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Co-adaptation of pheromone production and behavioural responses in *Drosophila melanogaster* males

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Summary

In *Drosophila melanogaster*, male courtship behaviour is genetically controlled and is influenced by sex pheromones. 7-tricosene (7-T) induces a dose-dependent inhibition of male–male courtship, whereas 7,11-dienes stimulate male courtship of females. There is a geographical quantitative variation in the production of two predominant male hydrocarbons, 7-T and 7-pentacosene (7-P). We have previously found that 7-P, the main hydrocarbon from males of West African strains, stimulates males that mainly produce 7-T. Using both 'natural' and genetically engineered strains, we find that genetic factors coding for low levels of 7-P in males have co-evolved with factor(s) coding for male responses to high levels of 7-P. These two phenotypes are coded by factors on different chromosomes: the intraspecific polymorphism for the production of 7-T and 7-P is largely controlled by chromosome 2, whereas the variation in courtship towards 7-P-rich males is largely controlled by chromosome 3. The polymorphism of male courtship towards 7-P-rich males shows no correlation with the variation in male responses to female flies.

1. Introduction

In laboratory conditions, *Drosophila melanogaster* can easily be induced to court both heterospecific and homotypic adult males (Cobb & Jallon, 1990; Balakireva *et al.*, 1998). Inter- and intraspecific male–male courtship seems to be induced by 25-carbon cuticular hydrocarbons, in particular 7-pentacosene (7-P), which is abundantly produced by males of some species (Jallon & David, 1987). This substance also forms a minor part of the *D. melanogaster* female cuticular hydrocarbon bouquet (Jallon, 1984).

D. melanogaster strains show a geographical variation in the amounts of hydrocarbons 7-P and 7-tricosene (7-T; 23C; Jallon, 1984) in males. Males from all strains produce both hydrocarbons, but at very different levels. For example, Canton-S (Cs, USA) males are 7-T-rich, whereas Tai (Ivory Coast) males are 7-P-rich. In general, males found in equatorial and subtropical areas predominantly produce 7-P, whereas males from other geographical areas mostly produce 7-T (J.-M. Jallon, unpublished data). This variation is under polygenic control (Scott & Richmond, 1988) and mainly depends on at least two genes segregating with chromosome 2 (Ferveur & Jallon, 1996). Males from various *D. melanogaster* strains show different responses to 7-P-rich males: for example, Cs males are excited, whereas Tai and Guadeloupe (Caribbean), both 7-P-rich strains, are not (Jallon, 1984; Cobb & Jallon, 1990). It is not known whether the replacement of 7-P by 7-T (or vice versa) on the male cuticle is genetically linked with the male response to 7-P-rich flies. The objective of the present study was to investigate the nature of the link between male production of 7-P and the male response to 7-P.

The male fly simultaneously perceives male and female pheromones: 7-T has a inhibitory effect while 7,11-dienes – produced only by females – stimulate male courtship (strain 55B-GAL4; Ferveur & Sureau, 1996). Ferveur & Sureau (1996) confirmed previous hypotheses as to the role of 7-T (Jallon, 1984; Scott, 1986) and 7,11-dienes (Antony & Jallon, 1982; Antony *et al.*, 1985), and determined the biological thresholds

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of these substances. Very low doses ($\approx$ 20 ng) of the main female pheromones (7,11-dienes) were sufficient to elicit strong male excitation. Males were dose-dependently inhibited by 7-T, with complete inhibition occurring at 500 ng 7-T. These studies also suggest that 7-P, which is found on both male and female cuticles, plays a stimulatory role in male courtship but at higher doses (790 ng). However, it should be noted that these thresholds almost certainly vary between strains.

In *D. melanogaster*, alteration of male courtship behaviour has mainly been studied using mutant genes showing various defects (Hall, 1994). The best-studied mutant, *fruitless* (*fru*), causes complex behavioural anomalies: males engage in a courtship chain in which each fly is simultaneously the courter and the courtee (Hall, 1978). The allele with the largest such effect (fru^1) shows a large decrease in quantities of 7-T (Cobb & Ferveur, 1996*a*). However, the fact that other *fru* alleles show normal pheromonal production but defective courtship behaviour (Ito *et al*., 1996; Ryner *et al*., 1996; J.-M. Jallon, unpublished data) suggests that these two sex-specific characters are controlled by distinct genetic factors within the *fru* gene complex (90C–91A; Gailey & Hall, 1989).

The recent development of targeted expression of transgenes in the nervous system allows more precise manipulation of male courtship behaviour. In particular, regional feminization of the male nervous system, performed with the female-spliced form of the sex-determination gene *transformer*, has produced brain-mosaic XY flies that indiscriminately court males and females (Ferveur *et al*., 1995; O'Dell *et al*., 1995). The same transgene ectopically expressed in the oenocytes leads to the feminization of sex pheromones in male flies. Such transformed XY flies can also induce male–male courtship (Ferveur *et al*., 1997). Ubiquitous expression of the *miniwhite* transgene causes males to produce courtship chains (Zhang & Odenwald, 1995) and to court other mutant males (Yin Hing & Carlson, 1996). However, it is not known whether these latter effects are produced by a defect in the transmission or/and in the reception of sensory messages (Ferveur, 1997).

