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# A set of female pheromones affects reproduction before, during and after mating in *Drosophila*

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**Summary**

**Sex pheromones are chemical signals used for mate attraction and discrimination in many invertebrate species. These compounds are often complex mixtures with different components having different effects. We tested live *Drosophila melanogaster* mutant female flies genetically depleted for unsaturated cuticular hydrocarbons, which were then perfumed with these substances to measure their influence on various aspects of reproduction. Female pheromones of the control Cs strain enhanced female attractivity, copulation duration and tended to decrease the number of female progeny of mutant females mated with Cs males, but no dose-dependent effect was found. Cs and variant males showed different response to Cs female pheromone, suggesting a strain-specific coadaptation of female and male characters. The fact that female pheromones induced reciprocal effects on the frequency of the genes contributed by females and males suggests that these substances regulate coevolutionary processes between the sexes.**

Key words: sex pheromone, copulation duration, pheromonal communication, *Drosophila*, *desat1*.

## Introduction

Chemical signals are among the most reliable biological cues and allow many organisms to accurately find landmarks in a environment rich in sensory interactions. For that reason, pheromones are frequently used in mate choice and discrimination (Bradbury and Vehrencamp, 1998). In many invertebrate species, sex pheromones are necessary for the rapprochement of sex partners, to elicit or prevent courtship and reproduction (Wyatt, 2003). Generally, each potential sexual partner emits chemical signals that must be correctly perceived by his/her mate to induce an adaptative behavioural response. With regard to reproduction, the specificity of pheromonal signals is of decisive importance and any change in coding or decoding the pheromonal signal can have dramatic consequences on species reproduction and formation (Boake, 1991). If the change in the signal is matched by the change in its reception, this variation could be at the origin of a new species (Roelofs et al., 2002). Despite an abundant literature on the communication by sex pheromones, there has been very little experimental investigation of the genetic architecture underlying this communication system (Panhuis et al., 2001).

The precise pheromonal influence of the molecular components of a complex bouquet is difficult to measure in live animals because the experimental methods involved in isolating and testing chemicals do not closely parallel the *in vivo* situation. In *Drosophila melanogaster*, two experimental *in vivo* approaches have been used to dissociate and measure the behavioral influence of some of the cuticular hydrocarbons (CHs) in the pheromonal bouquet of mature females (16 CHs) and males (12 CHs; Jallon and David, 1987). (1) Mutant strains with defective CHs have been induced (Ferveur and Sureau, 1996; Ferveur et al., 1997; Savarit and Ferveur, 2002), and (2) flies have been covered with foreign CHs from 'donor' flies of different genotypes (Coyne et al., 1994). Both approaches have been used separately to measure the influence of female and male CHs on male intra- or interspecific courtship and mating behaviours (Ferveur and Sureau, 1996; Coyne, 1996; Coyne and Charlesworth, 1997), or combined to produce mutant CH-depleted females perfumed with the CHs of donor females of different species (Savarit et al., 1999). Several studies revealed that 7,11-dienes in female *D. melanogaster* prevent interspecific courtship and copulation (Coyne and Oyama, 1995; Savarit et al., 1999).

*D. melanogaster* females show a natural polymorphism for the amount of two heptacosadiene (HD) isomers: 7,11-HD is predominant in most strains (e.g. Canton-S=Cs), whereas 5,9-HD levels are relatively high in Caribbean and sub-Saharan strains (e.g. Tai; Ferveur et al., 1996). The 7,11-HD:5,9-HD ratio is entirely controlled by *desat1* and *desat2*, two closely linked genes on chromosome 3 (Coyne et al., 1999; Dallerac et al., 2000). A mutation in the promoter of *desat2* has been correlated with variation in HD ratio (Takahashi et al., 2001), and a case of incipient speciation in Zimbabwe strains seems

to be caused by variation in *desat2* (Ting et al., 2001; Fang et al., 2002). We recently induced a mutation in *desat1* that drastically affects the production of all unsaturated CHs including the predominant sex pheromones in both sexes, together with male discrimination of these signals (F. Marcillac, Y. Grosjean and J.-F. Ferveur, unpublished observations). Moreover, the same *desat1* mutation affects the disengagement of mutant females after copulating with mutant males. The alteration of various reproductive characters induced by *desat1* suggests that this gene may be related to the evolution of pheromonal communication in *Drosophila*.

