Genetic Control of Pheromones in *Drosophila simulans*. II. *kété*, a Locus on the *X* Chromosome

Jean-François Ferveur and Jean-Marc Jallon

Laboratoire de Biologie et Génétique Evolutives, CNRS, 91198 Gif-sur-Yvette, France

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ABSTRACT

The production of Drosophila cuticular hydrocarbons, including contact pheromones, is under polygenic control. To investigate *X*-linked loci, EMS mutations were induced in *Drosophila simulans* flies. A mutant strain was discovered which in both sexes show a reduction in the biosynthesis of both 7-tricosene (7-T) the species contact pheromone and all other linear hydrocarbons. The locus controlling this effect, *kété*, is recessive and was localized to I, 18.5. Unlike a previously identified gene on the second chromosome of this species, *Ngbo*, *kété* does not affect the ratio of 7-T:7-pentacosene (7-P). Other reproductive characteristics are also affected, including egg-hatching. However, courtship behaviors in both sexes appear normal.

7-TRICOSENE is the main hydrocarbon on the cuticle of male and female flies in most strains of *Drosophila simulans* and of *Drosophila melanogaster* males of many strains (LUYTEN 1982; PECHINE *et al.* 1985). This substance has been shown to act as a pheromone, able to induce wing display in *D. simulans* males (JALLON 1984). The cuticle of *D. simulans* flies contains at least 13 hydrocarbons which vary quantitatively according to sex and strain. These molecules have 23, 25, 27 or 29 carbons and are linear hydrocarbons or 2-methyl-branched alkanes. The predominant linear hydrocarbons are monoenes with a single double bond in position 7: 7-tricosene (7-T, 23 carbons) and 7-pentacosene (7-P, 25 carbons) (JALLON 1984; PECHINE *et al.* 1985). Apart from 7-T, only one other *D. simulans* hydrocarbon, 7-P, is currently suspected of playing a role in mate recognition or stimulation in this species, perhaps via a synergetic interaction (COBB and JALLON 1990).

In a previous article we reported the existence of *Ngbo*, a major second chromosome locus in *D. simulans* which controls the ratio of 7-T:7-P by reciprocally changing the levels of both hydrocarbons (FERVEUR 1991). We also showed that females of most *D. simulans* strains have higher levels of 7-T than their homotypic males. This and other data suggest that genes on the *X* chromosome may be involved (BENAMAR and JALLON 1983; FERVEUR, COBB and JALLON 1989). SCOTT and RICHMOND (1988) argued that the *X* chromosome could modify levels of 7-T and 7-P in *D. melanogaster* males, although it is possible that genetic control of the 7-T:7-P ratio is different in the two species (FERVEUR 1991). To further investigate directly the role of the *X* chromosome on pheromone production we have used EMS mutagenesis to induce *X*-linked mutations in *D. simulans*.

MATERIALS AND METHODS

Drosophila stocks and crosses: All strains of *D. simulans* were kept on standard cornmeal and yeast medium under a 12:12-hr light:dark cycle at 25°. Three strains were used: (1) Seychelles, based on several inseminated females collected in the Seychelles archipelago in 1981; (2) *ywmf*, a strain carrying four recessive markers: *yellow* (I, 0.0), *white* (I, 1.5), *miniature* (I, 36.1) and *forked* (I, 56.7) (a gift of J. COYNE); and (3) *C(I)RM*, a strain with attached *X* chromosomes carrying *yellow* and *white* (a gift of T. WATANABE).

For regular crosses, five pairs of virgin 4-day-old flies were placed in vials with food. Progeny from these crosses were analyzed continuously throughout the course of the experiments reported here. For backcross experiments, isofemale lines were set up from females randomly collected over 14 generations and individually mated when 4–14 days old with 4-day-old males. In general, crosses set up for studying the inheritance of hydrocarbon phenotype were studied simultaneously.

Mutagenesis: Forty Seychelles males, 12–24 hr old, were fed overnight on saccharose supplemented with 0.1% EMS (LEWIS and BACHER 1968). These males were then mass-mated with 50 virgin *C(I)RM* females. This procedure was replicated 10 times. Six hundred and fifty F_1 males were produced and were individually backcrossed to four virgin *C(I)RM* females, producing F_2 males sharing the same *X* chromosome. Each line was kept inbred, except in the case of low fertility, where males were backcrossed to virgin *C(I)RM* females. Cuticular hydrocarbon profiles were compared for males from 295 lines.

Extraction and analysis of cuticular hydrocarbons: Cuticular hydrocarbons were extracted from individual flies with the method described in FERVEUR (1991), except for mutagenized lines, for which four males from each line were pooled at generations F_2 and F_3. This "pooling" procedure enabled us to exclude intraline hydrocarbon variability which was not controlled by the *X* chromosome. Individuals from lines showing *X*-linked hydrocarbon variation were subsequently tested separately.

