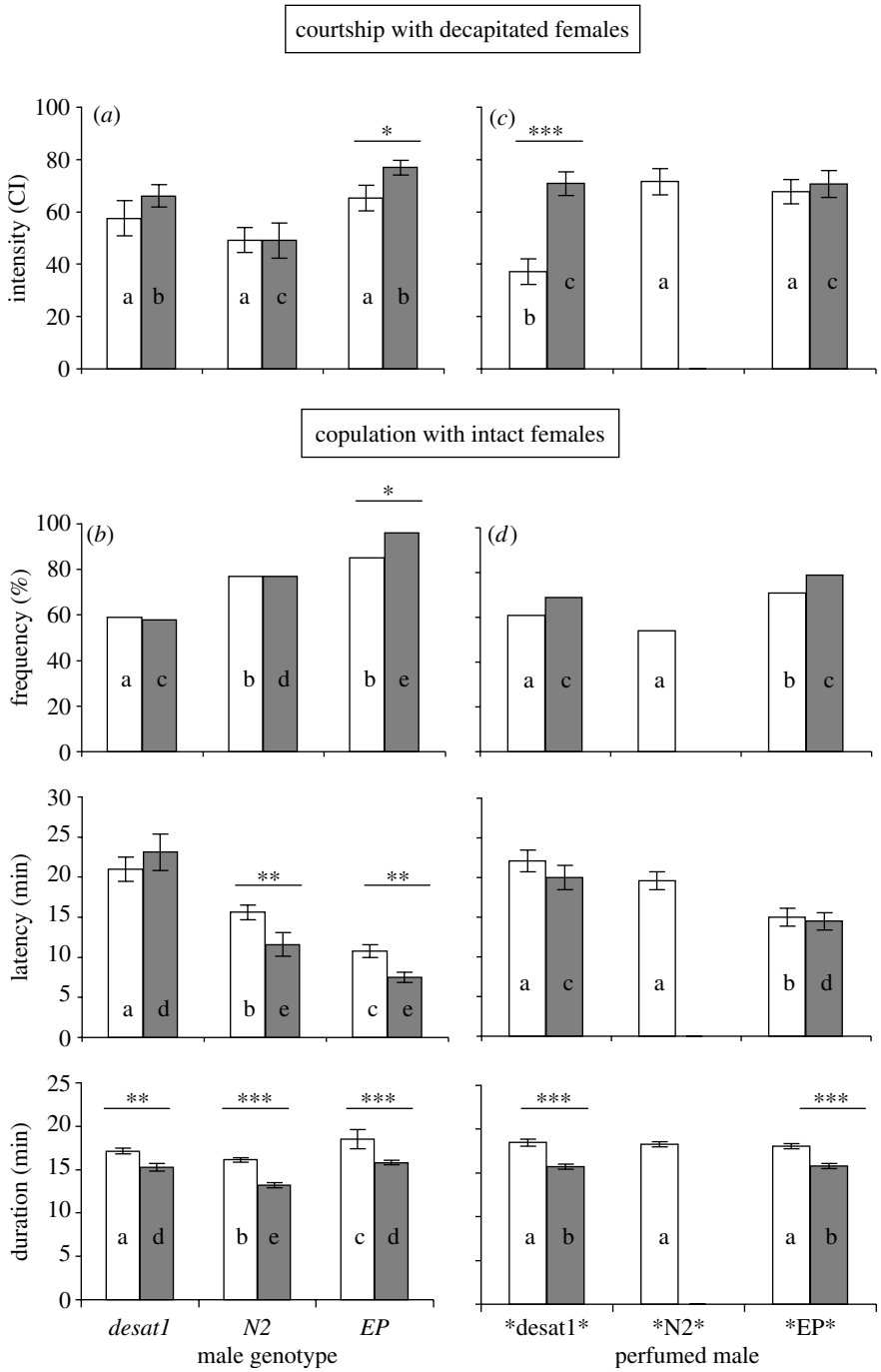

Figure 1. Male courtship and female mating response with various males. Individual 5 day old tester males were either paired (*a*, *c*) with a single decapitated female and their courtship index (CI) was measured, for 10 min, or (*b*, *d*) with an intact female and the frequency of mating, the mean (±s.e.m.) for copulation latency (copulation onset) and for copulation duration (in min) were measured for 60 min. Females were either homozygous for the $desat1^{1573\text{-}N2}$ allele (*N2*; white bars), or the $desat1^{1573\text{-}1}$ allele (*desat1*; grey bars). Tester males either belonged to the *desat1*, *N2*, *EP* genotypes (*a*, *b*) or were *desat1* males perfumed by donors of these three genotypes (*desat1*, *N2*, *EP*; *c*, *d*; see table 1). Statistical differences between different male types (genotype or perfume), measured separately for each female genotype, are shown by the letters inside the histogram bars. Significant differences between the two female genotypes, tested with ANOVA for the three male genotypes or for perfumed males, are indicated above bars as follows: $^{***}p<0.001$; $^{**}p<0.01$; $^{*}p<0.05$. (*a*) $n=22$–47; (*b*) 70–137; (*c*) 23–32; (*d*) 47–111.

followed a different pattern (*N2* > *desat1* > *EP*). Finally, *desat1* males showed higher amounts of linear alkanes (23Lin and 25Lin) than *EP* and *N2* males which had very similar levels. No significant difference was detected for any other CHs (not shown), and *desat1* males showed slightly increased SumCHs.

With decapitated *N2* females, *desat1* males showed a very reduced CI (37.1±4.9) that was nearly half that shown by *EP* and *N2* (with similar CIs: mean=69.6±4.8; figure 1*c*). Intact *N2* females showed an higher mating performance equal to 33% more matings and a copulation onset 8 min earlier—with *EP* males than with the two other perfumed males, which were not different (figure 1*d*). The comparison with decapitated females reveals that intact *N2* females can distinguish *EP* and *N2* (which directed similar CIs to decapitated females), but not *N2* and *desat1* (which showed different CIs). When the mating response of *N2* females