Here, we have measured the effect on reproduction of a set of female pheromones that includes 7,11-dienes. These pheromones, which are largely reduced in *desat1* mutant females, were replaced by contact with control Cs females. Their effect was evaluated on mate choice of Cs males, and on both copulation latency and duration of the males of various strains. The effect of Cs female pheromones was also measured on the number and sex-ratio of their offspring. Our results indicate that these substances affect several reproductive characters that occur before, during and after mating.

## Materials and methods

### *Strains and flies*

All experiments and fly husbandry took place at 24.5±0.5°C and 65±5% humidity.

Canton-S (Cs) is a widely used control strain of *Drosophila melanogaster*. Tai is a CH-variant strain from Ivory Coast that has been kept in the laboratory for over two decades (Jallon and Pechiné, 1989). The $desat1^{1573\text{-}1}$ mutant strain was induced by the insertion of a single P*GawB* (or P*Gal4*) transposon (Brand and Perrimon, 1993). The gene altered by the P*Gal4* transposon was mapped after cloning and sequencing of the two DNA fragments flanking the insertion point. The comparison with the BDGP database revealed that these fragments share a complete identity with two contiguous sequences of the *desat1* gene (Marcillac et al., in press). To generate derivative lines of $desat1^{1573\text{-}1}$, we jumped out the P*Gal4* transposon (Cooley et al., 1988). When these lines were stabilized, all $desat1^{1573\text{-}excision}$ alleles were outcrossed with the Cs strain for five generations to homogenize genetic background and to obtain transposon-less male flies that were used in behavioural tests. We tested five $desat1^{1573}$ alleles: $desat1^{1573\text{-}1}$, $desat1^{1573\text{-}J2}$ and $desat1^{1573\text{-}E1}$, which are affected for both their CH production and male discrimination, and $desat1^{1573\text{-}N2}$ and $desat1^{1573\text{-}D'1}$ alleles, which are totally rescued for their CH production and totally (N2) or partly (D′1) rescued for male ability to discriminate sex pheromones (F. Marcillac, Y. Grosjean and J.-F. Ferveur, unpublished observations).

### *Detection of cuticular hydrocarbons*

Extraction of cuticular hydrocarbons (CHs), gas chromatography of the extracts and estimation of CH quantities were performed on single 5-day-old flies washed in hexane following a standard procedure (Ferveur, 1991). Quantification of CHs was carried out by using a Varian CP3380 chromatograph (Walnut Creek, CA, USA), equipped with a Cp-sil 25 m/0.25 mm capillary column, with hydrogen as the carrier gas. CHs were calibrated with an added standard of hexacosane.

### *Transfer of hydrocarbons*

Virgin mutant females were crowded in a 4 ml space in a tube with fresh food together with Cs donor females, the day before the test. To change the proportion of unsaturated CHs, the total number of females was kept constant (~80), and the mutant: donor female's ratio varied between 40:40 and 65:15. The ratio of unsaturated: saturated CHs was used to measure the quantity of CHs transferred by donor Cs flies relative to the native CHs carried by the receiver mutant females. To control the effect of this experimental procedure, 80 *desat1* females were crowded using similar experimental conditions. Females were distinguished by wing clipping, which was rotated between genotypes. CH extraction and analysis were performed on some of the flies that were to be used the same day in behavioural tests. Choice tests were carried out with 16 independent samples (with $12 \leqslant N \leqslant 30$).

