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Genetic Control of Pheromones in *Drosophila simulans*. I. *Ngbo*, a Locus on the Second Chromosome

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ABSTRACT

7-Tricosene and 7-pentacosene are predominant hydrocarbons on the cuticle of both sexes in *Drosophila simulans*. The pheromonal role of 7-tricosene has been clearly established for conspecific males, while a synergistic effect for 7-pentacosene has been postulated. Interstrain variation for the production of both compounds is very marked, but similar for both sexes. The genetic basis of this polymorphism was investigated. A major role was found for the second chromosome, which controls the 7-tricosene:7-pentacosene ratio. The main locus involved in controlling this variation, *Ngbo*, was mapped to position 65.3 on the second chromosome. The production of 7-pentacosene is directly related to the *Ngbo* genotype, which is additively expressed with two known alleles, *Seychelles* and *Cameroon*. These alleles act codominantly and are, respectively, hypomorphic and hypermorphic with regard to their effect on 7-pentacosene production. The production of 7-tricosene, which is partially inversely related to that of 7-pentacosene, is also affected by secondary interactions with the second chromosome and with the autosomal background.

HYDROCARBONS on the cuticle of *Drosophila simulans* females act as pheromones capable of inducing conspecific male courtship (JALLON 1984). The hydrocarbon systems of mature flies of both sexes are virtually identical and mainly consist of long-chain hydrocarbons with one double bond, in position 7. The predominant cuticular hydrocarbons show a geographical polymorphism: 7-tricosene (7-T; 23 carbons), which has been shown to induce wing display in conspecific males (JALLON 1984), is the major hydrocarbon in both sexes of most strains. Flies from around the Benin Gulf in Africa have higher levels of 7-pentacosene (7-P; 25 carbons). This variation simultaneously affects both sexes (LUYTEN 1982).

7-T has been studied in bioassays with *D. simulans*. The role of 7-P, the predominant hydrocarbon in some African strains, is not yet known, although 7-P alone cannot stimulate precopulatory behavior in males from the 7-T-rich *D. simulans* Seychelles strain (J.-M. JALLON, unpublished results). On the basis of a study of inter- and intraspecific courtships, COBB and JALLON (1990) proposed that 7-P may also play a role in stimulating courtship in *D. simulans* males, perhaps through synergistic effects.

It has been suggested that 7-T or 7-P, which are predominant hydrocarbons in *Drosophila melanogaster* males, may play a strain-specific "anti-aphrodisiac" role in this species, following their transfer onto the female cuticle during mating (SCOTT and JACKSON 1988). Such an effect should not be expected in *D. simulans* because these compounds are present in both sexes in similar amounts.

BENAMAR and JALLON (1983) suggested that the level of 7-T in *D. simulans* was controlled by the *X* chromosome. FERVEUR, COBB and JALLON (1989) showed that one pair of autosomes played a major role in controlling the ratio of 7-T:7-P, and that the production of 7-T was also dependent on sex-linked factor(s). The genetic control of male-predominant compounds (7-T and 7-P) in *D. melanogaster* appears to be highly complex, possibly involving all chromosomes (SCOTT and RICHMOND 1988).

In order to further our understanding of genetic control of Drosophila pheromone production, we have undertaken an exhaustive investigation testing each potential hereditary factor. In this study we have chosen to concentrate on the relative and absolute production of 7-T and 7-P in *D. simulans*. We chose this species because the absence of a qualitative hydrocarbon sexual dimorphism allows both sexes to be analyzed and, unlike *D. melanogaster*, the interstrain hydrocarbon polymorphism is very marked, and may be controlled by only a few genes.

MATERIALS AND METHODS

Drosophila stocks and crosses: Strains of *Drosophila simulans* were kept at 25 ± 0.5°, under a 12:12 dark:light cycle, on standard cornmeal food. The Cameroon strain is derived from a small number of fertilized females collected in Yaounde in 1975. As with other *D. simulans* strains from the Benin Gulf area, both sexes of Cameroon flies produce more 7-P than 7-T. The Seychelles strain is derived from

several inseminated females collected in the Seychelles archipelago in 1981. As with most *D. simulans* strains (all except those from the Benin Gulf), both sexes produce more 7-T than 7-P.

The inheritance of hydrocarbon phenotype variation was studied by crosses between the two parental strains (Cameroon and Seychelles), based on flies bred in the same conditions and analyzed within a short period of time. Chromosomal localization of genes involved in hydrocarbon phenotype variation was carried out using the following marked strains: (1) G strain, which carried the recessive markers *garnet* (I,44.4), *net* (II,0.0) and *cinnabar* (II,57.5) (a gift of J. COYNE). (2) NBPSP or second chromosome recessive multimarker strain, which carries *net* (II,0.0) *black* (II,45.0) *pearly* (II,74.0) *spread* (II,80.0) and *plum* (II,108.0) (a gift of J. COYNE). (3) W strain, which carries the *Curly* mutation (II,6.8) (a gift of T. WATANABE). For crosses, five pairs of 4-day-old flies were placed in vials with food. Each cross was replicated at least nine times for each experiment.

