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A gene responsible for a cuticular hydrocarbon polymorphism in *Drosophila melanogaster*

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Summary

Drosophila melanogaster is polymorphic for the major cuticular hydrocarbon of females. In most populations this hydrocarbon is 7,11-heptacosadiene, but females from Africa and the Caribbean usually possess low levels of 7,11-heptacosadiene and high quantities of its position isomer 5,9-heptacosadiene. Genetic analysis shows that the difference between these two morphs is due to variation at a single segregating factor located on the right arm of chromosome 3 near map position 51·5 and cytological position 87C–D. This is precisely the position of a desaturase gene previously sequenced using primers derived from yeast and mouse, and localized by *in situ* hybridization to the polytene chromosomes of *D. melanogaster*. Alleles of this desaturase gene may therefore be responsible for producing the two hydrocarbon morphs. Mating tests following the transfer of these isomers between females of the two morphs show that, in contrast to previous studies, the hydrocarbon profiles have no detectable effect on mating behaviour or sexual isolation.

1. Introduction

Many molecular polymorphisms have been described in *Drosophila melanogaster*, but we know of only a few naturally occurring polymorphisms affecting observable properties of the phenotype. A rare example is the 'rover/sitter' polymorphism, affecting mobility of larvae and apparently based on alleles at a single locus (Bauer & Sokolowski, 1985; Osborne *et al.*, 1997). One of the more extensively studied polymorphisms is that affecting the composition of cuticular hydrocarbons in *D. melanogaster*. Females show two distinct morphs. One, fixed in populations outside Africa and the Caribbean, has high levels of *cis*,*cis*-7,11-heptacosadiene (henceforth '7,11-HD'), which comprises over 20% of total cuticular hydrocarbons. The other female morph, which reaches high frequencies only in African and Caribbean populations, has much lower levels of 7,11-HD but high levels of its isomer *cis*,*cis*-5,9-heptacosadiene (henceforth '5,9-HD'; Jallon & Péchiné, 1989; Ferveur *et al.*, 1996). This appears to be a true polymorphism in that no intermediate morphs have been described.

Two major aspects of this polymorphism remain unknown: its genetic basis and its adaptive significance. Chromosome substitution of the three major chromosomes shows that the hydrocarbon difference appears to reside entirely on chromosome 3, although the cytoplasm, fourth chromosome, and interchromosomal interactions were not tested (Ferveur *et al.*, 1996). It is thus possible that the polymorphism involves a single gene; but it could also be due to several genes on chromosome 3, particularly as that chromosome is polymorphic for many inversions that could harbour linked loci (Lemeunier & Aulard, 1992). As the cuticular hydrocarbons are not proteins, one should remember that 'hydrocarbon alleles' probably act by producing different forms of an enzyme that intervene in metabolic pathways to change the position of the heptacosadiene double bonds.

Hydrocarbon differences among species in the *D. melanogaster* group have been implicated in sexual isolation. Interspecific differences between females carrying either 7,11-HD or 7-tricosene (henceforth '7-T'), for example, lead to reduced courtship by males who do not normally encounter these compounds

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(Cobb & Jallon, 1990; Coyne *et al*., 1994; Coyne & Oyama, 1995; Coyne & Charlesworth, 1997). The significance of the polymorphism within *D. melanogaster*, however, is unknown. There has been speculation that this polymorphism also affects mating behaviour (Cobb *et al*., 1988), as females of strains having high levels of 7,11-HD appear to mate more rapidly than females from strains having lower levels of this compound – and presumably high levels of 5,9-HD (Ferveur *et al*., 1996). This observation, however, involved only three strains of each type and may have been due to genetic differences other than those affecting the hydrocarbon profile. Antony & Jallon (1982), Antony *et al*. (1985) and Ferveur & Sureau (1996) reported that over a threshold value of about 50 ng per female, higher levels of 7,11-HD act as a male sex attractant, increasing the amount of male courtship wing vibration in a dose-dependent manner.

The purpose of the present study was twofold. Our main aim was to pursue genetic analysis of the difference between the two hydrocarbon morphs, trying to determine whether it is due to allelic differences at a single locus. We have confirmed previous observations that the polymorphism is indeed located on the third chromosome and show that it maps to within one small region of 3R about 1·8 cM long, suggesting that the polymorphism is due to a single gene. Genetic and deficiency mapping further puts the locus near cytological position 87C, a location where Wicker-Thomas *et al*. (1977) placed a gene coding for a desaturase. As the sequence of this gene is known, further molecular studies may help answer questions about the ancestry and selective significance of the polymorphism.

Secondly, we report preliminary studies of the effect of the polymorphism on mating behaviour and sexual isolation using 'ruboff experiments' to transfer hydrocarbons between females of different hydrocarbon morphs. Any effect of the hydrocarbons on mating behaviour should be reflected by the courtship of males towards normal and chemically altered females. We found no effect of the hydrocarbon differences on mating behaviour or frequencies, and suggest that the polymorphism might be affected by forces other than selection on sexual behaviour.

2. Materials and methods

(i) Drosophila *stocks used*

The strains of *Drosophila melanogaster* used are described below. Mutations are followed by a description (in parentheses) of their genetic and cytological map positions taken from Lindsley & Zimm (1992). All mutations are recessive unless indicated otherwise. In parentheses following the name of wild-type stocks we give the abbreviation for that stock used in the data tables.

Zimbabwe 2 (Zim 2): An isofemale line from the Sengwa Wildlife Reserve, Zimbabwe, collected on 26 September 1990 by R. Ramey.

Tai 2: A stock composed of a mixture of isofemale lines collected in Tai, Ivory coast, in 1980. This strain contains the inversions In(3R)P (89C2–3; 96A18) and In(3R)K (86F1–87A1; 96F11–97A1), as well as other inversions on the left arm of the third chromosome (S. Aulard, personal communication). Because the line contained both hydrocarbon morphs, with a low frequency of the non-African high 7,11-HD morph, we used a subline of the strain derived from three generations of brother–sister mating. As the high 7,11-HD morph is recessive to the high 5,9-HD morph (see below), we verified by outcrossing that this subline (the stock called 'Tai 2' in our data tables) contained only the allele producing the high 5,9-HD morph. It is possible that the presence of the high 7,11-HD allele in this strain may have been due to contamination, but we consider this unlikely as such polymorphisms have been seen in other African strains (see below).

Tai-2-I3: A line derived from Tai 2 made by using the balancer *TM6B* to extract a homozygous Tai 2 third chromosome. This chromosome, which carried In(3R)P (89C2-3-96A18), proved to be homozygous lethal and was kept balanced against *TM6B*.

Ives: A stock homokaryotypic for the standard (ST) sequence on all chromosomes, made by combining 200 isofemale lines collected in 1975 in Amherst, Massachusetts, USA (for further information about this strain see Coyne *et al*., 1991).

Jaragua 3B (Jar 3B): A line containing a homozygous third chromosome extracted (using the *TM6B* balancer) from an isofemale line collected in Jaragua, Dominican Republic, in 1993. This chromosome carried the high 5,9-HD allele and was inversion-free on 3R, although it carried In(3L)P.

Princess Market Line 1A (P. Mkt. 1A): A line containing a homozygous third chromosome extracted (using the *TM6B* balancer) from an isofemale line collected in St Croix, Virgin Islands, in 1997. The line carried the ST sequence on both the left and right arms of the third chromosome and the allele for the high 5,9-HD hydrocarbon morph.

cu kar: A mutant stock carrying the standard (ST) chromosome sequence on the third chromosome and containing the third-chromosome mutant markers *curled* (wing mutation; 3–50·0; 86D1–4) and *karmoisin* (eye colour mutation; 3–51·7; 87C8).

cu kar ry: A stock carrying the same mutations and chromosome sequence as *cu kar*, but also including the eye colour mutation *rosy* (3–52·0; 87D8–12).