### *Courtship and mating tests*

Most experiments were carried out with mutant $desat1^{1573\text{-}1}$ females covered with exogenous CHs, either provided by Cs, *desat1* or Tai female donors. Male behaviour was tested using two control strains (Cs and Tai), and with males homozygous for the five $desat1^{1573}$ alleles described earlier. Flies were isolated 1–4 h after eclosion. All males were isolated in a food vial while females were kept in groups of five until 4 days old, and crowded 24 h before the test. All tests took place 1–4 h after lights on, which is the period during which flies are sexually more active (Tauber et al., 2003). Tester males were individually aspirated into an observation chamber (3.5 $cm^2$), and after 5 min, one or two females (for non-choice or choice experiments, respectively) were introduced. We measured the latency to copulate (time in minutes from the introduction of the female into the chamber until copulation) and the duration of copulation (time in minutes from the copulation onset until disengagement), during 1 h test experiments. In choice tests, target females were distinguished by wing-clipping, which was rotated within treatments. Wing-clipping induced no detectable effect on the behaviour tested here (data not shown).

The target flies used for the simultaneous discrimination test were decapitated a few minutes prior to the test. The simultaneous discrimination index (SDI) measures the difference of the courtship indices ($CI_1$–$CI_2$) that a single subject male directed towards two different headless flies (a female and a male of the same strain) during a 5 min observation period, under red light. CI is the cumulative amount of time in minutes that the male spends in active courtship (wing vibration, licking and attempting copulation). Experiments performed in red light with headless object flies

eliminated most of visual and acoustic signals and enhanced the role of pheromones.

### *Measure of fertility, fecundity, sex ratio and survival*

Immediately after mating, each female was transferred alone into a food vial and allowed to lay eggs for 1 week. A female was considered fertile when she yielded at least one viable adult offspring. To measure fecundity, male and female viable adult offspring were counted each day for the 8 days following the first day of adult eclosion. Same-sex flies were pooled, and the sex ratio (female:male) was calculated. The total progeny yielded by each female was also noted. One week after mating, isolated female flies were transferred and pooled by groups of 8–10 in a fresh food vial, according to experimental treatment. Females were transferred to a new food vial every 5 days, and the number of survivor(s) was noted at each transfer.

### *Statistical analysis*

The homogeneity of distribution between samples was tested using a $\chi^2$ test with a Yates correction. Treatments were compared using Student's *t*-test or ANOVA (for one condition over three groups or two conditions over two groups). The two-by-two comparison between the samples was performed with a *post-hoc* PLSD Fisher test (significance taken as $P<0.05$). A simple regression analysis was carried out to test the significance of CH ratio and mating preference between 16 independent groups.

## Results

Preliminary experiments showed that wild-type Cs virgin females mated faster than *desat1* mutant females in individual tests with a Cs male ($P=0.0097$; d.f.=277; $t=2.60$). Cs females also induced longer copulation duration than mutant females ($P=0.0004$; $t=3.61$). To measure directly the influence of Cs female CHs on male precopulatory and copulatory behaviours, *desat1* females genetically depleted for most of their desaturated CHs, were covered with the CHs of either control Cs females (desat1*Cs*=perfumed) or of mutant females (desat1*desat1*=non-perfumed). Perfumed and non-perfumed females were tested either in choice tests (2 females) with a single tester Cs male, or in no-choice tests with single males of various genotypes.

### *Effect of Cs female cuticular hydrocarbons on mate choice by Cs males*

In each choice test, two mutant females (one perfumed and one non-perfumed) competed for a single Cs male. Out of 250 tests, the first mating occurred more often with a perfumed female ($N=163$) than with a non-perfumed female ($N=87$; $P<0.0001$; binomial test). During the 1 h period, a second mating occurred with a perfumed female in 66 cases and with a non-perfumed female in 74 cases.