Hydrocarbon parameters: Thirteen hydrocarbons were detected in gas chromatography (GC) profiles and their quantities were measured. For the 295 mutagenized lines

the percentage of each hydrocarbon was measured relative to the sum of all hydrocarbons (∑Hc) for that line and compared to the percentages obtained from all lines pooled. The analysis of putative mutant lines was carried out using percentages and absolute amounts (Q) of hydrocarbons, in particular of the two main alkenes (7-T and 7-P) and two methyl branched hydrocarbons with 27 and 29 carbons (27 Br and 29 Br). Levels of other linear and branched hydrocarbons which showed lower absolute levels were separately pooled (∑lin and ∑Br). The ratios of 7-T:7-P and 7-T:27 Br were also measured.

Statistical tests: For mutagenesis screen each hydrocarbon parameter was compared to the mean ± 2 SD calculated from all the 295 mutagenized strains ($P < 0.05$). When one hydrocarbon parameter was compared between two independent samples, we used Student's *t* test. For comparisons between two or more samples differing for two conditions (genotype and/or age and/or mating status), we used a two factors ANOVA.

Behavioral and fertility tests: Virgin pairs of flies were observed under a watch glass for 2 hr. Courtship and copulation latencies and durations were noted for each pair. Mated females were individually transferred to a vial containing food colored with neutral red (0.05%; to enhance background contrast) and were allowed to oviposit for 24 hr before being discarded. At least 24 hr later the number of unhatched eggs was noted. The sex ratio of the resulting imagos was also recorded. The hydrocarbon profile of all flies was subsequently analyzed.

RESULTS

Genetic control of hydrocarbon variation: Males from 295 EMS-mutagenized lines were screened for their cuticular hydrocarbon profile. One strain (430), showed a significant quantitative variation: these flies showed the same number of GC peaks as wild-type individuals, with similar retention times, but the proportions of 7-T and of 7-P (41.6% and 2.5%, respectively) in males were lower than for all 295 mutagenized lines pooled (59.8 ± 6.5% and 5.4 ± 2.8%, respectively). Conversely, strain 430 males showed much higher proportions of both methyl branched hydrocarbons 27 Br and 29 Br (25.8% and 9%, respectively) as compared to males from all mutagenized strains pooled (9.0 ± 3.4% and 3.8 ± 2.1%). Thus strain 430 males have a much lower ratio of 7-T:27 Br than the other mutagenized strains (1.61 and 6.65 ± 0.20, respectively).

To assess whether the main hydrocarbon variation was linked to the *X* chromosome or not, males from the strain 430 were crossed with females from the strain *C(I)RM*. Absolute amounts of hydrocarbons were compared in Seychelles, strain 430 and *C(I)RM* flies (Table 1). Strain 430 males produced approximately 30% of the total amount of hydrocarbons (∑Hc) shown by Seychelles males. There was a similar decrease in the amount of 7-T and 7-P (Q7-T and Q7-P) and of all other linear compounds (∑lin). Strain 430 females carrying *C(I)RM* attached *X* chromosomes showed no such effect, suggesting that an *X*-linked factor is implicated in this difference. The 7-T:7-P ratio and the overall production of branched compounds (∑Br) were not affected in strain 430 males.

To localize the character(s) involved, cuticular hydrocarbons were analyzed from crosses between strain 430 and *ywmf* flies, which carry a multimarked *X* chromosome (Table 2). *ywmf* males and reciprocal F_1 males sharing the *ywmf X* chromosome all showed much higher levels of 7-monoenes than strain 430 males. The Q7-T value was approximately six times higher in males with a *ywmf X* chromosome than in strain 430 males.

However, *ywmf* males produced about 30% more 7-T than Seychelles flies while their 7-T:27 Br ratio was very close (6.04 and 6.6, respectively). For this reason, and because males with a *ywmf X* chromosome show a 7-T:27 Br ratio four times higher than strain 430 males (Table 2), we mapped the locus causing the hydrocarbon defect with a 7-T:27 Br ratio.

Recombinant males from backcrosses and F_2 strains were grouped according to their morphological phenotypes and were characterized by the two predominant hydrocarbons Q7-T and Q27 Br and by their ratio (7-T:27 Br) (Table 3).The 7-T:27 Br parameter gave the most consistent result. The factor controlling this parameter appears to segregate with *white* and *miniature* (6.42 ± 0.14 for w^-m^- as against 2.57 ± 0.11 for w^+m^+). On the basis of these distributions, an empirical cutoff point was established at 4.2, separating the two "7-T:27 Br" phenotypes with a low misclassification probability ($P = 0.056$) (Figure 1). With this classification criterion, the hydrocarbon profile of males showing only one of the two markers was studied. Overall, males expressing either w^- or m^- showed intermediate values; recombination between the two markers and the two 7-T:27 Br values (< 4.2 <) enabled us to map a single locus or a tightly linked group of loci on the *X* chromosome at 18.5 ± 1.8, which controls the ratio of 7-T:27 Br. We have called this locus *kété*, a word meaning "small quantity" in a West African dialect, following the mutant phenotype which shows low 7-T:27 Br value.

kété is the main factor involved in the control of 7-T level. F_2 recombinant $kété^+$ males showed significantly much larger amounts of 7-T than F_2 recombinant $kété^1$ males (1478 ± 37 ng and 606 ± 21 ng, respectively; $F = 428.4$; d.f. = 1, 458; $P < 0.001$). Q27 Br does not seem to be affected by *kété* in F_2 males (239 ± 6 ng and 226 ± 5 ng, respectively, for $kété^1$ and $kété^+$; $F = 1.82$; d.f. = 1, 458; $P > 0.05$).