C(1) RM *y w f* females, *dor* males: A stock whose females carried attached-X chromosomes bearing the mutations *yellow*, *white* and *forked* (1–0·0, 1B1; 1–1·5, 3C2; and 1–56·7, 15F1–3 respectively), while the male

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had a free X carrying the *dor* (deep orange) eye colour mutation (1–0·3; 2B11–12).

y; *bw*; *e*; *ci ey*^{R}: A stock with the ST cytological sequence and carrying mutant markers on each of the four chromosomes, which was used for localizing the 'hydrocarbon gene' to the third chromosome (see below). The markers included *yellow* (body colour; 1·0; 1B1), *brown* (eye colour; 2–14·5; 59E1–3), *ebony* (body colour; 3–70·7; 93D2–6), and the two fourth-chromosome markers, *cubitus interruptus* (4–0; 101F2–102A5) and *eyeless-Russian* (4–2·0).

ru h th st cu sr e ca: A stock with the ST cytological sequence and carrying the third-chromosome markers *roughoid* (3–0; 61F5–62A3), *hairy* (3–26·5; 66D15), *thread* (3–43·2; 72A2–72C1–2), *scarlet* (3–44·0; 73A3–4), *curled* (3–50·0; 86D1–4), *stripe* (3–62·0; 90D2–F7), *ebony* (3–70·7; 93D2–6) and *claret* (3–100·7; 99B5–9).

Df(3R) *Mk-X1TM3*, *Sb*^{1} *Ser*^{1}: A stock containing the *M-X1* deficiency (86C1; 87B5) balanced over a TM3 chromosome containing the dominant markers *Sb*^{1} and *Ser*^{1}.

Df(3R) *kar*-D2, In(3R) *AFA e*^{1}/*cu*^{1} *kar*^{1} *Sb*^{1}: A stock containing the *kar-D2* deficiency (87A7; 87D4–5) and an inversion balanced against a chromosome containing the dominant allele *Sb*^{1}.

Other stocks used in the study are described in the Appendix, while the crosses made to localize the gene are described in Section 3.

(ii) *Gas chromatography*

Extraction of cuticular hydrocarbons, gas chromatography of the extracts, and estimation of hydrocarbon quantities were performed as described previously (Coyne *et al*., 1994; adapted from Ferveur, 1991). Flies were analysed individually, and all data are presented as mean quantities or ratios of hydrocarbons per fly. Identification of peaks was done by co-migration of these peaks with those from strains whose main hydrocarbons had been identified by mass spectroscopy (Péchiné *et al*., 1985). Estimates of hydrocarbon quantities per fly were taken from integrated peak areas (proportional to the amount of the constituent hydrocarbon) compared with the peak area of a standard quantity of *n*-hexacosane (1200 ng) mixed into each sample.

While the 7,11-HD peak invariably appears by itself on gas chromatograms, in many cases (particularly in high 5,9-HD strains) the later-appearing 5,9-HD peak often co-migrates with another peak representing 2-methylhexane (henceforth '2-MH') (Ferveur *et al*., 1996). Hence, in our tables we usually present the estimated hydrocarbon quantities from two peaks: the 7,11-HD peak and the peak containing the two compounds 5,9-HD and 2-MH, which we call the 'CII peak'. The hydrocarbon profile of a given strain or genotype is generally represented as the ratio of these two peaks: CII/7,11-HD. This ratio had low standard errors among individuals within a strain or genotype, and so gives a reliable indication of the predominant female hydrocarbons.

In some samples we were able to distinguish the peak representing 5,9-HD from that representing 2-MH, and in one cross we present these values separately. As we show below, the polymorphism represented by the very different relative sizes of the 7,11-HD and CII peaks is due almost entirely to differences in quantity of the two isomers 7,11-HD and 5,9-HD, for the quantity of 2-MH varies very little between the two hydrocarbon morphs. As described by Jallon & Péchiné (1989) and confirmed by our own work, in high 5,9-HD strains the CII peak contains roughly 80–90% 5,9-HD and 10–20% 2-MH. In high 7,11-HD strains, on the other hand, the CII peak contains roughly 40% 5,9-HD and 60% 2-MH. Hence the difference between the morphs in their CII/7,11-HD ratios actually underestimates the difference in the true ratio of the isomers: 7,11-HD/5,9-HD.

For statistical comparison of the hydrocarbon profile among strains or genotypes, we used the non-parametric Mann–Whitney *U*-test on the CII/7,11-HD hydrocarbon ratios. The same test was used to compare courtship parameters between strains, except for the number of courtships and copulations, which were tested with simple chi-squared tests. One-tailed probabilities were calculated when testing *a priori* hypotheses involving differences between genotypes in expected directions.

(iii) *Hydrocarbon transfer experiments*

As described previously (Coyne *et al*., 1994; Coyne & Charlesworth, 1997), when a few adult females (called 'target females') are crowded into a small volume for 4 days with many females of a different hydrocarbon phenotype (called 'donor females'), the former acquire (probably by direct contact) substantial quantities of hydrocarbons from the latter. We used this technique as described in Coyne & Charlesworth (1997) to transfer hydrocarbons among high 7,11-HD and high 5,9-HD strains. Five 1-day-old virgin 'target' females, marked by wing-clipping, were crowded with 50 1-day-old 'donor' females or 4 days, and then immediately used for observations of courtship behaviour. Identically wing-clipped females were crowded at the same time with members of their own strain and used as controls. This crowding was able to effect substantial changes in the ratio of these hydrocarbons carried by females. We used such flies to determine whether altering a female's hydrocarbon

profile affected her sexual isolation from males of either a high 7,11-HD or a high 5,9-HD strain.

(iv) *Observations of courtship and copulation*

In these experiments we used two sets of 'no choice' experiments, placing individual males from either a high-7,11-HD strain (Ives) or a high 5,9-HD strain (Tai 2) with individual females. In the first experiment, single unaltered Ives males were observed when isolated with a single Ives female that had either been previously crowded with females of her own strain (these target female hence serving as controls) or with females from the Tai 2 strain (target females, with higher levels of 5,9-HD, serving as experimentals). Observations were made in pairs, with one observer scoring the mating behaviour of an Ives males confined with a control female and the other observer scoring the behaviour of an Ives male confined with an experimental female (both females had their wings clipped in an identical manner). Vials were coded so that observers did not know the identity of the females, and pairs were alternated among observers to minimize any differences in how behaviours were scored. Forty-two pairs were observed, for 20 min each.

A similar study was done observing Tai 2 males confined with either control Tai 2 females (target females crowded with other Tai 2 females) or experimental Tai 2 females (target females who had been previously crowded with Ives females and thus had acquired increased amounts of 7,11-HD).

In addition, similar observations were done on all possible pairwise combinations of males and females from the two pure strains to determine whether individuals from these strains initially showed non-random mating. In these latter studies, pairs of flies were also watched by pairs of observers who did not know the identities of the flies, with 20 replicate pairs watched per experiment.

In the 'ruboff experiments', samples of control and experimental females were subject to gas chromatography to determine the alteration in hydrocarbon profile induced by these experiments.

Male courtship behaviours were defined and measured as described by Cobb *et al.* (1985) and Coyne *et al.* (1994). These behaviours included:

(a) *Courtship latency*: time in minutes from introduction of the male into the female-containing vial until his first display of courtship (usually wing vibration).
(b) *Copulation latency*: time in minutes from introduction of the male into the vial until copulation.
(c) *Courtship duration*: the cumulative amount of time (in minutes) that a male spent courting a female, beginning when he was introduced into the vial and ending when he either copulated or the observation period ended.
(d) *Copulation attempts*: the number of times a male unsuccessfully attempted to mount a female, scored when the male curled his abdomen downwards and tried to make genital contact with the female.
(e) *Copulation attempts per minute*: the number of copulation attempts divided by the time from introduction of a male into a vial until either a mating occurred or the observation period ended.
(f) *Courtship intensity*: the total duration of courtship divided by the difference between the courtship latency and the copulation latency (or, in the few cases in which flies did not copulate, between the courtship latency and the end of the 20 min observation period). This represents the proportion of time between the initial courtship and copulation that is occupied by courtship behaviour.
(g) *Copulation*: whether or not a male successfully copulated (genital attachment to the female for more than 1 min). Failure to copulate was quite rare in this study.

3. Results

(i) *Pure strains and F1s.*

Table 1 shows the hydrocarbon quantities given as the mean nanograms per fly of each compound or mixture from the two peaks of interest. As noted above, the phenotype used was the ratio of the quantity of hydrocarbon represented by the CII peak divided by the quantity represented by the 7,11-HD peak. High values of this ratio characterize African and Caribbean strains, low values those strains from elsewhere, including the marker strains, which are all derived from non-African populations. As described previously (Ferveur *et al.*, 1996), our strains are easily divided into two groups: high 7,11-HD strains have 200–500 ng of 7,11-HD and less than 200 ng of CII hydrocarbons, whereas high 5,9-HD strains generally have less than 100 ng of 7,11-HD and between 300 and 500 ng of CII hydrocarbons. The strains can thus be roughly dichotomized by whether their CII/7,11-HD ratios are lower or higher than 1.