In the present study we show that male homo- and heterosexual courtship varies widely – from aversion to high excitation – in eight wild-type and 20 genetically engineered strains of *D. melanogaster*. We have dissociated the genetic factors that code for the production of 7-P and 7-T in males from the factor(s) that code for male courtship towards 7-P-rich males. Our results suggest that the factors coding for low levels of 7-P in males have co-evolved with the factor(s) that control vigorous courtship towards 7-P-rich males. The variation in male courtship towards 7-P-rich males shows no correlation with the variation in male courtship to female flies.

2. Methods

(i) Drosophila *stocks and feminization*

Flies that were used to induce a behavioural response were defined as object flies. Male flies whose behavioural response was measured in reaction to the object flies were defined as subject flies. Canton-S (Cs), Tai and 55B-GAL4 strains provided control male flies. Cs is a standard laboratory strain from the USA. Tai is a strain from the Ivory Coast and has been raised in our laboratory for more than a decade (Jallon, 1984). The courtship behaviour of males from the transgenic 55B-GAL4 strain has been described in detail (Ferveur & Sureau, 1996). Isofemale strains from Seychelles, Madagascar (Mdg) and Cotonou (Co; Benin) were collected between 1985 and 1987. The Bordeaux strain was collected in France in 1994. These four strains were provided by F. Lemeunier and S. Aulard (CNRS, Gif-sur-Yvette, France). The isofemale 'Malawi 62' and 'Guinea-Bissau 1′ strains were collected in Africa after 1994 and provided by M. Veuille and D. Higuet (University of Paris-6, France), respectively. Male flies from all these strains were used both as subject and object flies in our courtship tests. The shibire^{ts7} (Shi; Lindsley & Zimm, 1992) stock, when kept at 29 °C, yielded control virgin females that did not carry the shi^{ts7} thermosensitive mutation. Females from Shi and from wild-type strains were only tested as object flies. The balancer L strain carries SM1/*Pm*; Tm2/*Sb* autosomes. SM1 is marked with *Cy*; TM2 is marked with *Ubx*.

All other object flies were F1 male flies resulting from the cross between a P-GAL4 strain and the *UAS-transformer* strain (*UAS-tra*; Ferveur *et al*., 1995). These F1 flies can be more or less feminized for different sexual characters including their sex pheromones. For the procedure involved in screening and sexing mosaic flies, see Ferveur & Sureau (1996). All P-GAL4 strains were created by Brand & Perrimon (1993), except strain c62 which was generated in the laboratory of K. Kaiser (Glasgow University, Scotland).

Unless otherwise specified, all strains were kept at 25 °C under a 12:12 h day:night photoperiod. Substitution of chromosomes 2 and 3 was carried out using the L balancer strain following Bauer & Sokolowski (1985). This procedure allowed us to exchange the autosomes between two pairs of strains (Cs/Tai and Mdg/Co) diverging for pheromone production and pheromone perception.

(ii) *Behavioural tests and parameters*

Flies were isolated 1–4 h after eclosion and kept in food vials, in groups of five flies for crosses or to serve as pheromonal objects. Subject flies were kept alone.

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All tested flies were 4–5 days old. Behavioural experiments always took place 1–3 h after lights-on. Object flies were decapitated 30–60 min prior to observation. The advantages and disadvantages of using decapitated flies have been discussed elsewhere (Ferveur & Sureau, 1996). Briefly, decapitation allows behavioural observations to be standardized because no copulation occurs and courtship can be measured over a constant period. This procedure also eliminates most of the interactive signals that are normally exchanged between partners, and thus enhances the influence of chemical signals on male courtship behaviour (Ferveur *et al*., 1995).

Subject males were individually aspirated under a watch glass used as an observation chamber (1·6 cm^3). Ten minutes later, a decapitated object fly was aspirated under the glass. Each observation lasted 10 min. Two control and two experimental flies (see Section 2:i above) were simultaneously observed. Experiments with object flies of a given strain were performed over several days.

Stereotypical male courtship behaviours (tapping, wing vibration, licking and attempted copulation; Cobb *et al*., 1985) were noted. The courtship index (CI), calculated for each male, is the sum of the duration of all these courtship sequences. 'Heterosexual' and 'homosexual' orientations were not mutually exclusive: male flies tested here often courted both females and 7-P-rich males.

Locomotor activity was measured in similar environmental conditions. We averaged the total number of lines drawn under the mating chamber crossed by the fly (locomotor activity units = l.a.u.; Balakireva *et al*., 1998). For each experiment, four single flies were sequentially observed for five periods of 20 s, every 2 min, for 10 min. For all strains, $n = 20$.

(iii) *Hydrocarbon extraction and parameters*

Following testing, headless object and intact subject flies used in our behavioural assays were placed in fresh food vials and kept for 24 h before hydrocarbon extraction. This period normally allows a mated female to eliminate most of the molecules passively transferred by the male (Scott & Jackson, 1988). In our case, where no copulation was observed due to decapitation, and thus relatively little physical contact took place, this period would have been largely sufficient to avoid any artefactual result due to passive transfer.

Hydrocarbon extraction was carried out according to the standard procedure (Ferveur, 1991). The absolute quantities (in ng) of predominant hydrocarbons were noted for each fly: 7-tricosene (7-T, 23C), 7-pentacosene (7-P, 25C), 7,11-heptacosadiene (7,11-HD, 27C), 7,11-nonacosadiene (7,11-ND, 29C) and the total quantities of cuticular hydrocarbons (ΣHc). Absolute levels were quantified using a constant amount of standard hydrocarbon. Each molecule, corresponding to a peak on the chromatogram, was characterized by its retention time. Peaks were identified by co-migration with known standards complemented by mass spectrometry studies performed on pools of Cs flies (Antony & Jallon, 1982) and Tai flies (Pechiné *et al*., 1985; Jallon & Pechiné, 1989).