A similar effect of *kété* on the linear hydrocarbon production was also shown in females (see next section). Reciprocal female hybrids between strain 430 and *ywmf* strain showed no significant difference in Q7-T, nor were they significantly different from *ywmf* females, showing that the character is recessive (Table 4a).

TABLE 1

Ratio and absolute amounts of certain cuticular hydrocarbon in various strains of *D. simulans*

D. simulans strain	Sex	*N*	Q7-T (ng)	Q7-P (ng)	ΣLin (ng)	ΣBr (ng)	ΣHc (ng)	7-T:7-P (log *e*)
Seychelles	Male	18	970 ± 65	125 ± 12	265 ± 18	285 ± 15	1780 ± 120	2.05
	Female	15	1300 ± 110	130 ± 18	325 ± 24	290 ± 20	2300 ± 148	2.30
Strain 430	Male	17	300 ± 36	35 ± 4	85 ± 7	265 ± 18	670 ± 28	2.15
	Female	24	2250 ± 102	120 ± 8	455 ± 28	350 ± 16	3450 ± 172	2.93
C(I)RM	Female	39	1789 ± 113	108 ± 7	389 ± 24	342 ± 15	2811 ± 158	2.81

Unless otherwise specified, hydrocarbons were extracted from individual 4–5-day-old flies. Data shown are mean (±SE) of absolute amounts of 7-tricosene (Q7-T), 7-pentacosene (Q7-P), the sum of linear alkanes with 23 C, 25 C, and 27 C (ΣLin), the sum of branched (-C2-) compounds with 27 C and 29 C (ΣBr) and the sum of all hydrocarbons (ΣHc); 7-T:7-P ratio was logarithmically transformed. EMS-treated Seychelles males mass-crossed with *C(I)RM* females produced 650 F_1 males which were individually crossed with *C(I)RM* females. Out of 295 lines inbred from the F_2 generation, the strain 430 revealed significant hydrocarbon variations.

TABLE 2

Hydrocarbon production in male flies resulting from crosses between the strain 430 and the *ywmf* multimarked strain

Cross (female × male)	*N*	Q7-T (ng)	Q7-P (ng)	Q27 Br (ng)	Q29 Br (ng)	ΣHc (ng)	7-T:7-P (log *e*)	7-T:27 Br
(F_0) strain 430 × strain 430	73	283 ± 15 (*42.2*)	18 ± 2 (*2.9*)	159 ± 6 (*25.4*)	85 ± 12 (*12.9*)	650 ± 26	2.85 ± 0.08	1.74 ± 0.06
(F_0) *ywmf* × *ywmf*	33	1389 ± 95 (*55.8*)	128 ± 9 (*5.1*)	233 ± 15 (*9.6*)	169 ± 11 (*7.0*)	2485 ± 165	2.40 ± 0.02	6.04 ± 0.22
(F_1) *ywmf* × strain 430	20	1823 ± 89 (*59.2*)	185 ± 10 (*6.1*)	271 ± 11 (*9.0*)	168 ± 11 (*5.5*)	3067 ± 125	2.30 ± 0.05	6.77 ± 0.28
(F_1) strain 430 × *ywmf*	22	2143 ± 73 (*59.5*)	187 ± 6 (*5.2*)	278 ± 12 (*7.8*)	193 ± 9 (*5.4*)	3600 ± 120	2.44 ± 0.03	7.88 ± 0.32

Data shown are mean of absolute amounts (ng) ± SE of 7-tricosene (7-T), 7-pentacosene (7-P), 2-methyl-hexacosane (27 Br) and 2-methyloctacosane (29 Br). The percentage of each variable relatively to the sum of all hydrocarbons (ΣHc) is shown in parentheses. The 7-T:7-P ratio, logarithmically transformed, and the 7-T:27 Br ratio are also shown.

TABLE 3

Production of major hydrocarbons in recombinant males with different morphological phenotypes

Morphological phenotype	*N*	Q7-T (ng)	Q27 Br (ng)	7-T:27 Br
[ywmf]	52	1851 ± 78	298 ± 10	6.26 ± 0.20
[-wmf]	8	2005 ± 156	316 ± 15	6.37 ± 0.42
[ywm-]	33	1288 ± 61	198 ± 11	6.69 ± 0.23
[-wm-]	5	1085 ± 156	168 ± 23	6.43 ± 0.31
[yw-f]	23	1417 ± 111	259 ± 19	5.95 ± 0.58
[-w--]	6	998 ± 82	209 ± 23	5.17 ± 0.84
[yw--]	100	794 ± 35	180 ± 5	4.67 ± 0.22
[--mf]	72	1339 ± 77	301 ± 12	4.68 ± 0.28
[--m-]	30	928 ± 76	202 ± 11	4.69 ± 0.36
[y-mf]	4	1056 ± 254	239 ± 44	4.65 ± 1.20
[y---]	19	512 ± 54	179 ± 11	2.82 ± 0.21
[---f]	35	809 ± 58	303 ± 17	2.71 ± 0.14
[y--f]	3	835 ± 239	296 ± 30	2.77 ± 0.58
[+]	72	442 ± 34	186 ± 6	2.43 ± 0.18

For parameters, refer to Table 2. *ywmf* females were crossed with strain 430 males; resulting F_1 females were either crossed with F_1 males or backcrossed to strain 430 males producing two progenies (F_2 and backcross). Males from these crosses showed no significant difference in hydrocarbon production, were further pooled. Males were grouped according to their morphological phenotype: yellow (y), white (w), miniature (m) and forked (f).