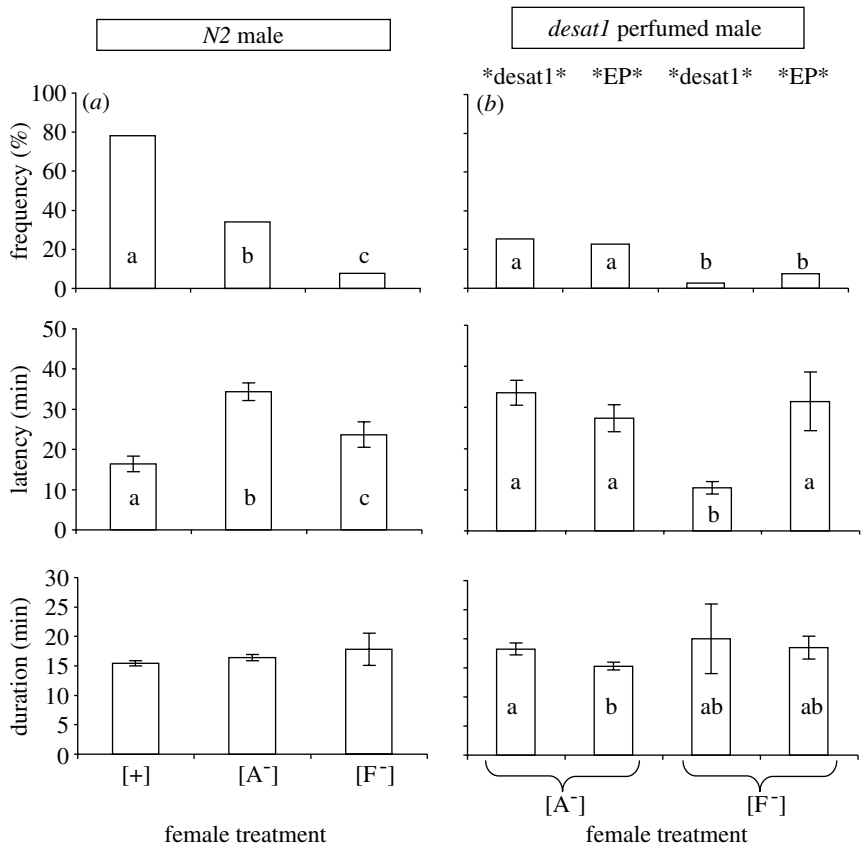

Figure 2. Effect of antennal ablation on female mating with control and perfumed males. Five day old females homozygous for the *desat1*$^{1573\text{-}N2}$ allele (*N2*) that were either intact ([+]), bilaterally ablated for their aristae ([A$^-$]), or for their funiculi ([F$^-$]) were either paired with (*a*) a same age *N2* male or (*b*) with a *desat1* male perfumed with the hydrocarbons of *desat1* (*desat1*), or of *EP* males (*EP*). Data shown represent the frequency of mating, and the mean (±s.e.m.) for copulation latency and duration (in min) measured during a 1 h period. For genotypes, see table 1; for statistics, see figure 1. n=63–82 except for [+] females with *N2* males (32).

obtained with the six male types (three genotypes and three perfumed males) was ranked by increasing efficiency (higher mating frequency combined with shorter latency: *EP*>*N2*>*EP*>*N2*≥*desat1*≥*desat1*), it followed the pattern for decreasing quantity of 7-T (table 1).

(c) desat1 *is partially involved in female perception of male pheromones*

We measured the effect of the *desat1* mutation in homozygous females that were tested (similarly to *N2* control females) with *EP*, *N2* and *desat1* males, and also with *desat1* perfumed males (*EP* and *desat1*; figure 1, grey bars).

With decapitated *desat1* females, males showed a CI pattern that resembled those directed to *N2* decapitated females, and *N2* males showed the weakest CI. However, if intact mutant females clearly distinguished *desat1* males (58% pairs copulated after 23 min) from *N2* and *EP* males, they showed only a slightly different response towards these two male genotypes: (i) their copulation latency was not different (and occurred 13 min earlier than with *desat1* males and (ii) more mutant females mated with *EP* (96%) than with *N2* males (78%), but this could reflect different male CI.

Then, we compared the attractivity of *desat1* females and their response to *desat1* versus *EP* males (*N2* males which had the same influence as *desat1* males on *N2* females were not tested). The two perfumed males directed similar CIs to decapitated *desat1* mutant females, and were poorly distinguished by intact *desat1* females: if mating was faster with *EP* males, both perfumed males induced similar mating frequencies. This result differs from that obtained with *N2* females which showed both more frequent and faster matings with *EP* males than with *desat1* males.

N2 males had a shorter copulation duration than *EP* and *desat1* males, but the CH transfer induced no difference. In all cases, copulation duration was shorter with mutant females than with *N2* females, and this variation follows the CH difference between the two female genotypes (*N2* are rich—and *desat1* are poor—in 7, 11-dienes; Marcillac *et al.* in press).

(d) *The female antenna is necessary for perception of male pheromones*

To determine the role of the female antenna in the perception of male courtship signals, the mating behaviour of control *N2* females bilaterally deprived either of their aristae (A$^-$) or of their third antennal segments, or funiculi (F$^-$), was measured.

First, to measure the effect of antennal ablation on female behaviour, control, A$^-$, and F$^-$ females were paired with control *N2* males (figure 2*a*). The mating frequency (77%) and the copulation latency (16 min) of intact *N2* females paired with homotypic *N2* males were both very close to the values previously obtained with similar genotypes (figure 1*b*). The two types of antennal ablation drastically decreased mating frequency, and F$^-$

females mated less frequently than A^- females (respectively, 8 and 34%). Although both operated females took more time to mate than intact ones, surprisingly, F^- females mated faster than A^- females.