F1 hybrids between high 7,11-HD and high 5,9-HD strains are generally intermediate between the parental strains in their CII/7,11-HD ratios (Table 1, genotypes 12–18), but display near-dominance for the quantities of CII hydrocarbons: hybrids show levels of these hydrocarbons nearly equal to those seen in high 5,9-HD parental strains, but have only 30–70 ng more 7,11-HD than observed in the high 5,9-HD parental strains. This semidominance was also reported by Ferveur *et al.* (1996).

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Table 1. *Hydrocarbons of females from various strains of* D. melanogaster *and their F1 hybrids*

Genotype	*N*	7,11-HD (ng)	CII (ng)	CII/7,11-HD
Pure lines				
1. Tai 2-I3	20	26·0 (1·6)	336·9 (12·6)	13·465 (0·559)
2. Zim 2	14	69·4 (2·3)	316·6 (8·5)	4·620 (0·134)
3. Jar 3B	16	55·9 (2·5)	569·3 (24·5)	10·333 (0·418)
4. *ru h th st cu sr e ca*	20	311·7 (13·2)	176·4 (9·81)	0·576 (0·033)
5. C(1) RM *y w f* (*dor* males)	15	385·7 (12·9)	141·8 (6·8)	0·372 (0·021)
6. *cu kar*	12	144·0 (5·4)	138·1 (8·1)	0·959 (0·046)
7. *cu kar ry*	15	455·0 (31·8)	141·5 (10·3)	0·486 (0·029)
8. *y*; *bw*; *e*; *ci ey*^{R}	15	237·5 (12·4)	91·5 (6·0)	0·391 (0·024)
9. Df(3R) *kar-D2* ln(3R) *AFA e*^{1}/*cu*^{1} *kar*^{1} *Sb*^{1}	12	195·2 (9·8)	200·7 (11·7)	1·026 (0·022)
10. Df(3R) *Mk-X1/TM3 Sb*^{1} *Ser*^{1}	18	257·2 (11·3)	106·7 (4·5)	0·419 (0·013)
11. Ives	15	652·7 (31·0)	168·7 (12·2)	0·260 (0·016)
F1 hybrids (female × male)				
12. Tai 2-I3 × *y*; *bw*; *e*; *ci ey*^{R}	15	62·0 (12·3)	283·7 (8·3)	4·804 (0·355)
13. Zim 2 × *dor*	15	124·8 (9·5)	379·1 (27·0)	3·104 (0·121)
14. C(1)RM *ywf* (*dor* males) × Zim 2	15	130·7 (3·7)	429·0 (13·1)	3·296 (0·093)
15. *ru h th st cu sr e ca* × Tai 2-I3	20	99·7 (5·3)	543·7 (23·9)	5·668 (0·301)
16. *ru h th st cu sr e ca* × Zim 2	6	93·9 (5·4)	354·7 (14·5)	3·804 (0·145)
17. *cu kar* × Zim 2	15	100·4 (3·6)	319·5 (10·9)	3·207 (0·112)
18. *cu kar ry* × Jar 3B	15	88·2 (5·1)	458·3 (21·0)	5·302 (0·208)

Values given are means of groups of females of sample size *N*; standard errors are given in parentheses.

Table 2. *Hydrocarbons in genotypes of female offspring in the backcross involving the* y; bw; e; ci ey^{R} *and Tai 2 strains* (*see text for details*)

Genotype (markers showing)	7,11-HD (ng)	CII (ng)	CII/7,11-HD
bw; *e*; *ci ey*^{r}	397·9 (25·0)	106·2 (11·0)	0·262 (0·059)
bw; *e*; + +	321·9 (26·5)	66·0 (9·2)	0·201 (0·012)
bw; +; *ci ey*^{R}	94·0 (6·3)	429·8 (13·8)	4·772 (0·248)
+; *e*; *ci ey*^{R}	296·8 (21·7)	69·9 (6·28)	0·266 (0·046)
+; *e*; + +	293·9 (10·71)	88·9 (6·1)	0·307 (0·023)
bw; +; + +	66·7 (5·3)	323·9 (19·6)	5·057 (0·269)
+; +; *ci ey*^{R}	101·9 (5·8)	394·4 (11·3)	4·075 (0·303)
+; +; + +	77·4 (6·0)	367·3 (18·0)	5·036 (0·359)

Values given are means of 15 females of each genotype; standard errors are given in parentheses.

(ii) *Localization of hydrocarbon polymorphism to chromosome 3*

Because of the dominance of CII hydrocarbon quantity in F1 hybrids, and because all marker stocks possess the high 7,11-HD phenotype, genetic localization of the polymorphism was carried out by making F1 hybrids between a high 5,9-HD strain and a high 7,11-HD marker strain, and then backcrossing the F1 hybrids to flies from the marker stock. Using chromosome substitution lines, Ferveur *et al.* (1996) localized the polymorphism to chromosome 3, but they did not examine either the small fourth chromosome or interactions between any chromosomes, nor did they attempt to distinguish cytoplasmic from X-chromosome effects. We repeated this localization using backcrosses to a multiple-marker stock instead of chromosome substitution.

In our reanalysis, we crossed males from the high 7,11-HD *y*; *bw*; *e*; *ci ey*^{R} marker strain to high 5,9-HD females from the Tai 2 strain. So that there would be no recombination within chromosomes, we then backcrossed the F1 hybrid males to *y*; *bw*; *e*; *ci ey*^{R} females, producing eight classes of recombinants carrying all combinations of autosomes from the two strains. (Because we used male hybrids, there was no segregation of the X chromosome; all backcross females had one X from the Tai 2 strain and one X from the marker strain. The X chromosome was examined separately as described below.) The results of this backcross are given in Tables 2 and 3; Table 2 gives the hydrocarbon profile of backcross offspring

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Table 3. *The subset of backcross data from Table 2* (*and the two pure strains from Table 1*) *in which the* '*CII*' *peak was separable into its constituents*: *2-MH and 5,9-HD*

Genotype	*N*	7,11-HD (ng)	2-MH (ng)	5,9-HD (ng)	5,9-HD/7,11-HD
Pure strains					
y; *bw*; *e*; *ci ey*^{R}	15	311·7 (13·2)	100·1 (5·4)	76·3 (4·8)	0·248 (0·015)
Tai 2	5	34·2 (3·4)	44·7 (5·4)	366·7 (43·2)	10·859 (1·055)
Backcross genotypes					
bw; *e*; *ci ey*^{R}	9	433·3 (35·6)	95·6 (11·0)	30·2 (4·6)	0·068 (0·006)
bw; *e*; + +	7	357·7 (50·8)	56·7 (15·0)	29·9 (3·4)	0·059 (0·005)
bw; +; *ci ey*^{R}	1	66·9 —	63·2 —	316·5 —	4·729 —
+; *e*; *ci ey*^{R}	7	309·5 (42·0)	63·5 (4·8)	25·1 (4·0)	0·092 (0·017)
+; *e*; + +	4	285·5 (12·9)	78·7 (8·2)	28·7 (3·6)	0·102 (0·015)
bw; +; + +	3	93·5 (8·2)	79·1 (4·4)	368·2 (16·7)	3·967 (0·162)
+; +; *ci ey*^{R}	6	110·4 (4·4)	76·0 (8·3)	317·7 (14·8)	2·890 (0·151)
+; +; + +	1	105·8 —	58·3 —	297·7 —	2·814 —

Standard errors are given in parentheses.

divided into their '7,11-HD' and 'CII' peaks, while Table 3 is a subset of the data from Table 2, consisting of all runs in which the CII peak was resolvable into its constituent 2-MH and 5,9-HD peaks.