Reproductive and behavioral effects of *kété*: In order to obtain homozygous *kété*^{1} females backcrosses were carried out. Hybrid females produced by crossing strain 430 (*kété*^{1}) with *ywmf* (*kété*$^+$) flies were individually backcrossed to strain 430 (*kété*^{1}) males over 14 subsequent generations. After 14 backcross generations the probability of a single female still containing the *kété*$^+$ allele should have been smaller than $(1/2)^{14}$. However, we found that among our samples both the *kété*$^+$ and the *kété*^{1} alleles were still present: Q7-T and 7-T:27 Br values were measured in virgin and in mated females (Table 4b) and could be related to the production of viable offspring (Figure 2). There were notable differences between females producing viable offspring (fecund females) and sterile females (Table 4c). Fecund females could be clearly distinguished by their 7-T:27 Br values which were always greater than the previously established empirical cutoff point of 4.2 for recombinant males (see Figure 1). The bimodal distributions of 7-T:27 Br suggest that female flies with 7-T:27 Br values <4.2 may be homozygous for *kété*^{1} while other individuals are heterozygous with a kété$^+$ phenotype. We still had a 1:1 ratio (estimated with a χ^2 goodness of fit test) of the two alleles in the samples of five different generations, therefore there

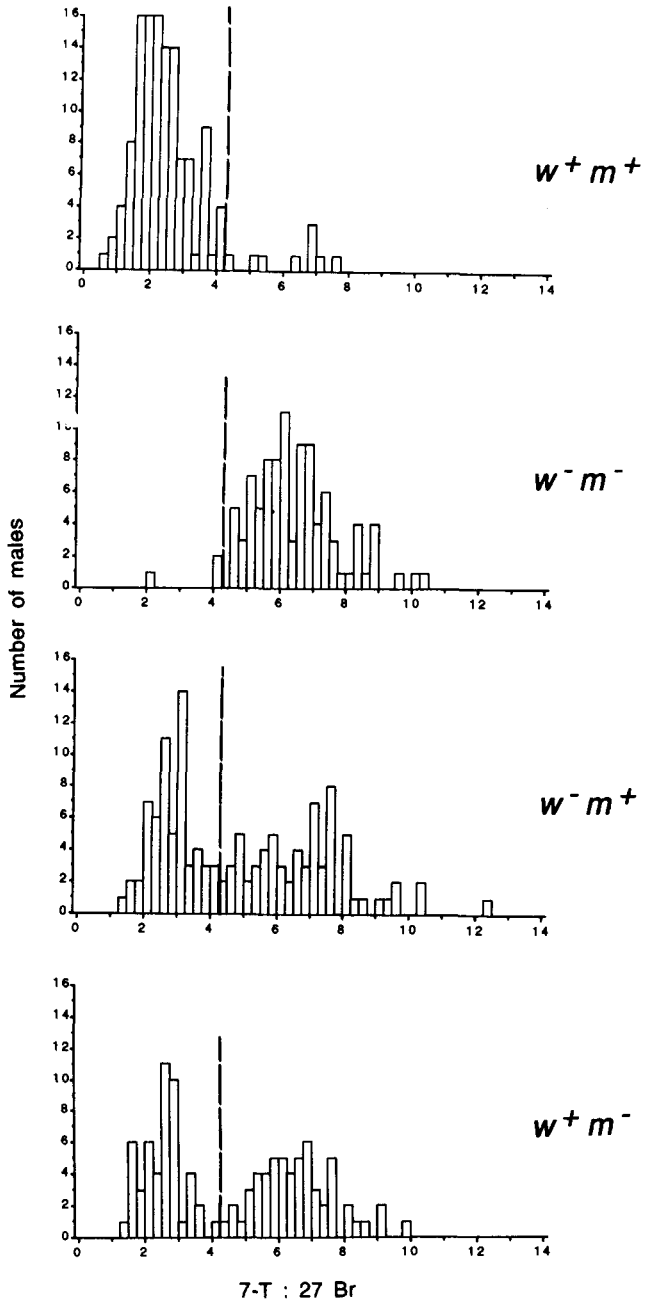

FIGURE 1.—Frequency distribution of 7-T:27 Br values for recombinant male flies showing combinations of the *white* (*w*) and *miniature* (*m*) markers of their *X* chromosome. *ywmf* (*X* chromosome multimarker) females were crossed with strain 430 males showing a mutant hydrocarbon phenotype. F_1 females were crossed with F_1 males (F_2 progeny) and with strain 430 males (backcross progeny). Both male progenies, which did not significantly differ for this hydrocarbon parameter, were subsequently pooled. Dotted lines indicate the empirical cutoff point (4.2) between mutant and wild-type hydrocarbon phenotypes (left and right, respectively). This value produces a misclassification probability of 0.056 (calculated from both groups w^+m^+ and w^-m^-). Out of 129 w^-m^+ flies, 67 were *kété*^{1} ($P = 0.48$). Out of 106 w^+m^- flies, 49 were *kété*^{1} ($P = 0.54$). Based on the position of *w* (1.5) and *m* (36.1), *kété* was mapped at 18.5 ± 1.8 (the standard error is derived from the misclassification probability).