To test for possible dose–response effects of Cs female CHs on mate choice, we changed the number of 'donor' Cs females relative to *desat1* mutant 'receiver' females. Depending upon the donor:receiver ratio, between 90 and 570 ng of 7,11-dienes were transferred (~350 ng in Cs females). The highest but non-significant correlation ($P=0.21$) was noted when the unsaturated:saturated CH ratio was plotted against the relative success of perfumed females in the choice test. This indicates that the relative abundance of Cs female CHs induced no dose–effect response in male preference.

In non-choice tests, Cs males also copulated more frequently with perfumed (88.7%; $N=283$) than with non-perfumed mutant females (77.5%, $N=227$; $P=0.0012$; $\chi^2=10.71$).

### *Effect of Cs female cuticular hydrocarbons on male courtship of various strains*

CHs from Cs females induced significant changes in the latency to copulate of Cs males, which mated faster with perfumed ($P=0.0056$; Table 1) than with non-perfumed desat1 females. Conversely, males of the other strains (Tai, *desat1* and four $desat1^{1573\text{-}excision}$ alleles with different sex pheromone discrimination abilities: *N2>D′1>E1>J2*; see Materials and methods) did not change their copulation latency, which was similar to perfumed and non-perfumed females (and also to desat1*Tai* perfumed females with Tai males; *P*=n.s.; Table 2).

We measured the quality of discrimination of sex pheromones in Tai males placed under red light and in the presence of two decapitated flies, one female and one male. Their simultaneous index of discrimination (SDI; see Materials and methods) was equally high with Cs and with Tai target flies (31.4±3.7 and 26.9±4.6, respectively; $N=40$). These values are close to the SDI shown by Cs males toward homotypic flies (33.9±5), indicating that both Cs and Tai males can discriminate sex-specific chemical signals, unlike mutant $desat1^{1573\text{-}1}$ males, which cannot discriminate the sex pheromones of Cs flies (F. Marcillac, Y. Grosjean and J.-F. Ferveur, unpublished observations).

### *Effect of female cuticular hydrocarbons on copulation duration*

In choice experiments with a single Cs male, CHs of donor Cs females induced a strong effect on the copulation duration (i.e. the time from the latency to copulate until disengagement) between 'first mating' females: copulation lasted longer with perfumed than with non-perfumed females ($P=0.0003$; Table 1). Moreover, a weaker effect (although non-significant) was also detected on the copulation duration of 'second mating' females.

In the no-choice experiment, the CHs of Cs females clearly increased the duration of copulation in males of most strains. The copulation of Cs males lasted longer with perfumed female than with non-perfumed $desat1^{1573\text{-}1}$ females ($P<0.0001$; Table 1). Tai males also copulated longer with perfumed desat1*Cs* and desat1*Tai* females than with non-perfumed females ($P<0.0001$; Table 2). Males with the four excision alleles (*N2*, *D′1*, *E1* and *J2*) also increased their copulation duration with perfumed females ($0.0006<P<0.0001$), but not $desat1^{1573\text{-}1}$ males (*P*=n.s.).

Table 1. *Influence of female pheromones on the mating of Cs males*

| Mating experiment with Cs | Female | | | Copulation time (min) | |
|---|---|---|---|---|---|
| | Mating rank | Source of perfume | *N* | Latency | Duration |
| No-choice | 1 | desat1 | 176 | 17.57±1.94 | 13.96±0.21 |
| | 1 | Cs | 136 | 10.90±1.05 | 15.74±0.22 |
| *t*-test d.f.=310 | | | | *P*=0.0056 | *P*<0.0001 |
| Choice | 1 | desat1 | 87 | 9.13±1.67 (a) | 13.17±0.28 (a) |
| | 1 | Cs | 163 | 9.32±1.40 (a) | 14.65±0.21 (b) |
| | 2 | desat1 | 74 | 33.89±3.14 (b) | 13.16±0.29 (a) |
| | 2 | Cs | 66 | 30.27±2.16 (b) | 13.92±0.46 (a,b) |
| ANOVA d.f.=386,1,1 | | | | | |
| A CH transfer | | | | *P*=0.41 | *P*=0.0003 |
| B Mating rank | | | | *P*<0.0001 | *P*=0.23 |
| A×B | | | | *P*=0.36 | *P*=0.24 |

Single Cs males were tested with either a single (no-choice), or two (choice) intact female(s), for 1 h. All tested females, homozygous for the *desat1* mutation and genetically depleted for most their unsaturated cuticular hydrocarbons (CHs), were covered by ruboff with the CHs of Cs females (perfumed) or with the CHs of desat1 females (non perfumed).