It is immediately evident from Table 2 that the difference between the two hydrocarbon morphs resides – as previously found by Ferveur *et al*. (1996) – on the third chromosome. All genotypes carrying the *ebony* marker show CII/7,11-HD ratios between 0·2 and 0·3, while flies lacking the *ebony* markers have ratios ranging between 4 and 5. The large effect of the third chromosome is borne out by an analysis of variance of the log-transformed CII/7,11-HD values taken from Table 2. Of all three autosomes, only chromosome 3, which carries the *ebony* marker, has a significant effect on the hydrocarbon ratios ($F_{1,112} = 3291$, $P < 0{\cdot}0001$, an effect more than 2 orders of magnitude larger than that of any single chromosome or interchromosomal interaction). Although two of the chromosome interactions (2×4 and 3×4) are also significant ($F_{1,112} = 6{\cdot}3$, $P < 0{\cdot}014$, and 10·1, $P = 0{\cdot}002$ respectively), these effects are tiny compared with the large effect of chromosome 3, and we will not consider them further.

Table 3 gives the data from individuals in the parental strains and backcross in which the 2-MH and 5,9-HD peaks were separable. The Tai 2 strain was unusual for a high 5,9-HD strain in that a substantial proportion of individuals showed this peak separation. In this strain only about 11% of the 'CII' peak consists of 2-MH, with the remaining 89% constituting 5,9-HD. In the *y*; *bw*; *e*; *ci ey*^{R} marker strain, the level of 2-MH was roughly 40% higher than that of 5,9-HD; these values are all close to those reported by Jallon & Péchiné (1989) for the same hydrocarbon morphs. These two strains thus differ by only about 60 ng in the amount of 2-MH, but by nearly 300 ng in the amounts of both 7,11-HD and 5,9-HD; this results in a 40-fold difference between the morphs in their 5,9-HD/7,11-HD ratios. The relative invariance of the quantity of 2-MH between morphs and among the backcross genotypes (whose means for this compound vary by less than 40 ng) confirms similar observations by Jallon & Péchiné (1989).

Even though Table 3 contains fewer backcross individuals than does Table 2, it is clear that the large effect of chromosome 3 on the composite 'CII' peak seen in Table 2 comes entirely from its effect on the relative amounts of 5,9-HD and 7,11-HD, and not on the amount of 2-MH. An analysis of variance on the log-transformed values of 5,9-HD/7,11-HD shows that only chromosome 3 has a significant effect ($F_{1,30} = 378$, $P < 0{\cdot}0001$), while the interaction of chromosomes 2 and 3 is also significant, though less so ($F_{1,30} = 7{\cdot}3$, $P = 0{\cdot}011$). We conclude that the difference between the two peaks of interest, 7,11-HD and CII, maps to the third chromosome, and that the gene or genes affecting the ratio of these two peaks does so by affecting the relative proportions of 7,11-HD and 5,9-HD.

As the X chromosome was not segregating among these progeny, its effects on hydrocarbon ratios was not resolvable. To evaluate any X-chromosome effects, we made reciprocal crosses between two strains. One was the high 7,11-HD stock in which females carried attached X chromosomes marked with the *y*, *w* and *f* alleles (strain 5 from Table 1), and the second strain was the high 5,9-HD strain Zim 2. Zim 2 males were crossed to C(1) RM *y w f* females, producing F1 hybrid females having both X chromosomes from the high 7,11-HD strain (genotype 14 in Table 1), while in the reciprocal cross (Zim 2 females × *dor* males), F1 hybrid females carry one X chromosome from the high 7,11-HD strain and one from the high 5,9-HD strain (genotype 13 in Table 1). Both female genotypes have similar CII/7,11-HD ratios (Table 1), and the

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Table 4. *Hydrocarbon ratios among females in the two backcrosses involving the* ru h th st cu sr e ca *stock and the Tai 2-I3 or Zim 2 strain* (*see text*)

Genotype (markers showing)	Tai 2-I3 cross		Zim 2 cross	
	N	CII/7,11-HD	*N*	CII/7,11-HD
ru h th st cu sr e ca	20	0·702 (0·053)	15	0·628 (0·132)
ru h th st cu sr e +	3	0·539 (0·016)	0	—
ru h th st cu sr ++	0	— —	0	—
ru h th st cu +++	4	0·545 (0·040)	11	0·502 (0·034)
**ru h th st cu* +++	1	3·850 —	2	3·060 (0·347)
ru h th st ++++	16	4·022 (0·219)	0	—
ru h th +++++	0	— —	0	—
ru h ++++++	15	3·916 (0·116)	0	—
ru +++++++	15	3·848 (0·197)	0	—
++++++++	13	5·621 (0·335)	19	3·568 (0·121)
++++++++ *ca*	8	5·387 (0·350)	22	3·056 (0·104)
++++++ *e ca*	0	— —	20	3·211 (0·074)
*+++++ *sr e ca*	2	5·213 (0·273)	52	3·699 (0·081)
+++++ *sr e ca*	0	— —	11	0·497 (0·023)
++++ *cu sr e ca*	15	0·596 (0·016)	25	0·457 (0·013)
+++ *st cu sr e ca*	0	— —	0	—
++ *th st cu sr e ca*	16	0·635 (0·034)	0	—
+ *h th st cu sr e ca*	14	0·634 (0·025)	0	—

N is the number of females analysed for each genotype; standard errors are given in parentheses. Asterisks indicate putative recombinants between the hydrocarbon allele of the marker strain and the closest marker allele (either *cu* or *sr*).

difference that does exist is not statistically significant under a two-tailed Mann–Whitney *U*-test ($Z = 1{\cdot}721$, $P = 0{\cdot}09$), and is in the wrong direction as well. Thus the X chromosome has no perceptible effect on the ratio of the two heptacosadiene isomers.

(iii) *Mapping the hydrocarbon gene on the third chromosome*

Initial localization of the hydrocarbon gene(s) was made by using the third-chromosome multiple marker strain *ru h th st cu sr e ca* (see Section 2). Two sets of backcrosses were done: *ru h th st cu sr e ca* females were crossed to either Tai2-I3 or Zim 2 males, and F1 females (in the case of Tai2-I3, those not showing the *Delta* allele on the TM6 chromosome) backcrossed to *ru h th st cu sr e ca* males. The association of particular markers with the high 7,11-HD or high 5,9-HD phenotype localizes the gene or genes causing the polymorphism. The Tai2-I3 strain was fixed for the In(3R)P inversion (89C2–3; 96A18–19), which includes both the *sr* and *e* loci), while the Zim 2 strain is known to carry the In(3R)K inversion in high frequency (86F1, 87A1; 96F11, 97A1, which includes the *e* locus) as well as some third chromosomes carrying the Standard sequence on 3R. We therefore expected to observe no recombinants between *sr* and *e* in offspring of the Tai2-I3 backcross, and few such recombinants in the backcross involving Zim 2.

The hydrocarbon profiles and CII/7,11-HD ratios for females from the marker strain, the Tai2-I3 and Zim 2 strains, and the relevant F1-hybrids are given in Table 1 (genotypes 1, 2, 15 and 16). As found in other crosses, the quantity of 'CII' from the high 5,9-HD strain was dominant in these hybrids.

Table 4 gives the hydrocarbon profile of a set of genotypes from each of the two backcrosses; recombinants, which occurred only in the *cu-sr* region, were identified by their pheromone profile and are indicated with asterisks. We did not analyse all possible genotypes because this was not necessary for localizing the hydrocarbon gene, but instead examined a subset of genotypes representing recombination in various regions of interest. Table 4 shows clearly that the chromosome region associated with the polymorphism lies between the loci *curled* (*cu*; at 50·0 cM, chromosome region 86D1–4) and *stripe* (*sr*; 62·0 cM, region 90D2–F7). Genotypes showing both the *cu* and *sr* alleles always have low CII/7,11-HD ratios, while possession of both alternative wild-type alleles always confers a high CII/7,11-HD ratio.