must exist a very strong selection against homozygosity for *kété*^{1}. These data strongly suggest that *kété*^{1} homozygous females are sterile. If the sterility mapped to a different locus on the *X* chromosome, recombination between the "sterile" locus and *kété* should have occurred in the backcrosses. However, in more than 200 female flies tested a sterility phenotype and low 7-T:27 Br ratio were correlated without exception (see below). It therefore appears that *kété*^{1} homozygous females are sterile.

Female flies, subsequently characterized by their hydrocarbon phenotypes as *kété*^{1} homo- or heterozygotes, were individually crossed for six hours with *kété*^{1} males. No significant differences were found for the number of courtships induced, courtship latency or copulation levels in a two hour period. The proportion of females laying eggs and the number of eggs laid per female also showed no significant differences between the two genotypes. Females did, however, differ for the proportion of eggs hatching: 87% of eggs laid by heterozygous females hatched, while no eggs laid by homozygotes were observed to hatch.

Hydrocarbon levels were compared in *kété*^{1}/*kété*^{1} and *kété*$^+$/*kété*^{1} females of different ages and reproductive states (Figure 3). Between 4 and 10 days old, heterozygous females showed a generalized increase in Q7-T (from 1716 ± 78 ng to 2532 ± 86 ng; $t = 8.74$, d.f. = 207, $P < 0.001$) and in Q27 Br (from 187 ± 7 to 299 ± 11 ng; $t = 12.47$, d.f. = 207, $P < 0.001$) with a stabilization for both Q7-T and Q27 Br between 10 and 14 days old. Homozygous females showed a similar increase of Q27 Br but their Q7-T remained always low (729 ± 34 ng and 645 ± 137 ng at 4 and 14 days old, respectively).

Moreover, a significant effect of mating was observed on Q7-T but not on Q27 Br (see Table 4b). The postmating decrease of Q7-T was only observed in heterozygous females of age 4 and 10 days.

DISCUSSION

The cuticle of male and female *D. simulans* flies contains long-chain hydrocarbons (23–29 carbons) branched (mainly with methyl on carbon 2) or linear (saturated or with one double bond mainly in position 7; JALLON 1984; PECHINE *et al.* 1985). There is no qualitative sexual dimorphism but females of most strains show higher levels of 7-tricosene, a species contact pheromone, than males. Our previous studies have suggested that variations in 7-T level in this species are under polygenic *X*-linked and autosomal control (FERVEUR, COBB and JALLON 1989). Using natural variants FERVEUR (1991) showed that an autosomal locus *Ngbo* (II, 65.3) controls the main polymorphic variation between the two major hydrocarbons 7-T and 7-P, in *D. simulans*.

In this study, we report the discovery of an EMS-

TABLE 4

Inheritance and mating effects of *kété* on hydrocarbon productions in 4-day-old female flies

	Strain female × male	Female status	*N*	Q7-T (ng)	Q27 Br (ng)	ΣHc (ng)	7-T:27 Br
a.	F_0						
	ywmf × *ywmf*	Virgin	9	1325 ± 191	159 ± 18	2478 ± 219	8.28 ± 0.93
	F_1						
	ywmf × strain 430	Virgin	18	1374 ± 75	159 ± 5	2117 ± 107	8.68 ± 0.37
	F_1						
	strain 430 × *ywmf*	Virgin	5	1553 ± 151	118 ± 7	2319 ± 172	13.30 ± 1.47
b.	Backcross F_4	Virgin	80	1334 ± 89	221 ± 8	2198 ± 125	6.35 ± 0.41
	Backcross F_4	Mated	105	981 ± 43	231 ± 9	1716 ± 63	4.85 ± 0.29
		t test	d.f. 183	3.86***	0.75 NS	3.70***	3.14**
c.	Backcross F_4	Mated and fecund	42	1329 ± 52	190 ± 8	2179 ± 76	7.29 ± 0.31
	Backcross F_4	Mated and nonfecund	63	760 ± 41	258 ± 13	1408 ± 69	3.22 ± 0.26
		t test	d.f. 103	8.78***	3.93***	7.41***	10.09***

Data given are mean absolute amount (±SE) and ratio of 7-T:27 Br; for symbols, refer to Tables 1 and 2. *ywmf* flies were reciprocally crossed with strain 430 flies (see Table 1). F_1 females resulting from *ywmf* females × strain 430 males cross were individually backcrossed to strain 430 males; resulting females (backcross F_1) and those of subsequent backcross generations were individually backcrossed to strain 430 males. Females analyzed here belong to the fourth backcross generation. Females were mated when 3 days old. Hydrocarbon productions were compared with a Student's *t*-test; Levels of significance: NS = not significant, * $P < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