1 and 2, respectively, represent the first mating and the second mating females of choice experiments.

Copulation latency, time from the beginning of the experiment until copulation; copulation duration, time from copulation onset until the end of copulation.

Values are means ± S.E.M. For the choice experiment, statistical difference (ANOVA; $P<0.05$) is represented by different letters (in parentheses).

### *Effect of cuticular hydrocarbons from Cs females on the number and sex ratio of the progeny*

Given that the CHs of Cs females increased copulation duration, we measured the consequence of this variation on fertility and noted the number of female and male adult offspring for each mated female, according to her CH status. The CHs of Cs females induced no effect on the fertility of all tested *desat1* females (0.896 and 0.878, respectively measured for 365 perfumed and 337 non-perfumed females; *P*=n.s.). However, the fecundity was significantly reduced in perfumed females compared to non-perfumed females, in both non-choice and choice tests. In non-choice tests, perfumed females produced significantly fewer daughters than non-perfumed females ($P=0.016$; Table 3) whereas male progeny did not change. When first and second-mating females used in the choice experiment were pooled, female CHs had a significant influence on fecundity ($P=0.0268$; d.f.=388; $t=2.22$). These perfumed females produced ~20% fewer viable daughters and their sex ratio was significantly lower ($P=0.034$; $t=2.12$) than in non-perfumed females (respectively, 0.978±0.046 and 1.16±0.083). A two-way ANOVA showed a significant interaction between the two factors (CH transfer and mating rank) and revealed that the CH effect on female progeny was only significant for 'second-mating' females ($P=0.0045$; d.f.=137; $t=2.88$). The difference in sex ratio between second-mating females was only just non significant ($P=0.062$; d.f.=137; $t=1.88$), with a tendency towards an excess of female offspring in non-perfumed females (1.26) as compared with perfumed females (0.95).

The total progeny (daughters + sons) was slightly different ($P=0.0318$) for second-mating females, probably because of the variation in daughter offspring.

We also measured (i) the frequency of female remating 14±1 days after the first mating, and (ii) the survival of mated females, and found no significant effect of CH transfer (data not shown).

## Discussion

### *Role of female pheromones*

We have used a new experimental strategy to reveal the role of female unsaturated hydrocarbons by replacing those that are present on *D. melanogaster* Cs females on the cuticle of genetically depleted *desat1* females. These data, obtained with a unique female genotype, allow us to evaluate precisely the behavioural effect of the substances replaced by ruboff. Cs female pheromones (largely composed of 7,11-dienes) affected different aspects of reproduction before, during and after copulation with Cs males. Our data suggest that these female substances can act both as attractant and 'copulation termination' pheromones, which can 'start' and 'stop' homotypic male mating behaviour.

Although the stimulatory effect of 7,11-dienes on male wing vibration was shown more than two decades ago (Antony and Jallon, 1982), this is the first demonstration that 7,11-dienes can increase the frequency and rapidity of Cs males to mate. Furthermore, we show that moderate amounts of female CHs are sufficient to elicit a maximal mating response because no dose–response effect was detected. This indicates that the threshold of detection by Cs males is low. This hypothesis is supported by the findings that a small amount (~50 ng) of 7,11-dienes can elicit a high courtship response in another 7-T rich male (55B-Gal4; Ferveur and Sureau, 1996), and that increased amounts of 7,11-dienes acquired by transfer did not increase

male courtship in 7-P-rich Tai and in 7-T-rich Ives strains (Coyne et al., 1999). However, our study did not mix the CHs of two wild-type strains, or of flies of both sexes, but instead replaced the unsaturated CHs that were quasi-absent in mutant *desat1* females.