Precise localization of the hydrocarbon gene in this region is not possible because of the presence of nearby inversions that inhibit recombination, but the locus appears to be closer to *cu* than to *sr*. Of the 63 individuals in the Zim 2 backcross showing the +++++ *sr e ca* phenotype, 52 carry the high CII/7,11-HD hydrocarbon phenotype characteristic of the wild-type line (i.e. these individuals were

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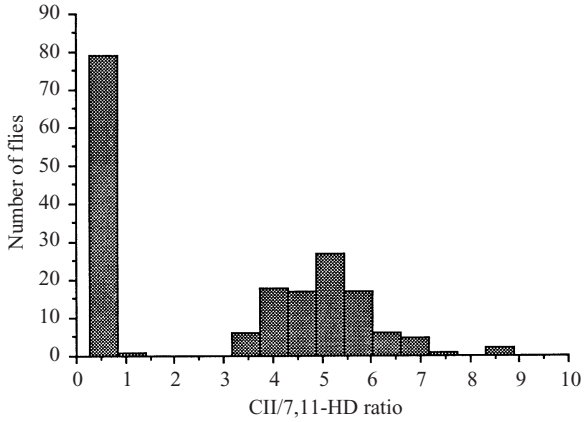

Fig. 1. CII/7,11-HD hydrocarbon ratios for the 179 offspring of the backcross between the high 5,9-HD Jar 3B strain and the high 7,11-HD *cu kar ry* strain (summary data given in Table 5). Bimodality of hydrocarbon ratios indicates a single segregating genetic factor.

Table 5. *Hydrocarbon ratios among female in the backcross involving the* cu kar ry *marker strain and the isochromosomal-3 Jar 3B strain* (*see text for details*)

Backcross genotype (markers showing)	*N*	CII/7,11-HD
cu kar ry	20	0·486 (0·017)
cu kar +	25	0·567 (0·026)
**cu* + +	51	5·041 (0·115)
cu + +	5	0·677 (0·091)
+ *kar ry*	29	0·433 (0·020)
* + *kar ry*	5	3·836 (0·107)
+ *kar* +	1	0·621 –
+ + *ry*	23	5·352 (0·225)
+ + +	20	5·296 (0·291)

N is the number of females analysed for each genotype; standard errors are given in parentheses. Asterisks give putative recombinants between the hydrocarbon allele of the marker strain and the closest marker allele (*cu* or *kar*).

recombinant between *cu* and the hydrocarbon allele in the marker strain). Moreover, in both backcrosses most individuals of the *ru h th st cu* + + + phenotype have the high 7,11-HD morph characteristic of the marker stock.

The proximity of the hydrocarbon gene to *cu* was estimated in two further sets of crosses. A preliminary analysis involved *cu kar* females crossed to Zim 2 males, and the F1 hybrid females backcrossed to *cu kar* males. The results of this cross (data not shown) indicated that the hydrocarbon polymorphism resided in the 1·7 cM region between the *cu* and *kar* markers, as both the *cu* + and + *kar* phenotypic classes showed some recombinants between the marker alleles and hydrocarbon loci. However, because of inversions segregating in the Zim 2 strain, we found only 42 recombinants between the *cu* and *kar* loci among thousands of backcross progeny. By inhibiting recombination, the inversions also make it impossible to map the hydrocarbon gene reliably in this cross.

To obviate these problems, we require a high CII/7,11-HD strain free of inversions on 3R. We were unable to produce such a strain using African third chromosomes because of the preponderance of lethal alleles and inversions on 3R. However, Caribbean lines contain many fewer inversions than do African strains. We produced a number of isochromosome-3 lines from Caribbean strains provided by H. Hollocher, and these lines were karyotyped by P. Sniegowski.

One of these lines, Jaragua 3B from the Dominican Republic (Table 1, strain 3; see also Section 2) was homozygous viable, showed the high 5,9-HD hydrocarbon phenotype, and was also free of inversions on 3R although it carried In(3L)P (63C;73E1–3). Males from the isochromosomal Jar 3B line were crossed to females from the marker stock *cu kar ry*, which had the high 7,11-HD phenotype. F1 females (showing the semidominant high 5,9-HD morph; see Table 1, genotype 18) were backcrossed to *cu kar ry* males. Table 5 gives the hydrocarbon ratios for the seven genotypes of females obtained in this backcross, and Fig. 1 shows the bimodality of hydrocarbon ratios among all progeny, indicating a single genetic factor segregating in the backcross. As with the previous cross, the data in Table 5 clearly show that the locus associated with the hydrocarbon polymorphism resides between *cu* and *kar*.

As in the previous cross using the Zim 2 strain, the hydrocarbon gene seems to be closer to *kar* then to *cu*, as the *kar* allele shows much tighter linkage to the 'high 7,11-HD' allele than does the *cu* allele (51 of 56 recombinants between *cu* and *kar* that carried only the *cu* marker also carried the non-parental high 5,9-HD phenotype). Because of the lack of nearby inversions, one can use these data to localize the hydrocarbon gene. It is roughly 5/34 of the distance from *kar* to *cu* (0·15) and roughly 51/56 of the distance from *cu* to *kar* (0·91). The weighted average of the distances from *cu* is (51 + 29)/90, or 0·89 of the total distance from *cu* to *kar*. As 1·7 cM separates these markers (*cu* is at 50·0 and *kar* at 51·7), the hydrocarbon locus therefore resides at approximately 50·0 + 0·89 (1·7), or 51·5 cM ± 0·06 cM (95 % CI). Using the correlated genetic and cytological maps provided by Lindsley & Zimm (1992), one can place this gene near position 87 C–D on the polytene chromosome map. Moreover, the similar results of backcrosses involving the Jar3B and Zim 2 strains show that the 'high 5,9-HD' allele in both Africa and the Caribbean reside at the same locus.

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Table 6. *Hydrocarbons in offspring of crosses between the two deficiency-containing strains and various wild strains* (*see text for details*)

Cross	Progeny	*N*	7,11-HD (ng)	CI (ng)	CII/7,11-HD
1. Df(3R)*Mk-X1*/*TM3*, *Sb*^{1} *Ser*^{1} × Jar 3B					
	Df/+	8	98·0 (7·0)	368·4 (24·6)	3·785 (0·172)
	+/+	8	55·6 (2·7)	506·1 (52·0)	9·060 (0·702)
2. Df(3R)*kar-D2* In(3R)*AFA e*^{1}/*cu*^{1} *kar*^{1} *Sb*^{1} × Jar 3B					
	Df/+	15	32·7 (2·7)	461·4 (20·6)	15·037 (1·028)***
	+/+	15	50·5 (2·1)	431·4 (18·1)	8·585 (0·231)
3. Df(3R)*kar-D2* In(3R)*AFA e*^{1}/*cu kar Sb* × P. Mkt. 1A					
	Df+	10	104·3 (7·0)	490·2 (24·3)	4·774 (0·175)***
	+/+	10	155·5 (8·5)	405·2 (19·0)	2·640 (0·341)
4. Df(3)*Mk-X1*/*TM3*, *Sb*^{1} *Ser*^{1} × P. Mkt. 1A					
	Df/+	13	224·0 (10·9)	315·9 (12·7)	1·436 (0·067)
	+/+	13	141·9 (8·2)	355·6 (24·4)	2·506 (0·131)
5. Df(3R)*kar-D2*, In(3R)*AFA e*^{1}/*cu kar Sb* × Tai 2					
	Df/+	12	49·3 (5·1)	521·0 (32·4)	11·168 (0·724)***
	+/+	12	94·3 (11·3)	499·5 (21·2)	5·992 (0·566)
6. Df(3R)*kar-D2*, In(3R)*AFA e*^{1}/*cu kar Sb* × Iv					
	Df/+	16	365·8 (112·0)	182·6 (8·4)	0·503 (0·023)***
	+/+	15	432·1 (22·9)	128·1 (6·6)	0·309 (0·024)
7. Df(3R)*Mk-X1*/*TM3*, *Sb*^{1} *Ser*^{1} × Iv					
	Df/+	15	463·8 (21·4)	139·9 (8·07)	0·304 (0·018)
	+/+	15	394·3 (24·5)	195·5 (11·7)	0·527 (0·051)

Values given are means of sample size *N*; standard errors are given in parentheses. Hydrocarbon ratios between genotypes resulting from each cross are compared with a one-tailed Mann–Whitney *U*-test.
*** $P < 0{\cdot}001$.

(iv) *Deficiency mapping of the hydrocarbon gene*

Despite the semidominance of the high 5,9-HD allele, we attempted to map the hydrocarbon gene using two deficiencies completely covering the region between *cu* and *kar*: Df(3R)*Mk-X1* and the smaller Df(3R) *kar-D2* (see Section 2). Each of the strains containing the deficiencies was crossed to a series of high 7,11-HD and high 5,9-HD strains, and gas chromatography was performed on female offspring carrying and lacking the deficiency (these are distinguishable by the dominant allele visible in the latter class). Our expectation was that if a deficiency contained the hydrocarbon locus, offspring carrying the deficiency would have lower amounts of 7,11-HD than their sibs lacking the deficiency.