(iv) *Statistical analysis*

The correlation between the levels of different pheromones and the behavioural responses (courtship, locomotor activity) was estimated with a non-parametric test (Spearman rank correlation coefficient), because the samples to be compared were the means for each strain and were not normally distributed. Differences between the CIs of two genotypes (Table 1) were tested with a non-parametric test (Mann–Whitney). The standard level of significance ($P = 0{\cdot}05$) was divided by nine (because nine comparisons were planned *a priori*) to produce a threshold significance level of 0·0055, a more conservative method than the Bonferroni correction for multiple comparisons (Rice, 1989).

3. Results

(i) *Intraspecific variation in the male courtship response*

Substantial variation in the courtship of *Drosophila melanogaster* males in response to different object flies was observed (Fig. 1). The courtship indices (CIs) of subject males from the Cs, Tai and 55B-GAL4 strains were very different in the presence of various decapitated object flies (Or-R, Cs and Tai males, XY mosaics from 6 P-GAL4-*tra* strains, and Shi females). Subject Cs males were moderately excited by Cs and c62-*tra* object males, but they were much more stimulated by Tai males and by the five other XY mosaic flies. Tai subject males were only moderately stimulated by 30B- and 32B-*tra* XY mosaic objects. Subject males from the transgenic 55B-GAL4 strain showed contrasting responses, as previously reported (Ferveur & Sureau, 1996). 55B-GAL4 males were inhibited ($CI < 5$) by high levels of 7-T (± 500 ng borne by Cs, 6J3-*tra* and 53B-*tra*). When the level of 7-T was much lower, they were stimulated either by high amounts of 7-P (541 ng; Tai) or by substantial amounts of 7,11-dienes (> 90 ng) borne by c62-, 10B-, 32B- and 30B-*tra* mosaics. The three types of subject males (Cs, Tai and 55B-GAL4) shared a strong heterosexual response with object Shi females and showed no excitation with Or-R males (7-T > 800 ng).

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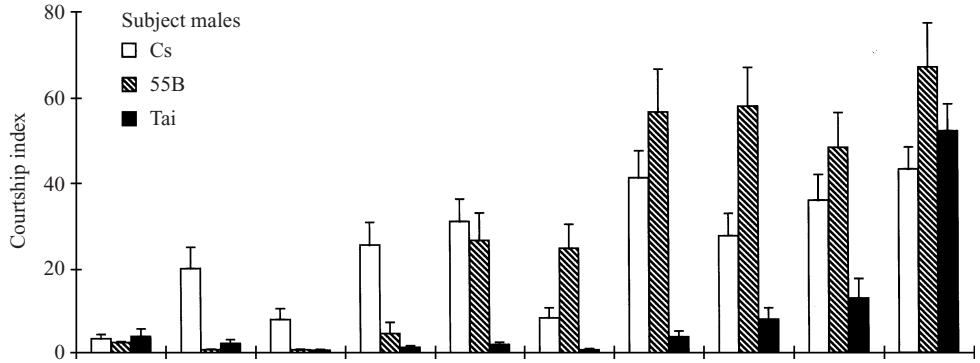

	Or.R	6J3	Cs	53B	Tai	c62	10B	32B	30B	Shi	
7-T	829	502	498	490	63	291	190	147	103	55	Object files
7-P	285	275	140	729	541	300	199	319	283	142	and their
7, 11-dienes	0	0	0	0	0	146	96	238	200	623	hydrocarbon
Σ Hc	2046	1407	1215	2147	1212	1552	990	1511	1339	1713	production (ng)

Fig. 1. Intraspecific variation in male courtship behaviour in various *D. melanogaster* strains. Mean courtship indices (±SE) of subject males of the three strains Cs (Canton-S), Tai and 55B (55B-GAL4) with decapitated flies of ten 'object' strains. Object strains consisted of Oregon-R (Or.R), Cs and Tai males, XY mosaics from six P-GAL4 *UAS-tra* (numbered strains; see Section 2) and Shi females. Each fly used for the courtship assay was tested only once. For Tai and Cs subject males: $15 > n > 27$; for 55B-GAL4 subject males: $7 > n > 23$. After testing, randomly chosen object flies were kept for subsequent hydrocarbon extraction. For each object strain, $n = 10$. The mean absolute quantities of 7-tricosene (7-T), 7-pentacosene (7-P), 7,11-heptacosadiene with 7,11-non acosadiene (pooled as 7,11-dienes) and of total quantities of cuticular hydrocarbons (ΣHc) are given in nanograms. Strains are ranked (from left to right) according to their absence/presence of 7,11-dienes combined with their decreasing amount of 7-T. Error bars represent the standard error of the mean.

The intraspecific variation in male courtship was studied on a larger number of subjects consisting of eight wild-type strains and the transgenic 55B-GAL4 strain. Male courtship with Shi and homotypic females, and with Tai, homotypic and Cs males was compared between these nine strains (Fig. 2). All subject males showed a strong heterosexual CI with Shi females (CI > 35), except Cotonou males (CI = 20). In homotypic crosses, heterosexual courtship (with homotypic (Htp) females; not shown) was always more frequent than homosexual courtship, which occurred very rarely. Htp females induced CIs that were intermediate between those induced by Shi females and Tai males. Canton-S (Cs) males induced weak responses (data not shown) that were very similar to the responses induced by Htp males, whereas Tai males elicited significant homosexual courtship by all subject males except those from Guinea-Bissau, Tai and Cotonou.