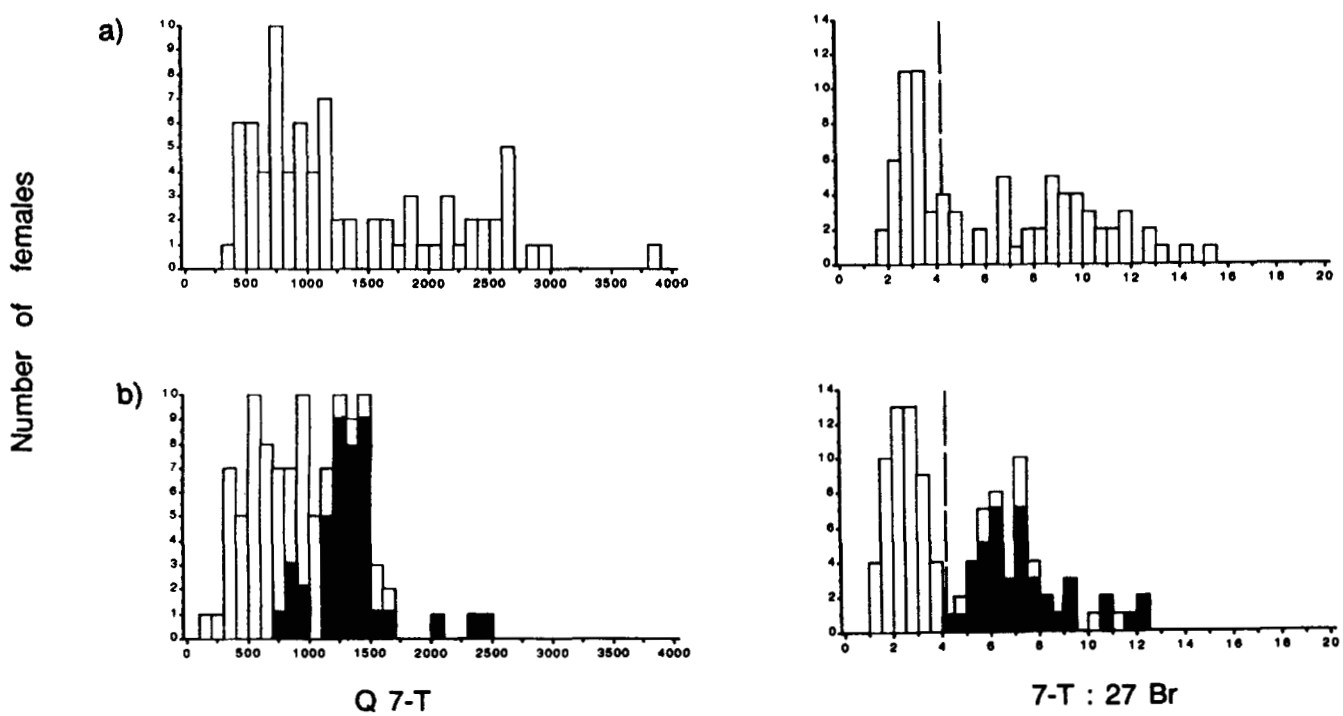

FIGURE 2.—Frequency distribution of Q7-T, 7-T:27 Br for virgin (a) or mated (b) female flies. Fecund flies producing viable progeny are shaded. Females were collected at backcross F_4 generation (see Table 4). *kété* genotypes were defined according to their 7-T:27 Br values (homozygote < 4.2 < heterozygote).

induced mutation on the *X* chromosome which affects the production of linear hydrocarbons. This mutation, *kété*^{1}, mapped at 18.5 ± 1.8, produces a similar decrease of the amount of the pheromone 7-T in flies of both sexes and of different genetic backgrounds: strain 430 males show a 69% reduction as compared to Seychelles males; *kété*^{1} hemizygotes show a 59% reduction in F2 recombinant males as compared to *kété*$^+$ males, and there is a 58% reduction in homozygous *kété*^{1} backcross females as compared to heterozygous females.

kété seems to affect not only the production of Q7-T but also that of Q7-P (−72% from Seychelles to strain 430 males), without significantly affecting their ratio. The production of all other detected linear hydrocarbons is also reduced. Except in 4-day-old females, no significant effect of *kété* was found on the production of branched hydrocarbons (27 Br).