Only the Df(3R) *kar-D2* deficiency produced such differences between offspring genotypes (Table 6). In all four crosses, the differences between the two classes of offspring were significant and in the direction expected if the Df(3R) *kar-D2* deficiency carried the hydrocarbon gene. For Df(3R) *Mk-X1*, on the other hand, crosses to two of the same high 5,9-HD strains and to one high 7,11-HD strain produced differences between offspring in the direction opposite to what was expected, giving no evidence that Df(3R) *Mk-X1* includes the hydrocarbon gene. (The differences in the 'wrong' direction, which are significant using two-tailed statistics, may be due to alleles on the balancer chromosome.) It thus seems likely that the hydrocarbon gene is contained within Df(3R)*kar-D2* (87A7;87D4–5). This conclusion supports the genetic mapping experiments placing the locus in the 87C,D region. One should also note the unusually high CII/7,11-HD ratio observed in the Df(3R) *kar-D2* balancer strain itself (Table 1, genotype 9). This is the highest hydrocarbon ratio seen in any other high-7,11 HD strain, and may be due to the reduced amount of 7,11-HD caused by the presence of only one copy of the high 7,11-HD hydrocarbon allele.

(v) *Association of the hydrocarbon gene with third-chromosome inversions*

Because the hydrocarbon gene is close to the breakpoints of several inversions on the right arm of chromosome 3, it was of interest to know whether the high 5,9-HD allele was consistently associated with any particular arrangement on that chromosome arm (this cannot be true for the high 7,11-HD allele, which is fixed in all populations outside of Africa and the Caribbean despite the extensive third-chromosomal polymorphism in these populations: Lemeunier & Aulard, 1992). Table 7 shows the hydrocarbon phenotype of several African and Caribbean lines made homozygous for chromosome 3 and scored for inversions on 3R. It is clear that the high 5,9-HD allele is associated with at least three 3R sequences in Africa

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Table 7. *Isochromosomal lines with known 3R inversions and hydrocarbon phenotypes*

Line	Collection data	Inversion on 3R	Hydrocarbon allele
Zimb 6B	1991, Zimbabwe, Sengwa Wildlife Reserve	In(3R)K	High 5,9-HD
Zim 6C	1991, Zimbabwe, Sengwa Wildlife Reserve	ST	High 5,9-HD
Zim 30B	1991, Zimbabwe, Sengwa Wildlife Reserve	In(3R)K	High 5,9-HD
Zim 11	1991, Zimbabwe, Sengwa Wildlife Reserve	In(3R)K	High 7,11-HD
Tai 2-I3	1980 Tai, Ivory Coast	In(3R)P	High 5,9-HD
FR9A	1993, Finca Mora, Dominican Republic	In(3R)P	High 5,9-HD
Jar 3B	1993, Jaragua, Dominican Republic	ST	High 5,9-HD
Jar 1	1993, Jaragua, Dominican Republic	In(3R)P	High 5,9-HD
EB12	1991, El Boniato, Cuba	In(3R)P	High 5,9-HD
SCPM1B	1997, Princess Market, St Croix	ST	High 5,9-HD
SCCBA	1997, Cane Bay, St Croix	ST	High 5,9-HD

The boundaries of In(3R)K are (86F1–87A1; 96F11–97A1), and of In(3R)P are (89C2–3; 96A18–19).

and two in the Caribbean. The gene is thus not absolutely linked to a particular inversion, but more comprehensive work will be needed to determine whether there is significant linkage disequilibrium between chromosome sequences and alleles at the hydrocarbon locus.

(vi) *Polymorphism of the hydrocarbon locus within populations*

In their world-wide survey of hydrocarbon phenotypes, Ferveur *et al.* (1996) reported that the low 7,11-HD phenotype is fixed in sub-Saharan Africa and on Caribbean islands, while the high 7,11-HD phenotype is fixed in North and South America, Europe, Asia, and islands in the South Pacific. (As Ferveur *et al.* could not reliably separate 5,9-HD from 2-MH, their 'low 7,11-HD morph' is equivalent to our 'high 5,9-HD morph'.) Because of the dominance of the hydrocarbon profile conferred by the high 5,9-HD allele, however, the allele producing the high 7,11-HD morph may nevertheless still occur in low frequencies in Africa and the Caribbean, but might not have been detected by Ferveur *et al.* (1996) since it would be masked in heterozygotes. We surveyed a number of Caribbean and African lines for this polymorphism, sampling flies directly from isofemale lines or crossing flies from high 5,9-HD strains to a high 7,11-HD tester stock (Ives) and surveying a sample of five or more offspring for the recessive high 7,11-HD phenotype. The results are given in the Appendix. Although all samples taken from outside Africa and the Caribbean are fixed for the high 7,11-HD allele, nearly all African populations and several Caribbean populations contain both alleles. Although one cannot rule out the possibility that some of these strains have been contaminated by laboratory stocks containing the high 7,11-HD allele, this seems improbable because many of these wild lines were collected within the last 7 years. It is more likely that many populations in Africa and the Caribbean are polymorphic at the hydrocarbon locus.

(vii) *Effect of hydrocarbon differences on sexual behaviour*

To determine whether there was any assortative mating between the Ives strain (high 7,11-HD) and the Tai 2 strain (high 5,9-HD), we watched male/female pairs of these flies in all possible combinations. This thus yielded four pairs of comparisons, each involving seven measured parameters of courtship and copulation (Table 8, top section). These data show neither positive nor negative assortative mating between these strains. Of 24 comparisons between pairs of courtship parameters, only two are significantly different, and only one of these (the lower number of copulation attempts per minute shown by Tai 2 males towards Ives females than towards Tai 2 females) remains significant under Rice's (1989) suggested Bonferroni correction of probability levels. Nor is there any consistent direction in differences between courtship parameters that would suggest that the strains differ in courtship vigour or female receptivity. These results are somewhat in contrast to those of Ferveur *et al.* (1996) who found – using three strains fixed for each of the two hydrocarbon morphs – that high 7,11-HD females mated more rapidly with all males than did high 5,9-HD females. However, their observations of courtship lasted only 10 min,

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Table 8. *Mating parameters of individual pairs of flies from the pure Ives and Tai 2 strains, and from the* '*ruboff*' *experiment*

		N	Courtship latency	*N*	Courtship duration	*N*	Copulation attempts	*N*	Copulation latency	*N*	Courtship intensity	*N*	Copulation attempts/minute
Pure species experiment													
Female	*Male*												
Ives	Ives	20	1·46 (0·73)	20	5·18 (0·98)	20	5·60 (2·07)	18	7·72 (1·05)	20	0·71 (0·04)	20	1·12 (0·20)
Tai 2	Ives	20	1·78 (0·17)	20	5·26 (0·98)	20	5·30 (1·48)	15	6·78 (0·62)	20	0·62 (0·04)	20	1·18 (0·20)
Ives	Tai 2	20	1·34 (0·17)	20	5·72 (1·28)	20	1·95 (0·71)	17	7·05 (1·28)	20	0·73 (0·04)	20	0·27 (0·08)**
Tai 2	Tai 2	20	1·98 (0·27)	19	2·89 (0·55)	19	3·36 (0·79)	19	6·86 (0·88)	19	0·64 (0·04)	19	1·32 (0·24)
Ives	Ives	20	2·10 (0·19)*	20	3·77 (0·99)	20	6·65 (2·90)	18	5·99 (0·91)	20	0·75 (0·05)	20	1·35 (0·31)
Ives	Tai 2	20	1·69 (0·40)	20	5·61 (1·05)	20	2·75 (0·75)	18	7·80 (1·02)	20	0·74 (0·03)	20	0·45 (0·10)
Tai 2	Ives	20	2·10 (0·20)*	20	3·85 (0·82)	20	5·60 (0·17)	18	7·27 (0·14)	20	0·61 (0·05)	20	1·40 (0·28)
Tai 2	Tai 2	20	1·61 (0·32)	20	4·68 (1·12)	20	4·45 (1·28)	18	7·55 (1·33)	20	0·70 (0·04)	20	1·40 (0·38)
Ruboff experiment													
Target female	*Donor female*												
Ives male													
Ives	Ives	42	1·71 (0·16)	42	2·88 (0·58)	42	4·76 (1·37)	40	5·01 (0·50)	42	0·74 (0·04)	42	1·28 (0·24)
Ives	Tai 2	42	2·02 (0·25)	42	4·03 (0·70)	42	6·76 (1·29)	37	5·72 (0·55)	42	0·81 (0·04)	42	1·18 (0·15)
Tai 2 male													
Tai 2	Tai 2	42	1·39 (0·16)	42	2·76 (0·54)	42	5·69 (1·31)*	41	4·69 (0·51)	42	0·76 (0·03)	42	1·81 (0·21)*
Tai 2	Ives	42	1·73 (0·28)	42	2·64 (0·59)	42	3·19 (0·64)	39	4·29 (0·53)	42	0·79 (0·31)	42	1·27 (0·20)