(ii) *The relation between 7-P produced and 7-P perceived by subject males*

In the eight wild-type strains studied here, we found a significant negative correlation between the amount of 7-P carried by subject males and their response to 7-P-rich object males (Tai; $r = 0{\cdot}738$; $n = 8$; $P = 0{\cdot}036$). Locomotor activity was also measured on single subject males that were not tested in the courtship assay (data not shown); their values (which varied from 42 l.a.u. in Malawi to 75 l.a.u. in Cs) were not correlated with any CIs.

The correlation between the production and the aversive response towards 7-P was genetically dissected twice: chromosomes 2 and 3 were exchanged between two pairs of strains (Cs/Tai and Madagascar (Mdg)/Cotonou (Co); Table 1). These strains were selected because they differed both in their production of 7-P and in their courtship of Tai males. Cs and Mdg strains have 7-T-rich males that are excited by Tai object males, whereas Tai and Co strains have 7-P-rich males that are not excited by Tai males (see also Fig. 2). The L strain, used for balancer chromosomes 2 and 3, has 7-T-rich males showing strong courtship towards Tai males.

Results from F1 males from both kinds of reciprocal crosses suggest that the 7-T/7-P ratio is under different genetic control in each pair of strains: Cs and Tai autosomes are co-dominant with regard to this character whereas Co autosomes are semidominant over Mdg autosomes. The balance of production between 7-T and 7-P seems to depend almost entirely on the origin of chromosome 2, as previously shown (Ferveur & Jallon, 1996). F3 and F5 male flies with chromosome 2 from a 7-T-rich strain (Cs, Mdg or L) predominantly produced 7-T. When chromosome 2

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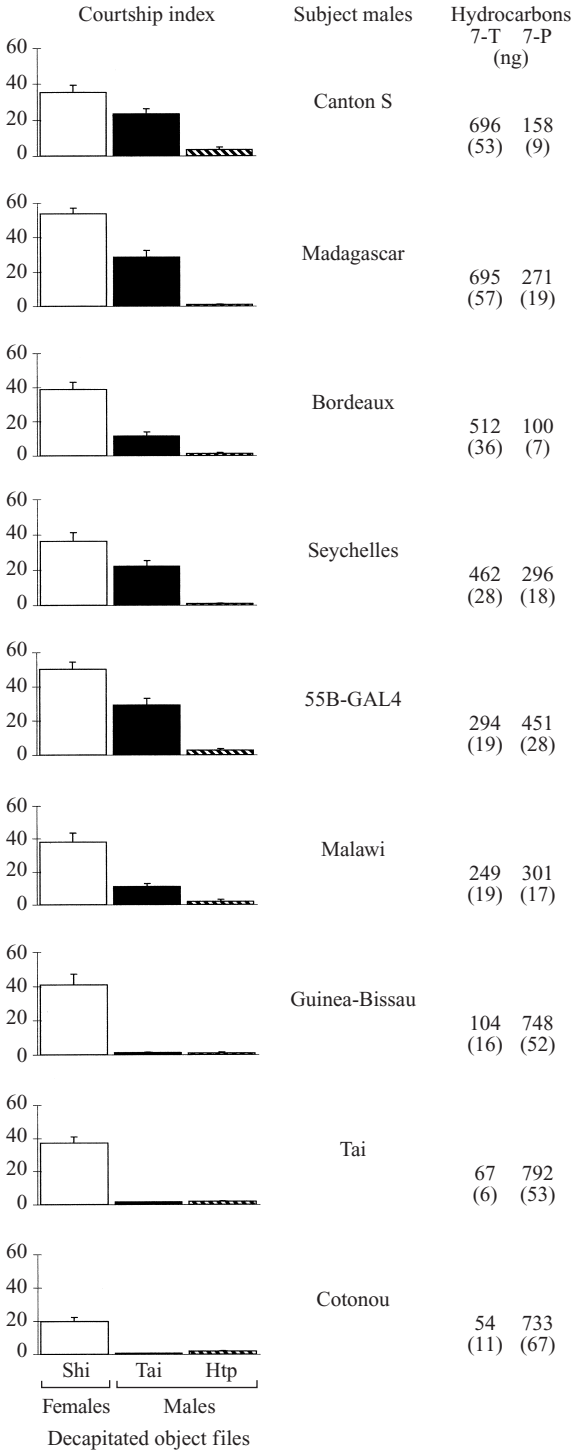

Fig. 2. Intraspecific variation of male courtship index (CI) and hydrocarbon production. Mean CIs (±SE) directed to decapitated object shibire (Shi) females, and to Tai and homotypic (Htp) males were measured on subject males from nine strains (eight wild-type strains and the transgenic 55B-GAL4 strain). With Shi females: $20 > n > 33$; with Tai males: $26 > n > 41$ (except for Malawi = 21; for Guinea-Bissau = 10); with Htp males: $5 > n > 17$. Hydrocarbon extraction was performed on randomly chosen subject males following their courtship test ($18 > n > 36$). Mean absolute amounts (±SE) of 7-tricosene (7-T) and 7-pentacosene (7-P) are shown. Strains are ranked according to their decreasing amounts of 7-T.

came from a 7-P-rich strain (Tai or Co), F3 and F5 males predominantly produced 7-P. Chromosome 3 seems to exert a secondary effect on the balance between 7-T and 7-P. The influence of each pair of autosomes on the 7-T/7-P ratio was tested: chromosome 2 controls roughly two-thirds of the total variation between Cs and Tai strains (Table 2).