There are several reasons why 7,11-dienes are often found in much higher levels than necessary to stimulate courtship and mating of homotypic males. These substances play a strong role in sexual isolation by preventing male interspecific courtship and copulation (Coyne and Oyama, 1995; Savarit et al., 1999)

Table 2. *Influence of female pheromones and of male genotype on mating performance*

| Male | Female, source of perfume | *N* | Copulation time (min) | |
|---|---|---|---|---|
| | | | Latency | Duration |
| Tai | desat1 | 59 | 11.46±1.27 | 9.90±0.22 (a) |
| | Cs | 67 | 13.04±1.74 | 11.01±0.21 (b) |
| | Tai | 38 | 11.53±1.24 | 11.21±0.23 (b) |
| | | ANOVA d.f.=161, 2 | *P*=0.69 | *P*<0.0001 |
| *desat1*$^{1573\text{-}1}$ | desat1 | 70 | 14.66±1.85 | 17.67±0.36 |
| | Cs | 91 | 13.91±1.49 | 17.21±0.33 |
| | | *t*-test d.f.=159 | *P*=0.75 | *P*=0.27 |
| *desat1*$^{1573\text{-}N2}$ | desat1 | 60 | 11.02±1.31 | 13.45±0.25 |
| | Cs | 69 | 11.48±0.85 | 16.14±0.34 |
| | | *t*-test d.f.=127 | *P*=0.76 | *P*<0.0001 |
| *desat1*$^{1573\text{-}D'1}$ | desat1 | 62 | 9.34±1.18 | 14.79±0.47 |
| | Cs | 65 | 11.32±1.13 | 16.77±0.32 |
| | | *t*-test d.f.=125 | *P*=0.28 | *P*=0.0006 |
| *desat1*$^{1573\text{-}E1}$ | desat1 | 60 | 12.61±1.30 | 14.47±0.34 |
| | Cs | 60 | 12.25±1.60 | 17.40±0.39 |
| | | *t*-test d.f.=118 | *P*=0.86 | *P*<0.0001 |
| *desat1*$^{1573\text{-}J2}$ | desat1 | 60 | 11.47±1.37 | 13.87±0.28 |
| | Cs | 60 | 10.87±1.06 | 15.63±0.40 |
| | | *t*-test d.f.=118 | *P*=0.73 | *P*=0.0005 |

For all male genotypes, the effect of female pheromones, transferred by ruboff, was measured on copulation latency and duration (see Table 1). Tai males were also paired with *desat1* females perfumed with the CHs of Tai females.

*desat1*$^{1573\text{-}1}$ are homozygous for a mutation caused by a P*Gal4* transposon. The other four *desat1*$^{1573}$ alleles result of a precise (*N2*) or imprecise excision of this transposon (*D′1, E1, J2*; Marcillac et al., in press).