In the pure-strain analysis, 20 observations were made for each pair. In the 'ruboff experiment', mating parameters of Ives or Tai 2 males were tested against control and experimental females of their own strain. Forty-two observations were made for each male–female pair, and pairs were tested simultaneously. For both experiments, *N* is the number of pairs displaying a particular behaviour, values given are means among observations, and standard errors are in parentheses. All times are given in minutes. Each of the seven courtship parameters was compared between each tested pair using either chi-squared (number of copulations) or Mann–Whitney *U*-tests with two-tailed probability values.
* $P < 0{\cdot}05$; ** $P < 0{\cdot}001$.

Table 9. *Hydrocarbons of pure strains used in ruboff experiment and of* '*target*' *flies crowded with* '*donor*' *flies for 4 days* (*see text for details*)

Strain		*N*	7,11-HD (ng)	CII (ng)	CII/7,11-HD
Pure species					
Tai 2	—	20	59·3 (5·9)	400·0 (26·8)	7·384 (0·585)
Ives	—	20	652·7 (31·0)	168·7 (12·2)	0·260 (0·016)
Target	Donor				
Tai 2	Tai 2	13	46·5 (12·2)	344·1 (19·3)	9·884 (0·861)
Tai 2	Ives	13	197·7 (18·4)	218·1 (21·2)	1·569 (0·569)
Ives	Ives	13	499·2 (27·5)	102·1 (6·1)	0·205 (0·008)
Ives	Tai 2	13	290·6 (24·1)	207·5 (9·3)	0·743 (0·034)

Values given are the mean of each group of sample size *N*; standard errors are given in parentheses.

while ours lasted 20 min. This discrepancy in methodology, however, cannot account for the disparity of results, as re-analysis of our data using only the first 10 min of observation (data not shown) also revealed no difference between the tested pairs in either number of copulations or time until copulation.

Although the observations of pure strains give no evidence for disassortative mating or sexual isolation, we cannot therefore assume that the difference in hydrocarbon profile plays no role in mate discrimination. These two strains may differ genetically in many ways that might affect courtship, and such differences may mask any effect of the hydrocarbons on sexual behaviour. To isolate the effect of the hydrocarbons themselves, we performed 'ruboff' experiments (see Section 2), transferring hydrocarbons among females of the pure strains.

Table 9 shows the alteration of hydrocarbon profile of females of the two pure strains and of females subjected to crowding experiments. Crowding of individuals with other members of their own strain had very little effect on the quantity or ratios of their

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hydrocarbons, but target females crowded with donor females of a different hydrocarbon morph experienced considerable change in the amounts and ratios of hydrocarbons. Crowding of adults was, as observed in our previous studies (Coyne *et al*., 1994; Coyne & Charlesworth, 1997), unable to effect a complete interconversion of hydrocarbon morphs, but in these previous studies was able to induce substantial changes in the mating behaviour of males exposed to the altered females.

Two sets of courtship experiments were performed using hydrocarbon-altered females and controls: the first involved Ives males exposed to Ives females crowded with either Ives females or with Tai 2 females; the second experiment involved Tai 2 males exposed to Tai 2 females crowded with either Tai 2 females or Ives females.

The results of these experiments (summarized in the bottom section of Table 8) show that altering the hydrocarbon profile of either Ives or Tai 2 females has little effect on their courtship or copulation by males of either strain. Only two marginally significant differences are seen among the 12 pairwise comparisons, and neither of these remains significant under the sequential Bonferroni correction. Compared with the profound effect of hydrocarbon transfer on sexual isolation *between* species (Coyne *et al*., 1994; Coyne & Charlesworth, 1997), the results are not impressive. In these tests, at least, we have found no evidence that the two hydrocarbon morphs have any effect on mating behaviour.

4. Discussion

The gene or genes responsible for the female hydrocarbon polymorphism in *Drosophila melanogaster* reside on the right arm of the third chromosome between the loci *curled* and *karmoisin*, at approximately 51·5 cM on the genetic map and 87C–D on the cytological map. It is surely not a coincidence that 87C is precisely the location of the *desat* locus, a fatty acid desaturase gene studied by Wicker-Thomas *et al*. (1997). Using primers from a Δ9 desaturase gene cloned in yeast and rat, these workers found only one cDNA but two genomic fragments in the Canton-S strain of *D. melanogaster*. Both genomic fragments were localized to chromosome region 87C and have the same open reading frame, but one was without introns and thus is likely to be a pseudogene. We are further studying genomic sequences to determine whether the *desat* locus has one or several active genes.

Because of our mapping results, it is extremely likely that the hydrocarbon polymorphism is determined by at least one of the two sequences studied by Wicker-Thomas *et al*. (1997). Although it is possible that this polymorphism is due to alleles of two or more closely linked genes, in the absence of contrary information we assume that the polymorphism is based on two alleles at a single *desat* locus. It is not yet clear how these alleles produce different relative amounts of 7,11-HD and 5,9-HD. Little is known about the pathways leading to the synthesis of cuticular hydrocarbons in *Drosophila*, although Jallon (1984) proposed a scheme whereby these long-chain compounds are synthesized by the addition of successive two-carbon units to long-chain fatty acids, followed by two successive desaturation steps (at the 16- and 18-carbon stage) and then a final female-specific decarboxylation. Wicker-Thomas *et al*. (1997) theorize that the *desat* locus produces an enzyme catalysing the first of two desaturation steps, and that the high 5,9-HD allele produces an enzyme preferentially desaturating myristate (14 carbons) rather than palmitate (16 carbons), producing a double bond in the 5 rather than the 7 position. If a second enzyme then produces a double bond four carbons away from the first, followed by normal elongation and decarboxylation, then a change in the *desat* locus could cause the observed change in the heptacosadienes.

Males of *D. melanogaster*, as well as both males and females in most other species in its subgroup, possess high levels of the 23-carbon compound 7-tricosene (7-T) and very low amounts of 7,11-heptacosadiene (Jallon & David, 1987). Wicker-Thomas *et al*. (1997) note that *desat* is expressed in male *D. melanogaster* as well as in males and females of the sibling species *D. simulans*, both producing 7-T as their major cuticular hydrocarbon. Jallon (1984) posits that this compound is produced by another pathway that branches from the 7,11-HD synthetic pathway after the first desaturation. One might then predict that males of the African and Caribbean strains might have high levels of 5-tricosene instead of 7-tricosene but this is not true. These males generally have high levels of 7-pentacosene and low levels of 7-tricosene; however, strains having high 5,9-HD females do not always have high 7-pentacosene males (Jallon, unpublished data). The *desat* gene may act differently in males and females (possibly regulated differentially by sex-specific factors), or the synthetic pathway may differ from that suggested by Jallon (1984).