'Reconstituted' F5 males with Cs autosomes (cross # 10), Tai autosomes (# 13) or Co autosomes (# 21) showed homosexual CIs that were not significantly different from the CIs of their respective parental males (# 2, # 3 and # 15 strains, respectively). However, this was not the case for reconstituted male progeny carrying Mdg autosomes (# 18 vs # 14), indicating that the genetic control of their stimulation by 7-P-rich males may also depend on the X chromosome or indeed chromosome 4. For this reason, we focused our genetic analysis of 'homosexual' courtship on male progenies yielded by crosses between Cs and Tai strains (Table 1).

Both types of reciprocal F1 male (# 4 and # 5) showed homosexual CIs that were intermediate between parental CIs, suggesting a co-dominant control by autosomal factors. Low homosexual excitation in F3 and F5 males mainly segregated with the Tai chromosome 3 because the CI differences between # 9 vs # 6, 7 and 8 were significant ($P = 0{\cdot}0003$–$0{\cdot}0016$), as were the differences between # 10 and # 11 (d.f. = 48; $z = 3{\cdot}56$; $P = 0{\cdot}0004$) and between # 12 and # 13 (d.f. = 48; $z = 4{\cdot}62$; $P = 0{\cdot}000\,001$). There is also an additional significant effect of the Tai chromosome 2 (# 10 vs # 12: d.f. = 48; $z = 3{\cdot}63$; $P = 0{\cdot}0003$, and # 11 vs # 13: d.f. = 48; $z = 4{\cdot}81$; $P = 0{\cdot}000\,02$). Chromosome 3 controls roughly two-thirds of the difference in CI towards Tai males shown by Cs and Tai males (Table 2).

The data obtained with wild-type strains show that high levels of 7-P carried by subject males are correlated with their aversion towards 7-P-rich object males (Fig. 2), which suggests that the production of 7-P and the response to 7-P are two partially independent characters that are co-adapted in nature. Results from the chromosome exchange experiment support this finding: when strains contained only autosomes from either 7-T-rich strains (Table 1; # 1, 2, 6, 7, 10, 14 and 18) or 7-P-rich strains (# 3, 13, 15 and 21), they showed a highly significant correlation between their 7-P production and their CI towards Tai males ($r = 0{\cdot}873$; $n = 11$; $P = 0{\cdot}0004$). However, when strains contained autosomes of mixed origins (7-T- and 7-P-rich strains: # 4, 5, 8, 9, 11, 12, 16, 17, 19 and 20), they showed no correlation between the two phenotypes ($r = 0{\cdot}139$; $n = 10$; $P = 0{\cdot}70$). The dissociation of the two phenotypes was clearly observed in two strains where males produced a high level of 7-P and also showed a significant response to Tai males (Table 1; genotypes # 12 and # 20).

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Table 1. *Courtship indices (CI) and hydrocarbon production in subject males of various genotypes*

Cross #	Generation	Chromosomes			Hydrocarbons (ng)				Courtship with Tai male		Courtship with Shi female	
		X	2	3	7-T	7-P	ΣHC	7-T/7-P	CI	% CI	CI	% CI
1	F0	L	S/Pm	T/Sb	1067	347	2196	3·37	18	95	29	100
2	F0	Cs	Cs	Cs	696	158	1599	4·60	23	85	35	84
3	F0	Tai	Tai	Tai	67	792	1567	0·09	1	3	37	88
4	F1	Cs	Cs/Tai	Cs/Tai	488	431	1858	1·18	11	75	48	93
5	F1	Tai	Cs/Tai	Cs/Tai	521	673	2282	0·79	9	52	37	87
6	F3	L	Cs	T/Sb	1565	418	3169	3·96	17	79	22	100
7	F3	L	S/Pm	Cs	949	299	2277	3·28	17	74	28	100
8	F3	L	Tai	T/Sb	672	793	2507	1·01	18	88	26	100
9	F3	L	S/Pm	Tai	703	401	2048	1·88	7	40	21	93
10	F5	L	Cs	Cs	552	232	1418	2·72	25	95	36	100
11	F5	L	Cs	Tai	552	309	1487	1·95	12	70	30	100
12	F5	L	Tai	Cs	383	670	1754	0·59	11	77	38	100
13	F5	L	Tai	Tai	108	1054	1864	0·10	3	5	32	100
14	F0	Mdg	Mdg	Mdg	695	271	1736	2·63	29	91	54	97
15	F0	Co	Co	Co	54	733	1521	0·07	1	0	20	84
16	F1	Mdg	Mdg/Co	Mdg/Co	221	597	1483	0·39	3	13	33	93
17	F1	Co	Mdg/Co	Mdg/Co	294	492	1471	0·59	2	0	50	100
18	F5	L	Mdg	Mdg	870	468	2056	1·89	16	95	41	100
19	F5	L	Mdg	Co	549	377	1513	1·57	11	88	28	95
20	F5	L	Co	Mdg	182	875	1713	0·21	9	72	35	100
21	F5	L	Co	Co	44	583	1164	0·08	2	0	25	91

Chromosomes 2 and 3 were substituted over five generations of genetic crosses either between Canton-S (Cs) and Tai strains (# 2–13), or between Madagascar (Mdg) and Cotonou (Co) strains (# 14–21). L strain (# 1) carries balancer chromosomes *SM1* (S) and *TM3* (T) against dominant morphological markers *Plum* (Pm) and *Stubble* (Sb) borne by chromosomes 2 and 3, respectively. Balancer chromosomes, which were used in the chromosome substitution, appear in the F3 generation (# 6–9).