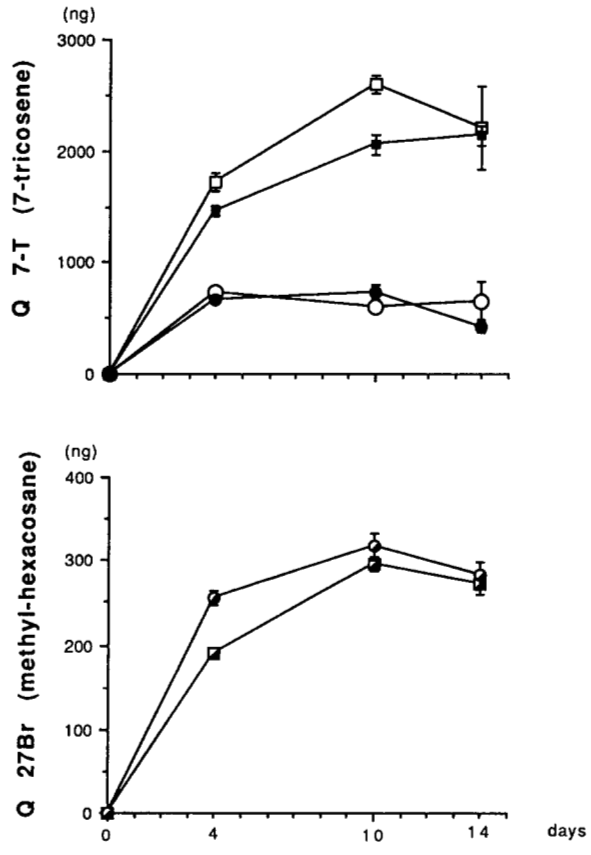

FIGURE 3.—Effect of *kété* on hydrocarbon ontogeny in females. Females of different backcross generations were pooled according to age and mating status. Homozygous (circles) and heterozygous females (squares) were analyzed separately depending on their mating status (virgin = empty; mated = filled) for 7-tricosene production. Virgin and mated females were pooled for the analysis of 2-methylhexacosane.

In order to map *kété* in males, flies carrying this mutation were crossed with flies carrying the *ywmf* marked *X* chromosome. The 7-T:27 Br ratio seems controlled mainly by *kété*. This parameter identified females of different genotypes: there was a strong correlation between the distribution of this hydrocarbon ratio and fecundity. Other minor loci on *X* chromosome affected Q7-T or/and Q27 Br levels. Those loci are probably responsible for differences detected either between Seychelles and *ywmf* strains (see RESULTS) or between strain 430 ($kété^1$) males and F_2 recombinant $kété^1$ males where Q7-T seems different (283 ± 15 ng and 606 ± 21 ng, respectively). The polygenic control of Q7-T on the *X* chromosome could explain the continuous variation observed between different recombinants for this chromosome (Table 3). The higher levels of Q27 Br seemed to segregate with *forked*. No significant correlation was found between Q7-T and Q27 Br variations.

We have previously described a locus in this species, *Ngbo* (II, 65.3), which controls the ratio of Q7-T and Q7-P (FERVEUR 1991). The production of 7-P is directly related to the *Ngbo* genotype, which is additively expressed with two known alleles. The production of 7-tricosene is dependent on *Ngbo* and on other autosomal and *X* chromosome loci (FERVEUR, COBB and JALLON 1989). We have previously suggested that different alleles of *Ngbo* might change the relationship between the speed of either elongation or decarboxylation during hydrocarbon biosynthesis (FERVEUR 1991).

In most insects, including Drosophila, linear and branched hydrocarbons seem to be derived by decarboxylation from long-chain fatty acids resulting from repeated elongations of medium size fatty acids (C12–C18) (BLOMQUIST, DILLWITH and ADAMS 1987; PENNANEC'H *et al.* 1991). The enzymes involved in homologous elongations and decarboxylations might be more or less specific for linear or branched substrates. Moreover, the methyl-branched group seems to be introduced early in chain synthesis. Several fatty acid synthetases (FAS) display various substrates specificities (especially for linear and branched chain initiators) and tissue specificities (BLOMQUIST, DILLWITH and ADAMS 1987; JUAREZ, CHASE and BLOMQUIST 1992). In Drosophila a soluble FAS has been characterized and semipurified. It is also able to produce shorter medium size fatty acids (C12–C14) as well as the longer ones (C16–C18) but cannot use methylmalonyl CoA, the main chain initiator leading to branched fatty acids, alone as a substrate (DE RENOBALES, WOPODIN and BLOMQUIST 1986). We suggest that *kété* might act on the early biosynthetic steps especially the fatty acid synthesis of linear compounds.

De novo hydrocarbon biosynthesis is greatest between 2 and 4 days in *D. melanogaster* flies of both sexes (CHAN-YONG and JALLON 1986). It seems that this active biosynthesis continues over 10 days in *D. simulans* females. Depending on the *kété* genotype, Q7-T seems to reflect changes in reproductive physiology of heterozygous females with increasing age and following mating. Homozygous $kété^1$ females produce low amounts of Q7-T which remain constant throughout the life of the fly, irrespective of mating status. The 7-T production may thus depend on two genetic systems: one including *kété*, which is physiologically regulated, and another one which is independent of this regulation and includes loci like *Ngbo*. Preliminary data show that $kété^1/Y$; $Ngbo^{Cam}/Ngbo^{Cam}$ flies have defects in both viability and fertility. Presumably the double mutant has very little if any 7-T. This reduction of fitness therefore supports the hypothesis that a minimal synthesis of 7-tricosene, or its precursors, is required for survival in *D. simulans*.

Although no major behavioral anomaly was found to be linked to the $kété^1$ hydrocarbon phenotype, a more detailed analysis of male and female courtship behaviors would be necessary in order to definitely exclude any behavioral effect of the mutation. It is

clear that the amount of 7-T synthesized by kété[1] flies is sufficient to elicit a response in sexual partners.