If the hydrocarbon polymorphism indeed resides at the *desat* locus, it will be of great interest to determine the sequence of the two alleles. This could answer a number of questions. Do the allelic differences reside at a coding position or in a control region? The sequence of the *desat* open reading frame is now known for the Tai strain of *D. melanogaster* (Wicker-Thomas, unpublished results). The Tai and Canton S sequences of *D. melanogaster* show some differences, and sequence analysis of other hydrocarbon alleles, *in vitro* assays, and transformation of lines will be needed to determine whether the allelic difference

affecting the hydrocarbon produce resides at a coding position or in a control region. Which is the ancestral allele: the high 7,11-HD allele or the high 5,9-HD allele? Because the latter predominates in Africa, where *D. melanogaster* is thought to have originated (David & Capy, 1988), Ferveur *et al.* (1996) suggested that the low 7,11-HD allele (our 'high 5,9-HD' allele) is ancestral. Sequencing these alleles may provide an answer, and also allow us to estimate how long ago these alleles diverged. Sequencing may in addition help resolve the general phylogeny of female hydrocarbons in this group, which is puzzling. For example, sexually dimorphic hydrocarbons (high 7,11-HD females, high 7-T in males) occur in both *D. melanogaster* and *D. sechellia*, and we do not know whether this represents an independent origin or a synapomorphy (see Coyne & Charlesworth, 1997). Is the polymorphism a balanced one? Most African and Caribbean populations appear to contain both alleles, with the high 7,11-HD allele present in low frequencies. Sequence analysis, especially around the site causing the phenotypic difference, may show genetic patterns implicating a balanced polymorphism (e.g. Kreitman & Hudson, 1991). Finally, is the hydrocarbon polymorphism in the Caribbean due to the same alleles as those present in Africa? We have shown that the differences map to the same genetic position in each locality, but this does not mean that the alleles are identical at the nucleotide site causing the hydrocarbon difference. Ferveur *et al.* (1996) speculated that the high frequency of the low 7,11-HD allele in the Caribbean reflects a historical association between commensal flies carried with the slave trade from Africa; and other workers (e.g. David & Capy, 1988) have also posited such migration. Such a hypothesis, of course, cannot explain the fixation for high 7,11-HD alleles in North and South America (Ferveur *et al.*, 1996), which would then require assuming either separate introductions of 7,11-HD alleles into North and South America or selection against high 5,9-HD alleles on these continents. In theory, the 'slave trade' hypothesis could be settled by sequencing alleles from these different areas.

Although sequence analysis may indicate whether the polymorphism might be a balanced one, the selective significance of this polymorphism – if any – can be understood only through studying the effects of the different hydrocarbons on the fitness of flies. Our analysis involving transfer of hydrocarbons gives little evidence that the polymorphism affects mating behaviour. As noted above, our results are in contrast to those of Ferveur *et al.* (1996), who found that high 7,11-HD females mated more rapidly with all males than did high 5,9-HD females. We have no evidence for this from our study of either the pure strains or those with altered hydrocarbons, but the discrepancy may have resulted from other genetic differences between Ferveur *et al.*'s African versus non-African strains that did not involve cuticular hydrocarbons. Moreover, our results contrast somewhat with Antony *et al.*'s observation (1985) that increasing amounts of 7,11-HD placed on dummy females induced increasing amounts of courtship wing vibration by males, as well as Ferveur & Sureau's (1996) similar observation derived from flies genetically manipulated with the *transformer* locus. We found no difference in courtship duration or intensity between either the pure strains whose females had very different hydrocarbon profiles or between the control and chemically altered 'ruboff' females, whose hydrocarbon profiles differed in the same direction but to a lesser degree than those of the pure strains.

7,11-HD is known to inhibit male courtship when transferred to females of other species that normally lack this compound (Coyne *et al.*, 1994; Coyne & Oyama, 1995; Coyne & Charlesworth, 1997). Jallon (1984) pointed out that, as an intraspecific 'aphrodisiac', the presence of 7,11-HD is a necessary but not sufficient condition for the expression of male courtship behaviour, and other signals (both chemical and non-chemical) are required for the male to perform his full courtship repertoire. It should also be noted that our hydrocarbon-transfer experiments involved just two strains, and also did not effect a complete transformation of the female's hydrocarbon profile. It is possible that more radical alterations may have produced changes in male courtship. However, the increase in the amount of 7,11-HD experienced by Tai females crowded with Ives females (an increase from about 50 ng per female in control flies to about 200 ng per female in experimental flies) represents more than enough of the compound to significantly increase the amount of male wing vibration according to the studies of Antony *et al.* (1985) and Ferveur & Sureau (1996). In the study of Antony *et al.* (1985) on dummy flies, does of 7,11-HD of less than 50 ng per fly yielded no wing-vibration response in males, while doses larger than this gave increasing male responses up to the maximum response seen with 200 ng per female. In the study by Ferveur & Sureau (1996) on live flies, females carrying 50 ng of 7,11-HD produced roughly half the wing-vibration response of females carrying 200–300 ng of the compound.

At present, then, we do not know the significance of the hydrocarbon polymorphism in *D. melanogaster*. In some species, including *D. psudoobscura* (Howard & Blomquist, 1982; Toolson, 1988), long-chain cuticular hydrocarbons serve to reduce desiccation as well as to attract males. It is possible that high levels of 5,9-HD are better adapted for reducing desiccation in areas where the relevant allele reaches high frequencies, but it is hard to believe that the environments of both Africa and the Caribbean are similar in this way and differ from all other world-

wide locations, in which flies are fixed for the high 7,11-HD allele. Moreover, it is difficult to believe that two hydrocarbons differing only slightly in the positions of the double bonds could cause a significant difference in organismal desiccation.

Finally, it is no surprise that a phenotypic polymorphism without intermediate forms maps to a single genetic region. This was also the case in the polymorphism involving the relative quantities of 7-tricosene and 7-pentacosene (7-P) in both male and female *Drosophila simulans* (Ferveur, 1991). Most worldwide populations of *D. simulans* show high levels of 7-T and low levels of 7-P in both sexes, while African populations around the Gulf of Benin contain a morph producing low levels of 7-T and high levels of 7-P. This difference maps largely to a locus called *Ngbo*, near genetic position 65 cM on the second chromosome (Ferveur, 1991). The geographic variation among male *D. melanogaster* in the ratios of 7-T to 7-P, however, is due to differences in at least four genes (Ferveur & Jallon, 1996).

Interspecific differences in *Drosophila* female hydrocarbons, on the other hand, seem to be more polygenic. Three of these have been studied in detail. The difference in relative amounts of 7-T and 7,11-HD between females of *D. melanogaster* and *D. simulans* are due to at least five genes on the third chromosome, while the other three chromosomes have no effect (Coyne, 1996*b*). Similarly, the difference between the same two hydrocarbons in females of *D. sechellia* (high 7,11-HD) and *D. mauritiana* (high 7-T) is due to at least six loci on the third chromosome, with the other three chromosomes again having no effect. Finally, the difference in *male* cuticular hydrocarbons between *D. simulans* (with high levels of 7-T) and *D. sechellia* (with low levels of 7-T but high levels of its position isomer 6-tricosene) is due to at least five genes spread among all major chromosome arms (Coyne 1996a). It is worth noting that every gene affecting intraspecific polymorphisms or interspecific differences in hydrocarbons of females in the *D. melanogaster* group is situated on chromosome 3. Localizing and sequencing these genes will ultimately reveal whether the same loci are involved in both intraspecific polymorphisms and interspecific differences among species in female cuticular hydrocarbons.

Appendix

Hydrocarbon constitution of isofemale lines. For all lines except those indicated with asterisks, crosses were made to a high 7,11-HD line and five offspring were analysed (see Section 3). For lines with asterisks, only one female per line was analysed.

Population	Year collected	Lines analysed	Pure 5,9-HD	Pure 7,11-HD	Segregating
Zimbabwe, Sengwa Wildlife Refuge	1991	6	5	0	1
Ivory Coast, Tai	1980	1	0	0	1
Mali	Not known	3	3	0	0
Kenya	Not known	3	2	0	1
Benin, Cotonou	1985	1	0	0	1
South Africa	Not known	3	2	0	1
Tunisia	1991–2	4	0	3	1
Mauritius	1992	1	1	0	0
Cuba*	1991	4	3	1	0
Dominican Republic, Finca Mora*	1993	2	2	0	0
Dominican Republic, Jaragua*	1993	1	1	0	0
Granada, Grand Etang*	1995	2	2	0	0
Puerto Rico, Tornado*	1995	2	2	0	0
Puerto Rico, Toro Negro*	1995	2	0	2	0
Puerto Rico, Barranquitas*	1995	2	1	1	0
St Croix, Cane Bay*	1997	2	2	0	0
St Vincent, Vermont*	1996	3	3	0	0
Honduras, Belize	1991–2	2	0	2	0
Colombia	1991–2	3	0	3	0
Israel	1991–2	3	0	3	0
Australia, Melbourne	1992	2	0	2	0
Vietnam, Ho Chi Minh City	1980	3	0	3	0
China, Beijing	Not known	3	0	3	0

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