For each subject strain, the mean absolute quantities of 7-tricosene (7-T), 7-pentacosene (7-P) and total quantities of cuticular hydrocarbons (ΣHc) are given in nanograms. The ratio between 7-T and 7-P is also shown. Hydrocarbon extraction was performed on flies following their courtship assay: $14 > n > 36$. Courtship indices were measured with decapitated object Tai males ($19 > n > 32$; except for # 2 = 40; and for # 16 and # 17 = 15) or with decapitated object shibire (Shi) females ($14 > n > 33$). For the frequency of courtship, only males with a CI > 5 (courting more than 30 s during 10 min) were included. Locomotor activity was also measured on crosses # 1–13 (data not shown).

(iii) *Behavioural specificity of courtship towards 7-P-rich males*

Our data suggest that male courtship behaviour towards 7-P-rich object males is under different genetic control from that of courtship towards females. In most of the strains studied here, the variation in CIs towards Tai males and the variation in CIs towards Shi females did not co-segregate. For example, Cs and Tai males showed similar CIs with Shi females but different CIs with Tai males, whereas the situation was different between Mdg and Co (see Table 1). Note that heterosexual CIs of males with 'reconstituted' Cs, Tai and Co autosomes (# 10, 13 and 21) had values very close to those of their respective parents (# 2, 3, 15), whereas males with 'reconstituted' Mdg autosomes (# 18) were much less excited than Mdg parental males (# 14). This suggests that the Mdg chromosomes X and 4 may be also involved in controlling courtship towards females.

In male progeny resulting from the Cs/Tai crosses, we found that locomotor activity (data not shown) was correlated with the CI towards Shi females ($r = 0{\cdot}588$; $n = 13$; $P = 0{\cdot}034$) but not with the CI towards Tai males ($r = 0{\cdot}142$; $n = 13$; $P = 0{\cdot}641$).

Discussion

Male homosexual courtship, which rarely occurs between wild-type *D. melanogaster* individuals from the same strain, can frequently be observed in heterotypic male–male interactions. Our data suggest that male–male courtship in wild-type strains is a polymorphic character which seems to be mediated by 7-pentacosene (7-P), as such courtship is always induced by 7-P-rich object males. In wild-type males the variation in response to 7-P males segregates with the variation in the level of 7-P: males producing a low amount of 7-P show a robust courtship toward 7-P-rich flies, and vice versa. The fact that the greatest

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Table 2. *Relative effect of each autosome on male courtship and on hydrocarbon ratio*

Chromosome	Origin	CI with Tai male	7-T/7-P ratio
2	Tai	11·1	0·61
	Cs/L	15·6	2·84
3	Tai	7·8	1·37
	Cs/L	17	2·46

Individual male courtship responses to Tai males (CI) and the 7-T/7-P ratio were averaged with regard to the origin of a single pair of chromosomes (2 or 3; see Table 1). Only crosses between Cs and Tai were analysed because of their high sample size. Chromosomes from L and Cs strains (Cs/L) were considered as being of the same origin and were thus pooled (L and Cs strains showed the same 7-T/7-P ratios and produced very similar courtship responses to Tai males; see Table 1). Chromosome 3 exert an effect on courtship with Tai males that is roughly twice the effect of chromosome 2 (difference between Tai and Cs/L: 9·2 and 4·5, respectively), whereas this relation is reversed with regard to the 7-T/7-P ratio (2·23 for chromosome 2 and 1·09 for chromosome 3).

part (2/3) of the two characters segregate on different chromosomes (production of 7-P on chromosome 2, and response to 7-P on chromosome 3) suggests that they are co-adapted in wild-type strains. While this observation implies the existence of a genetic linkage between the emission and the perception of the same signal (7-P) within a given *D. melanogaster* strain, it is probable that other genes involved in pheromonal communication are co-adapted in both sexes, as shown for acoustic communication in a cricket species (Ritchie, 1996).

Previous experiments have reported the existence of an intraspecific variation in male courtship in *D. melanogaster* (Jallon, 1984; Cobb & Jallon, 1990), and the three types of subject males used in the present study (Cs, Tai and 55B-GAL4) show very different courtship behaviours in response to objects carrying different pheromonal profiles (Fig. 1). Our data suggest that male perception of different pheromones is variable between these strains. For example, the threshold of total inhibition by 7-T seems to be higher for Cs males ($\approx$ 800 ng, borne by Or-R males) than for 55B-GAL4 males ($\approx$ 500 ng). Also, male flies have differing response thresholds to female, 7,11-dienes: $\approx$ 200 ng for Tai males and less than 50 ng for 55B-GAL4 males (Ferveur & Sureau, 1996). Our results indicate that 7-P, which is a predominant hydrocarbon found on the cuticle of *D. melanogaster* males from West Africa and which also occurs at lower doses on females of all strains, can elicit variable male courtship, depending upon the strain (Fig. 2). Previous experiments have shown that males can simultaneously perceive male and female pheromones (Ferveur & Sureau, 1996), which are processed in different areas of the fly nervous system (Ferveur *et al.*, 1995; Balakireva *et al.*, 1998). The present study suggests that the male response towards male and female pheromones is controlled by distinct genetic factors. Our data also provide strong evidence that the variation in courtship performance between males from different wild-type strains can be influenced by factors other than the receptivity of their partner (Table 1; Cobb & Ferveur, 1996*b*; Scott, 1994, 1996) because sexual receptivity is controlled by the brain (Hall, 1979; Tompkins & Hall, 1983) and our object flies are decapitated.