Although homozygous females copulate and lay eggs, the mutation appears to exert a maternal effect which prevents egg hatching. Despite 14 generations of backcrosses in isofemale lines, it was not possible to separate the hydrocarbon phenotype associated with *kété* from the egg lethality. This strongly suggests that the various phenotypes either depend on a single pleiotropic locus or on several very tightly linked loci. *kété* might belong to the B1 class of *X*-linked female sterile mutations as defined in *D. melanogaster* by PERRIMON *et al.* (1986). The egg sterility is due to a maternal defect certainly related to oogenesis while the second phenotype is zygotically expressed by a reduction in Q7-T. Under this assumption, the *kété*[1] mutation might be an amorphic or hypomorphic allele of a nonvital gene.

As yet we have no data about the morphogenetic defects responsible for egg lethality in *kété*[1] mutants. A link between the two mutant phenotypes might be the implication of common biochemical intermediates in both hydrocarbon biosynthesis and the lipid part of the egg yolk and/or chorion lipoproteins.

If *kété* is not itself a sex-determination gene, it might be triggered by sex-determination genes in order to control the structural genes of pheromone biosynthesis enzymes in *D. simulans*. The gene *kété* appears to control at least two different sex-specific characters in *D. simulans*. Although 7-T production shows no qualitative differences between males and females, it clearly acts to induce male wing vibration (JALLON 1984). It is also possible that female-specific behaviors are induced by this substance. In *D. melanogaster* 7-T is a male-specific product; females primarily produce dienes with 27 and 29 carbons. Given the phenotypic homology in the production of 7-monoenes between these two sibling species, and given the quantitatively important effect of *kété*, we suggest that this locus might control an ancestral hydrocarbon character. However, hydrocarbon phenotypes do not follow the evolutionary relationship between closely related species in the melanogaster subgroup (COBB and JALLON 1990) and characters which may have been subject to reinforcing selection are generally not suitable for establishing phylogenetic relationships.

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LITERATURE CITED

BENAMAR, O., and J.-M. JALLON, 1983 The production of 7-tricosene, an aphrodisiac of *Drosophila simulans*, is under the control of one gene on the X chromosome (abstr.) Behav. Genet. **13:** 533.

BLOMQUIST, G. J., J. W. DILLWITH and T. S. ADAMS, 1987 Biosynthesis and endocrine regulation of sex pheromone production in Diptera, pp. 217–243 in *Pheromone Biochemistry*, edited by G. PRESTWICH and G. J. BLOMQUIST. Academic Press, San Diego, Calif.

CHAN-YONG, T. P., and J.-M. JALLON, 1986 Synthèse *de novo* de hydrocarbures potentiellement aphrodisiaques chez les Drosophiles. C. R. Acad. Sci. **303:** 197–202.

COBB, M. J., and J.-M. JALLON, 1990 Pheromones, mate recognition and courtship stimulation in the *Drosophila melanogaster* species sub-group. Anim. Behav. **39:** 1058–1067.

DE RENOBALES, M., T. S. WOPODIN and G. J. BLOMQUIST, 1986 *Drosophila melanogaster* fatty acid synthetase. Insect Biochem. **16:** 887–894.

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., M. J. COBB and J.-M. JALLON, 1989 Complex chemical messages in *Drosophila*, pp. 397–409 in *Neurobiology of Sensory Systems*, edited by R. N. SINGH and N. J. STRAUSFELD. Plenum Press, New York.

JALLON, J.-M., 1984 A few chemical words exchanged by *Drosophila* during courtship. Behav. Genet. **14:** 441–478.

JUAREZ, P., J. CHASE and G. J. BLOMQUIST, 1992 A microsomal fatty acid synthetase from the integument of *Blattella germanica* synthesizes methyl-branched fatty acids, precursors to hydrocarbon and contact sex pheromone. Arch. Biochem. Biophys. **293:** 333–341.

LEWIS, E. B., and F. BACHER, 1968 Methods of feeding EMS to *Drosophila* males. Drosophila Inform. Serv. **43:** 193.

LUYTEN, I., 1982 Variation intraspécifiques et interspécifiques des hydrocarbures cuticulaires chez *Drosophila simulans*. C. R. Acad. Sci. **295:** 733–736.

PECHINE, J. M., F. PEREZ, C. ANTONY and J.-M. JALLON, 1985 A further characterization of *Drosophila* cuticular monoenes using a mass spectrometry method to localize double bonds in complex mixtures. Anal. Biochem. **145:** 177–182.

PENNANEC H, M., J.-F. FERVEUR, D. B. PHO and J.-M. JALLON, 1991 Insect fatty acid related pheromones. Ann. Soc. Entomol. Fr. **27:** 245–263.

PERRIMON, N., D. MOHLER, L. ENGSTROM and A. P. MAHOWALD, 1986 X-linked female-sterile loci in *Drosophila melanogaster*. Genetics **113:** 695–712.

SCOTT, D., and R. C. RICHMOND, 1988 A genetic analysis of male-predominant pheromones of *Drosophila melanogaster*. Genetics **119:** 639–646.

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