Table 3. *Effect of Cs female pheromones on progeny and sex ratio*

| Mating experiment with Cs male | Female | | *N* | Progeny | | | Sex ratio (female:male) |
|---|---|---|---|---|---|---|---|
| | Mating rank | Source of perfume | | Female | Male | Total | |
| No choice | 1 | desat1 | 150 | 13.36±1.23 | 13.71±1.24 | 27.07±2.44 | 1.10±0.08 |
| | 1 | Cs | 121 | 9.57±0.84 | 11.21±0.88 | 20.78±1.67 | 0.98±0.09 |
| *t*-test d.f.=269 | | | | *P*=0.016 | *P*=0.117 | *P*=0.044 | *P*=0.336 |
| Choice | 1 | desat1 | 87 | 10.04±0.99 | 10.69±1.05 | 20.74±1.97 | 1.03±0.09 |
| | 1 | Cs | 163 | 9.82±0.62 | 11.36±0.70 | 21.18±1.26 | 0.97±0.06 |
| | 2 | desat1 | 74 | 13.62±1.31 | 13.63±1.27 | 28.41±2.50 | 1.26±0.14 |
| | 2 | Cs | 66 | 8.91±0.91 | 10.53±1.06 | 21.38±1.93 | 0.95±0.06 |
| ANOVA d.f.=386,1,1 | | | | | | | |
| A CH transfer | | | | *P*=0.0135 | *P*=0.273 | *P*=0.0663 | *P*=0.0324 |
| B mating rank | | | | *P*=0.196 | *P*=0.347 | *P*=0.247 | *P*=0.4536 |
| A×B | | | | *P*=0.0252 | *P*=0.080 | *P*=0.0384 | *P*=0.2346 |

All tests were carried out with a single Cs male with perfumed and/or non-perfumed *desat1* females, either in choice or in no-choice experiments (see Table 1). The total female and male progenies of each mated female were pooled during the 8 days following the first day of adult eclosion.

The total progeny and sex ratio represent the sum and the ratio of female and male progenies.

Values are means ± S.E.M.

and also have a role in sexual selection (this report). It is possible that large amounts of 7,11-dienes are the result of a runaway selection process, as shown for secondary characters as pheromones in many organisms (Wyatt, 2003). CHs acting as sex pheromones that reinforce mate recognition were shown to be selected in other *Drosophila* species (Higgie et al., 2000). Finally, the dienes present on *D. melanogaster* cuticle have long carbon chain-lengths that could serve to reduce desiccation in warmer drier environments (Gibbs, 1998).

Surprisingly, the same set of female pheromones acted on copulation termination: the CHs of Cs females increased the copulation duration of all males except for *desat1*$^{1573\text{-}1}$ mutants. This absence of effect indicates that the *desat1* mutation can also alter male perception of sex pheromones during copulation. We do not know how the male fly perceives female pheromones during mating, but the difference between Cs and variant strains suggest that the system of perception of female pheromone(s) during copulation differs from that required during courtship. Alternatively, it is also possible that the substances perceived before and during copulation are not identical and that both sets of pheromones are absent in mutant *desat1* females.

One possible consequence of the variation for copulation duration was the sex ratio effect. The presence of Cs female pheromones was correlated with decreased frequency of daughters in the progeny fathered by Cs males. A similar tendency towards a male-biased sex ratio was noted in the progeny from matings between *desat1* females and Tai males (1.55±0.34 and 0.88±0.13, respectively, for non-perfumed and perfumed; $P$=0.034; d.f.=39; $t$=2.22). Moreover, when the sex ratio obtained individual *desat1* mutant females mated with Cs males was plotted against the duration of copulation of each pair, the regression analysis showed a slight negative correlation ($P$=0.0129; d.f.=494.1; $F$=6.224). This indicates that the relative number of females decreased slightly in the progeny of parents with longer copulation durations.

Similar effects have been found in cockroaches. The manipulation of cues involved in male social dominance in the cockroach *Nauphoeta cinerea* allowed females to discriminate between male cues, but these females produced fewer male progeny than randomly mated females (Moore et al., 2001). We found a reciprocal situation in *Drosophila*: the manipulation of female pheromones increased the propensity of male mating and tended to decrease female viable offspring. Given that in both species the homogametic sex is female, the gender difference observed between the two sets of results could be a consequence of the subsocial interactions that exist in cockroaches but not in flies. Although we have no experimental evidence to explain sex ratio variation, it is possible that a longer copulatory contact facilitates the transfer of substances that will favour egg fertilization by the spermatozoa carrying a Y chromosome (and yielding male offspring).