We are now trying to assess directly the pheromonal role of 7-P in male courtship. 7-P has been postulated to stimulate inter- and intraspecific male–female and male–male courtship (Jallon, 1984; Antony *et al.*, 1985; Cobb & Jallon, 1990) and, in synergy with 9-pentacosene (its minor isomer), to increase the time that a male spends in attempting copulation (Ferveur & Sureau, 1996). Although the latter study failed to detect any significant effect for 7-P, close examination of one strain (18B-*tra*) suggested that this compound does indeed play a stimulatory role in male courtship when present at relatively high doses (790 ng). 7-P has also been proposed to play an inhibitory role in 7-P-rich males (Scott & Jackson, 1988), but no data distinguish between an active inhibitory effect of 7-P and the absence of stimulation of 7-P-rich males due to the masking of another substance.

This study clearly demonstrates that the level of 7-P mainly depends upon chromosome 2, supporting previous results (Ferveur & Jallon, 1996), whereas male courtship of 7-P-rich object males is principally controlled by factor(s) borne by chromosome 3 (we are currently mapping these factor(s)). Although the high production of male 7-P segregates with male aversion of 7-P-rich males of wild-type strains, we have been able genetically to dissociate the phenotypes in two laboratory strains (Table 1, genotypes # 12 and # 20) where 7-P-rich males are excited by other 7-P-rich males. We are now studying these two recombinant strains to see whether their pheromones and the male response thereof remain stable over many generations. This will allow us to test whether the production of a new pheromonal signal (male inhibitory 7-T) has preceded the change in male courtship preference (stimulation by 7-P), as proposed by Coyne *et al.* (1994). Alternatively, the replacement of 7-P by 7-T could have weakened selection against a male response to 7-P. These hypotheses also raise the question of the quantitative polymorphism for 7-T and 7-P in *Drosophila melanogaster* males and in *D. simulans*. Flies from both species show 7-P-rich morphs in the Benin Gulf area, in West Africa. However, the fact that the variation in the 7-T/7-P ratio is under different genetic control in the two

species suggests that the quantitative variation in 7-monoenes is a product of convergent evolution, perhaps corresponding to an adaptation to environmental conditions (Ferveur, 1991; Ferveur & Jallon, 1996). We hope that these findings will enable us (i) to determine the influence of 7-pentacosene during the evolution of sexual communication, and (ii) to characterize the gene(s) and the neural structure(s) that are involved in the perception of the 7-P pheromone.

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References

Antony, C. & Jallon, J.-M. (1982). The chemical basis for sex recognition in *Drosophila melanogaster*. *Journal of Insect Physiology* **28**, 873–880.

Antony, C., Davis, T. L., Carlson, D. A., Pechiné, J.-M. & Jallon, J.-M. (1985). Compared behavioral responses of male *Drosophila melanogaster* (Canton S) to natural and synthetic aphrodisiacs. *Journal of Chemical Ecology* **11**, 1617–1629.

Balakireva, M., Stocker, R. F., Gendre, N. & Ferveur, J.-F. (1998). *Voila*, a new Drosophila courtship variant that affects the nervous system: behavioral, neural and genetic characterization. *Journal of Neuroscience* **18**, 4335–4343.

Bauer, S. J. & Sokolowski, M. B. (1985). A genetic analysis of path length and pupaison height in a natural population of *Drosophila melanogaster*. *Canadian Journal of Genetics and Cytology* **27**, 334–340.

Brand, A. H. & Perrimon, N. (1993). Targeted gene expression as a mean of altering cell fates and generating dominant phenotypes. *Development* **118**, 401–415.

Cobb, M. & Ferveur, J.-F. (1996*a*). Evolution and genetic control of mate recognition and stimulation in Drosophila. *Behavioural Processes* **35**, 35–54.

Cobb, M. & Ferveur, J.-F. (1996*b*). Female mate discrimination or male response to female stimulation? *Evolution* **50**, 1719–1720.

Cobb, M. & Jallon, J.-M. (1990). Pheromones, mate recognition and courtship stimulation in the *Drosophila melanogaster* species sub-group. *Animal Behaviour* **39**, 1058–1067.

Cobb, M., Connolly, K. & Burnet, B. (1985). Courtship behaviour in the *melanogaster* species sub-group of *Drosophila*. *Behaviour* **95**, 203–231.

Coyne, J. A., Mah, K. & Crittenden, A. P. (1994). Pheromonal hydrocarbons and sexual isolation in *Drosophila*. *Science* **265**, 1461–1464.

Ferveur, J.-F. (1991). Genetic control of pheromones in *Drosophila simulans*. I. *Ngbo*, a locus on the second chromosome. *Genetics* **128**, 293–301.