To assess the role of the female antenna on the perception of male pheromone, *N2* females which had been operated on were either paired with perfumed *EP* or *desat1* males, and their mating performance was compared (figure 2*b*). With both perfumed males, females which had been operated on showed mating frequencies very close to those obtained with *N2* males: only 24% A^- and 6% F^- females mated. However, very few—if any—of the females which had been operated on distinguished the two perfumed males. This contrasts with the discriminatory response of intact *N2* females paired with similarly perfumed males (figure 1*d*), and suggests that antennal ablation affected female perception of the male pheromone. As with *N2* males (figure 2*a*), F^- females mated faster than A^- females with *desat1* males. Finally, antennal ablation had no or very little effect on copulation duration.

4. DISCUSSION

Darwin (1874) postulated that if the most odoriferous males were the most successful in winning females, male odours should constitute a sexually selected trait. The sexual selection of a male scent was initially proposed to explain the 'rare-male' effect observed between strains of *Drosophila pseudoobscura* (Leonard *et al.* 1974; Leonard & Ehrman 1976). However, the active substance(s) was not identified, and the rare-male effect may be explained by an experimental artefact (Bryant *et al.* 1980; Partridge 1988). More recently, a functional coupling between acoustic and chemical signals obtained with transgenic males depleted for CHs and surgically deprived of wings was postulated (Rybak *et al.* 2002). Again, the nature of the chemical signal(s) was not revealed, but the variation of male behaviour could also be a by-product of the experimental treatment, which severely affected survival and behaviour in these flies (Savarit *et al.* 1999; Savarit & Ferveur 2002).

(a) *Female perception of male pheromones*

Our data suggest that 7-tricosene (7-T) is a male-specific trait preferred by *D. melanogaster* females. A clear relationship was found between the amount of 7-T carried by the tester male (table 1) and his mating efficiency with control females, and this effect was not related to the variation of male courtship (with decapitated females; figure 1). The fact that control females could detect an increase of 20–40 ng of 7-T transferred to the cuticle of *desat1* males suggests that 7-T was not masked by closely related unsaturated CHs like 7-P whose production was drastically decreased in *desat1* males (Marcillac *et al.* in press). Indeed, the more successful types of male had generally a higher 7-T : 7-P ratio (2.90) than the less successful ones (1.42). If this is true, this means that *Drosophila* females can discriminate male pheromones based on the ratio between the principal component (7-T) and less abundant related molecules (7-P), similarly to moths (reviewed in Wyatt 2003).

With different tools, we impaired female discrimination of male pheromones: *desat1* females mated faster (and more frequently) than genetically related control *N2* females, whereas funiculi-less females mated faster (but less frequently) than aristae-less females. This shows that the female latency to copulate is, by itself, not a reliable indicator of receptivity. The effect of 7-T on female willingness to mate seems to be better reflected when both the latency and the mating frequency were examined together. With *N2* females, 7-T seems to induce a dose-dependent effect above a 70–90 ng threshold: no behavioural variation was observed between males carrying less than 70 ng of 7-T (with *desat1*, *desat1* and *N2* males, 54–61% matings occurred after a 20–22 min latency), but with doses of 7-T increasing from 90 to 1300 ng, females proportionally enhanced their mating frequency (71–85% with *EP*, *N2* and *EP* males) and decreased their mating latency (15–10 min). If the mating frequency of aristae-less females was close to the values obtained in previous studies (Petit 1958; Manning 1967), 'our' funiculi-less females mated less frequently than aristae-less females, and this difference was not previously reported. This indicates that the aristae and the funiculi both exert additive effects on female perception of male-specific signals. We believe that aristae-less females mated less frequently and showed a longer latency than control females, probably because they were much less receptive to—but still perceived—male signals. The faster matings observed with funiculi-less females indicate that in rare cases, males forced copulation with an unreceptive female. However, our results do not indicate whether other chemosensory structures are also involved in male pheromone perception.

Finally, the fact that *desat1* males—but not other perfumed or non-perfumed males—showed a much lower courtship of *N2* females (but not of *desat1* females) suggest that males can perceive the CHs transferred on their own cuticle and compare them to the CHs carried by the female.

