

Revisited Roles of *Drosophila* Female Pheromones

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Introduction

Chemical signals, or sex pheromones, are often required for the rapprochement of sex partners and to elicit or prevent courtship and reproduction (Wyatt, 2003). In many invertebrate species, sex pheromones are a part of complex mixtures because they are often associated with (or made of) multiple components inducing different effects (Cardé and Minks, 1997). The precise pheromonal influence of the various components of a complex bouquet is difficult to determine in live animals because the experimental methods involved in isolating and testing are invasive and do not parallel the 'in vivo' situation. *Drosophila melanogaster* is a favorable species to study pheromonal communication because the comparison of live transgenic flies producing various blends allows the evaluation of the 'in vivo' role of the pheromonal components that vary. Another non-invasive method allows for the 'perfuming' of flies by passive transfer of pheromones between flies of different genotypes (Coyne *et al.*, 1994).

In *D. melanogaster* females of most strains (such as Canton-S = Cs), two cuticular hydrocarbons (CHs) are quantitatively predominant [7,11-heptacosadiene (7,11-HD = 400 ng) and 7,11-nonacosadiene (7,11-ND = 200 ng)] (Antony and Jallon, 1982; Antony *et al.*, 1985). However, females of the variant strains collected in Caribbean and sub-Saharan areas produce much less 7,11-dienes (<100 ng; Ferveur *et al.*, 1996). The genetic and molecular bases of this variation, together with its possible evolutionary and adaptative implications, were recently revealed (Coyne *et al.*, 1999; Takahashi *et al.*, 2001; Fang *et al.*, 2002; Greenberg *et al.*, 2003). These substances may play a role in sexual isolation because they are present in *D. melanogaster* and *D. sechellia* females, but not in females of the two closely related species *D. simulans* and *D. mauritiana* (Jallon and David, 1987).

Two studies carried out with the two transgenic female genotypes, *hsp70 x UAS-transformer (hs-tra)* and *desat1*, which respectively produce no or low amounts of 7,11-dienes (Savarit *et al.*, 1999; Marcillac *et al.*, 2004) revealed the multiple roles that these pheromones play in inter- and intraspecific male courtship and mating.

Results

7,11-Dienes strongly prevent interspecific male courtship and mating

Flies with relatively high levels of 7,11-dienes induce no or very low courtship in *D. simulans* males, indicating that these substances are involved in reproductive isolation (Coyne *et al.*, 1994; Coyne and Oyama, 1995). The strong inhibitory role of *D. melanogaster* female pheromones to *D. simulans* and *D. mauritiana* males, but not to *D. sechellia* males, was clearly shown with *hs-tra* females that were, or were not, perfumed with dienes (Savarit *et al.*, 1999).

To measure the threshold of inhibition, mutant *desat1 D. melanogaster* females that were, or were not, perfumed with female pheromones of the control Cs strain were tested with males of three species (Table 1). The difference between non-perfumed and perfumed females [with, respectively, 46 ± 5 ng (*n* = 26) and 108 ± 12 ng (*n* = 34) of 7,11-dienes] was apparently detected by *D. mauritiana* males that courted and mated more often with non-perfumed than with perfumed *desat1* females. Both frequencies of male courtship and mating were apparently lower than those induced by totally deprived females (*hs-tra*; Savarit *et al.*, 1999) suggesting that *D. mauritiana* males are dose-dependently inhibited by quantities of 7,11-dienes ranging between 0 and 100 ng. Conversely, *D. simulans* males could be more sensitive to these substances because they were completely inhibited by non-perfumed females.

Table 1 Courtship and mating of various females with males of three species

Male	Female	Homotypic			<i>Desat1</i> non-perfumed			<i>Desat1</i> perfumed			Cs		
		<i>n</i>	Court	Mate	<i>n</i>	Court	Mate	<i>n</i>	Court	Mate	(<i>n</i>)	Court	Mate
<i>D. melanogaster</i>	Intact	34	85.3	67.6	41	78.1	53.7	42	88.1	69	(=homotypic)		
	Decapitated	34	70.6	–	42	73.8	–	38	86.8	–			
<i>D. mauritiana</i>	Intact	80	90	31.2	112	32.1	10.7	79	6.3	0	(57)	0	–
	Decapitated	21	81	–	26	7.7	–	25	0	–	(20)	10	–
<i>D. simulans</i>	Intact	71	83.1	19.7	41	0	–	24	0	–	(20)	0	–
	Decapitated	22	63.6	–	28	0	–	20	0	–	(21)	4.8	–