Ferveur, J.-F. (1997). The pheromonal role of cuticular hydrocarbons in *Drosophila melanogaster*. *BioEssays* **19**, 353–358.

Ferveur, J.-F. & Sureau, G. (1996). Simultaneous influence on male courtship of stimulatory and inhibitory pheromones, produced by live sex-mosaic *Drosophila melanogaster*. *Proceedings of the Royal Society of London*, *Series B* **263**, 967–973.

Ferveur, J.-F. & Jallon, J.-M. (1996). Genetic control of male cuticular hydrocarbons in *Drosophila melanogaster*. *Genetical Research* **67**, 211–218.

Ferveur, J.-F., Stortkühl, K. F., Stocker, R. F. & Greenspan, R. J. (1995). Genetic feminization of brain structures and changed sexual orientation in male *Drosophila melanogaster*. *Science* **267**, 902–905.

Ferveur, J.-F., Savarit, F., O'Kane, C. J., Sureau, G., Greenspan, R. J. & Jallon, J.-M. (1997). Genetic feminization of pheromones and its behavioral consequences in *Drosophila* males. *Science* **276**, 1555–1558.

Gailey, D. A. & Hall, J. C. (1989). Behavior and cytogenetics of *fruitless* in *Drosophila melanogaster*: different courtship defects caused by separate, closely linked lesions. *Genetics* **121**, 773–785.

Hall, J. C. (1978). Courtship among males due to a male-sterile mutation in *Drosophila melanogaster*. *Behavior Genetics* **8**, 125–141.

Hall, J. C. (1979). Control of male reproductive behavior by the central nervous system of *Drosophila*: dissection of a courtship pathway by genetic mosaics. *Genetics* **92**, 437–457.

Hall, J. C. (1994). The mating of a fly. *Science* **264**, 1702–1714.

Ito, H., Fujitani, K., Usui, K., Shimizu-Nishikawa, K., Tanaka, S. & Yamamoto, D. (1996). Sexual orientation in *Drosophila* is altered by the *satori* mutation in the sex-determination gene *fruitless* that encodes a zinc finger protein with a BTB domain. *Proceedings of the National Academy of Sciences of the USA* **93**, 9687–9692.

Jallon, J.-M. (1984). A few chemical words exchanged by *Drosophila* during courtship. *Behavior Genetics* **14**, 441–478.

Jallon, J.-M. & David, J. R. (1987). Variations in cuticular hydrocarbons along the eight species of the *Drosophila melanogaster* subgroup. *Evolution* **41**, 487–502.

Jallon, J.-M. & Pechiné, J. M. (1989). Une autre race de chimique *Drosophila melanogaster* en Afrique. *Comptes Rendus de l'Académie des Sciences de Paris* **309**, 1551–1556.

Lindsley, D. L. & Zimm, G. G. (1992). *The Genome of* Drosophila melanogaster. London: Academic Press.

O'Dell, K. M. C., Armstrong, J. D., Yang, M. Y. & Kaiser, K. (1995). Functional dissection of the *Drosophila* mushroom bodies by selective feminization of genetically defined subcompartments. *Neuron* **15**, 55–61.

Pechiné, J. M., Perez, F., Antony, C. & Jallon, J.-M. (1985). A further characterization of *Drosophila* cuticular monoenes using mass spectrometry method to localize double bonds in complex mixtures. *Analytic Biochemistry* **145**, 177–182.

Rice, W. (1989). Analysing tables of statistical tests. *Evolution* **43**, 223–225.

Ritchie, M. G. (1996). The shape of female mating preferences. *Proceedings of the National Academy of Sciences of the USA* **93**, 14628–14631.

Ryner, L. C., Goodwin, S. F., Castrillon, D. H., Anand, A., Villella, A., Baker, B. S., Hall, J. C., Taylor, B. J. & Wasserman, S. A. (1996). Control of male sexual behavior and sexual orientation in *Drosophila* by the *fruitless* gene. *Cell* **87**, 1079–1089.

Scott, D. (1986). Sexual mimicry regulates the attractiveness of mated *Drosophila melanogaster* females. *Proceedings of the National Academy of Sciences of the USA* **83**, 8429–8433.

Scott, D. (1994). Genetic variation for female mate

discrimination in *Drosophila melanogaster*. *Evolution* **48**, 112–121.

Scott, D. (1996). Reply to: Female mate discrimination or male response to female stimulation? *Evolution* **50**, 1720–1723.

Scott, D. & Jackson, L. L. (1988). Interstrain comparison of male-predominant antiaphrodisiacs in *Drosophila melanogaster*. *Journal of Insect Physiology* **34**, 863–871.

Scott, D. & Richmond, R. C. (1988). A genetic analysis of male-predominant pheromones of *Drosophila melanogaster*. *Genetics* **119**, 639–646.

Tompkins, L. & Hall, J. C. (1983). Identification of brain sites controlling female receptivity in mosaics of *Drosophila melanogaster*. *Genetics* **103**, 179–195.

Yin Hing, A. L. & Carlson, J. R. (1996). Male–male courtship behavior induced by ectopic expression of the *Drosophila white* gene: role of sensory function and age. *Journal of Neurobiology* **30**, 454–464.

Zhang, S.-D. & Odenwald, W. F. (1995). Misexpression of the *white* (*w*) gene triggers male–male courtship in *Drosophila*. *Proceedings of the National Academy of Sciences of the USA* **92**, 5525–5529.