(b) *Evolution of pheromonal communication*

Could the amount of 7-T be used by the *Drosophila* female as a indicator of the 'good genetic quality' of the male (Williams 1966) as shown for a male acoustic signal: the pulse song (PS)? This male trait, which provides an indication of male quality, was preferred by females: PS frequency was correlated with the survival rate of male's progeny in *Drosophila montana* (Hoikkala *et al.* 1998), and the amount of PS offered during courtship was positively correlated with increased mating in *D. melanogaster* (Talyn & Dowse 2004). We hypothesize that the high proportion of 7-T in males reflects their high degree of genetic and physiological homeostasis: 7-T was often decreased in males with altered genes or facing environmental stress (Ferveur & Jallon 1993*a*,*b*; Cobb & Ferveur 1996*a*; Sureau & Ferveur 1999; Savarit & Ferveur 2002).

If 7-T exerts such a marked effect on female preference, this substance should be abundant on the cuticle of all *D. melanogaster* males. This is not the case and males show an important quantitative variation for their 7-T/7-P ratio: in strains collected in temperate area, males have a much higher ratio (4.5 in Cs males) than males of tropical and equatorial strains (0.1 in Tai males; Sureau & Ferveur 1999). The persistence of 7-T-poor males could be explained if some females do not prefer males with higher

doses of 7-T. This is consistent with the observation that Tai-like females were less accurate than Cs-like females in discriminating males with different CH profiles (Scott 1994; Haerty *et al.* 2002). However, as these strains are diverging for many genetic loci (Ferveur & Jallon 1996; Haerty *et al.* 2003), this pheromonal difference may not be related only to the behavioural variation, but could also reflects adaptation to variable climatic conditions (Rouault *et al.* 2004).

Males with low levels of 7-T (and high levels of 7-P) are mostly found in Africa, where the *D. melanogaster* ancestor is supposed to have arisen, 3–4 Myr ago (Lemeunier *et al.* 1986; David & Capy 1988). The expansion of this species to a colder environment could have relaxed the selection pressure that kept 7-T at a low level (in relation to its possible primary effect on desiccation; Gibbs 1998), subsequently allowing males of some populations to increase their 7-T production. In theory, a new variation of a signal could either reinforce a gradual divergence that already exists between two allopatric populations with postmating incompatibility (Dobzhansky 1937), or induce a rapid divergence if a high correlation between the emission and reception of the signal exists in a metapopulation (Lande 1981). In the latter case, a high positive assortative mating could reduce the gene flow with the principal population (Butlin 1987). This fits well with the effect of *desat1*: this gene changes (i) the amount of the principal female and male pheromones (7,11-dienes and 7-T), two critical signals for mate choice by conspecifics together with (ii) male and female perception of these sex pheromones (Marcillac *et al.* 2005*a*,*b*, in press). The present study also suggests that *desat1* females perceived the difference between the three males less accurately than *N2* females and supports the hypothesis that *desat1* females have partially—but not totally—lost their ability to discriminate male differences including the variation of male CHs.

We do not know whether the pleiotropic effect of *desat1* is a unique example, but several aspects of the pheromonal communication system described in the *D. melanogaster* subgroup of species resembles those discovered in *Drosophila serrata* and *Drosophila birchi*. In these species, male and female flies use different CHs for mate recognition, and these pheromones are subject to directional sexual selection of a similar strength (Blows & Allan 1998; Chenoweth & Blows 2003). The genetic correlation between male and female components of the mate recognition system can rapidly coevolve suggesting that these traits are controlled by a small number of major genes (Blows 1999; Higgie *et al.* 2000). Therefore, subtle molecular changes in *desat1*, which links pheromonal emission and reception, could be related to examples of incipient speciation such as in the Zimbabwe area: Zimbabwean flies tend to mate in homogamy (Wu *et al.* 1995), and the gene(s) segregating with that behavioural variation have been mapped to a cytogenetic region that includes *desat1* (Fang *et al.* 2002). Moreover, Zimbabwean flies also differ for the *desat2* gene (flanking *desat1*) which affects cold adaptation in the fly (Greenberg *et al.* 2003).

In summary, we show that the principal *Drosophila* male cuticular pheromone (7-tricosene) can change female receptivity and mating behaviour. This finding should help us to better understand the implication of pheromonal communication in sexual selection and isolation.

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