All tests involved a pair of 5-day-old male and female (intact or decapitated) flies. Females were 'homotypic' (same species and strain as the tested male: *D. melanogaster*, Cs strain; *D. mauritiana*, 163.1 strain; *D. simulans*, Seychelles strain), 'desat1 non-perfumed' (*D. melanogaster desat1* mutant), 'perfumed' (*desat1* with transfer of Cs females pheromones), or 'Cs' (*D. melanogaster* control strain). Data shown are the frequencies of courtship (with both intact and decapitated females) and of mating (with intact females), within a 1 h observation period and were calculated from the total number of tested pairs (shown in brackets). *D. mauritiana* males courted ($\chi^2 = 16.81$, $P < 0.001$) and mated ($\chi^2 = 8.83$, $P < 0.01$) more frequently with intact *desat1* non-perfumed than with *desat1* perfumed females.

7,11-Dienes are not required to induce intraspecific male courtship and mating behaviors

Early experiments performed with natural fractions of CHs extracts tested on dead dummies suggested that 100 ng of 7,11-HD and 400 ng of 7,11-ND can induce the maximum excitation in Cs males (Antony *et al.*, 1985). A study with living sex mosaic flies producing variable mixtures of female and male predominant CHs, suggested that <30 ng 7,11-dienes can elicit the maximal male courtship intensity (Ferveur and Sureau, 1996).

The hypothesis that 7,11-dienes are not required to induce strong male courtship was clear when intact *hs-tra* females (totally deprived of 7,11-dienes) and Cs females induced a similarly strong excitation in Cs males (Savarit *et al.*, 1999). However, male flies mated less often (–25%) with intact *hs-tra* females and directed a weaker courtship (–30%) to decapitated *hs-tra* females if compared to control females. This suggests that the multiple sensory signals emitted by intact females could make up for the absence of 7,11-dienes; nevertheless, these substances seem to increase male propensity to mate.

7,11-Dienes enhance mate choice between homotypic partners, without any dose-dependent effect

To clearly assess the role on mating of female pheromones, live *desat1* females were or were not perfumed (by crowding with Cs donor females) with 7,11-dienes. In both choice and non-choice experiments (carried out, respectively, with two or one *desat1* females and one Cs male), perfumed females mated more often than non-perfumed *desat1* females. Moreover, Cs males, but not Tai males, mated faster with perfumed than with non-perfumed females (Marcillac and Ferveur, 2004).

To measure for dose-dependent effect of 7,11-dienes, the ratio between donor Cs and receiver *desat1* females was changed. Choice tests involving a Cs male with both a non-perfumed female and a perfumed female (carrying between 90 and 570 ng of 7,11-dienes) showed no dose effect for female preference and mating speed.

The presence of 7,11-dienes is correlated with increased copulation duration and decreased sex-ratio of the progeny

The transfer of Cs female's pheromones on mutant *desat1* females increased their copulation duration with Cs and Tai males. Furthermore, the presence of 7,11-dienes and longer copulation duration were both associated with a decreased production of female offspring in mating tests with Cs males. Perfumed females significantly produced fewer daughters, but a similar number of sons, than non-perfumed females. Therefore, the sex ratio in the progeny of perfumed females was biased towards an excess of sons whereas that of non-perfumed females was close to one. Moreover, individual females with the higher proportion of daughters showed the shorter copulation durations (Marcillac and Ferveur, 2004). These findings suggest that female pheromones cause reciprocal effects on the genes contributed by females and males that may have a consequence in sexual conflict (Rice, 1996).

Conclusion

The predominant cuticular hydrocarbons (CHs) of *D. melanogaster* female (likely 7,11-dienes) play a much more complex pheromonal role than hitherto suspected. According to their quantity, these substances play a dual role on male inter- and intraspecific mating behavior. Less than 100 ng can efficiently prevent male interspecific mating and strongly reinforce sexual isolation. More than 100 ng of 7,11-dienes are sufficient to regulate several aspects of intraspecific mating, with strain differences. If these female pheromones did not increase the overall intensity of male courtship, they enhanced both

mating speed and frequency, at least in homotypic situation. The fact that a large majority of males preferred to mate first with a perfumed female can explain the selection of the factor(s) coding for these pheromones. Females of many strains produce amounts of dienes that largely exceed the threshold required for mate preference and it is not yet clear whether increasing doses of female pheromones can proportionally increase copulation duration and decrease the number of daughter in the progeny. The high level of female pheromones could also reflect the fisherian selection process of this secondary sexual character (Fisher, 1930), or serve as a yet unknown signal during inter-individual communication both at strain and at species levels. Finally, the significant role played by 7,11-dienes on various sequences of reproduction that occur before, during and after mating suggest that these pheromones help to finely adjust the co-adaptation mechanisms between the sexes.

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