The interpretation of some of the data obtained in the choice experiment is less straightforward, probably because of the interaction between the two competing females. A biological dose of 7,11-dienes transferred on mutant females highly increased their probability of mating first with a Cs male. The fact that the copulation latency with the first mating female (irrespective of her CHs) was very similar to that shown by perfumed females tested in the no-choice experiment indicates that Cs males were initially aroused by the presence of some Cs female pheromones, without distinguishing the perfumed female. Later during courtship, and before copulation, males could distinguish the pheromones of both females by gustatory contact. On the other hand, the effect of female CHs on the sex ratio of the progeny was only detected with second mating females. Given that the time interval between the two matings was relatively short (13.2±1.8 min), it is possible that the female pheromones that were perceived during the first copulation persisted long enough on male receptors or on his cuticle to influence his second mating.

### *Role of* desat1 *on the evolution of pheromonal communication*

The *desat1* gene may play an important role in the evolution of *Drosophila* pheromonal communication because when it is mutated, several reproductive characters are altered, including (i) the production of female and male pheromones (Marcillac et al., in press), (ii) male discrimination of these pheromones, (iii) genital disengagement after copulation (F. Marcillac, Y. Grosjean and J.-F. Ferveur, unpublished observations) and (iv) male perception of female pheromone during copulation (this report). Given that *desat1* expression can be detected in specific regions of the antenna, legs and proboscis, which probably detect pheromones during different phases of mating (Robertson, 1983; Boll and Noll, 2002), and that the *desat1*$^{1573}$ alleles studied here showed different effects on the various pheromonal phenotypes (this report; F. Marcillac, Y. Grosjean and J.-F. Ferveur, unpublished observations), it is possible that the variable expression of *desat1* in these chemosensory organs affects specific components of male pheromonal perception. We do not know whether the strong genetic linkage between pheromonal emission and perception is a situation exceptional to *Drosophila*, but it has not yet been found in other species such as the well-studied European corn borer moth *Ostrinia nubilalis*, where the production and the perception of sex pheromones are controlled by distinct genes (Loefstedt et al., 1989).

Together with abnormal male perception and discrimination of sex pheromones, the natural variation of female pheromones could reflect intraspecific variation for expression of the *desat1* gene. This view is partly supported by the incipient speciation process discovered in Zimbabwe strains of *D. melanogaster* (Wu et al., 1995), which seems to be related to a mutation in the *desat2* gene (Fang et al., 2002). Like Zimbabwe, Tai is a variant strain in which females produce low levels of 7,11-dienes and high amounts of 5,9-dienes (Takahashi et al., 2001). Tai and Cs males are already known to differ in their response to CHs (probably 7-pentacosene), and the genetic factors responsible for this polymorphism have been mapped to limited regions of the chromosome III (Sureau and Ferveur, 1999; McMahon et al., 2002), including some of the candidate genes that affect male discrimination and/or responses to female signals during courtship (Ting et al., 2001).

In the present study, we found that Tai and Cs males

responded differently to the variation of 7,11-dienes. Tai males normally detect 7,11-dienes during copulation with an intact female (this study) and before copulation on an immobilized female (Sureau and Ferveur, 1999). However, the fact that they did not show a faster copulation latency with intact perfumed female indicates that Tai males do not perceive (or react to) 7,11-dienes as early as Cs males do, whereas their response becomes similar during copulation. This supports the hypothesis that, as in mutant *desat1* alleles, the variation for expression of *desat1* and *desat2* genes in chemosensory organs of Tai males could change specific aspects of their response to female pheromones.

We have proposed that the *desat1* gene plays a critical role in the genetic architecture underlying some of the mechanisms involved in pheromonal communication (Marcillac et al., in press). Here, we show that the female pheromones coded by *desat1* can influence multiple aspects of reproduction, and cause reciprocal effects on the genes contributed by females and males that may have a consequence in sexual conflict (Rice, 1996). These findings support the hypothesis that *desat1* finely regulates the coevolution between the sexes in *Drosophila*.

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