Extraction and analysis of cuticular hydrocarbons: For each experiment, flies from different strains or crosses were collected and bred simultaneously. Flies were sexed under light etherization shortly after eclosion and were kept in groups of five on fresh food. Following the method of ANTONY and JALLON (1982), 4-day-old flies were individually washed in 50 μl hexane in a microtube and agitated for 1 min. Washed flies were immediately removed from each microtube. In certain samples, 20 μl of a hexane solution containing 800 ng of hexacosane (26C) as an internal standard were added to each tube. When the solvent was fully evaporated, each microtube, now containing a dry extract, was sealed with a Teflon cap and stored at $-20°$ until analysis.

Immediately before analysis 20 μl hexane were added to each microtube, and after gentle agitation 5 μl of the resultant solution were injected into a Girdel 300 gas chromatograph equipped with a C.P. Sil 5 capillary column (l = 25 m; ϕ = 0.23 mm) with nitrogen as the vector gas (P = 0.5 bar). During analysis the temperature increased from 170 to 250° at a rate of 2°/min. Peak detection was carried out using a Flame Ionization Detector coupled with an SP 4270 Spectraphysics integrator which yields retention times and areas under each peak. Extracts of different phenotypes were injected in varying order to randomize experimental variability.

The major hydrocarbon compounds of *D. simulans* Seychelles have been chemically identified by PECHINÉ *et al.* (1985) and show characteristic constant retention times (±0.2% under constant conditions) over many generations.

For each fly spectrum, we calculated the total quantities of hydrocarbon using the summed areas of 13 compounds which are always detected in this species. Each hydrocarbon was characterized by its percentage relative to the total hydrocarbon sum. By comparing this figure to that of the internal hexacosane standard, the absolute quantity in ng could also be calculated.

Hydrocarbon parameters: To localize genetic factors involved in the 7-monoene polymorphism, different hydrocarbon parameters were used. The percentages of 7-T and 7-P showed significant differences between parental strains, a low intrastrain variation and a marked stability over many generations. Percentages of 7-T and 7-P were used as measures of hydrocarbon phenotype for studying the role of various hereditary components. More than 1500 flies were individually analyzed. We chose those parameters shown to be the most stable over time. The ratio of 7-T:7-P produced clear interstrain differences and was used for the localization of genetic control of 7-monoene variation. This ratio was logarithmically transformed ($\log_e$) in order to reduce intrastrain variations in 7-T and 7-P levels. The variation in 7-T:7-P levels was compared with changes in absolute amounts of 7-T and 7-P in small samples of flies raised together. These results suggested that absolute amounts of the two substances would not be a suitable parameter for studying genetic determination, as significant intrastrain variation could occur within a short space of time (see RESULTS).

RESULTS

Hereditary control of hydrocarbon variation: Flies were characterized by their hydrocarbon phenotype (S or non-S) based on two quantitative characters: % 7-T and % 7-P (see Figure 1). Mean 7-T and 7-P percentages were also calculated for each group of flies. Results of chromatographic analysis of males and females from all sixteen strains obtained from the two parental strains, Seychelles and Cameroon, their F_1, backcrosses and F_2 are shown in Table 1.

When strains differed for some of their autosomes they showed significant differences either for S/non-S characterization (SS *vs.* strain 1 males, SS *vs.* strain 5 females), or for their mean percentage 7-T and 7-P levels within the non-S class (CC *vs.* strain 2 females; CC *vs.* strain 6 males). These differences were composed of two effects. The hydrocarbon phenotype classification differentiated flies homozygous for *Seychelles* autosomes from other genotypes; variations in mean % 7-T and % 7-P within the non-S class must be caused by the varying autosomal genotypes present in this class.

Other hereditary components (X chromosomes tested only between females, Y chromosome and cytoplasmic factors) showed no significant effects. Comparison of S/non-S distributions between males of strains 1 and 5 was significant ($P < 0.001$). This effect was initially attributed to the Y chromosome but upon further investigation was revealed to be a sampling effect caused by delayed eclosion of certain hydrocarbon phenotypes.

Although the production of 7-T and 7-P varied in different strains, their sum (% 7-T + % 7-P) for all strains pooled remained remarkably constant both for males (57.5 ± 0.2; n = 719) and for females (62.3 ± 0.2; n = 750). Within the sample as a whole, and for all strains except S × S, there was a significant negative correlation between the proportions of 7-T and of 7-P in individual flies. The absence of a significant correlation in Seychelles parental flies was probably due to the very low variability of 7-P percentage in both sexes (see Figure 1).

Genetic control of 7-T:7-P ratio: Strains with the same autosomes which showed no significant differences for their hydrocarbons parameters (see Table 1) were pooled. Populations of flies were characterized by their hydrocarbon phenotype, S or non-S. The

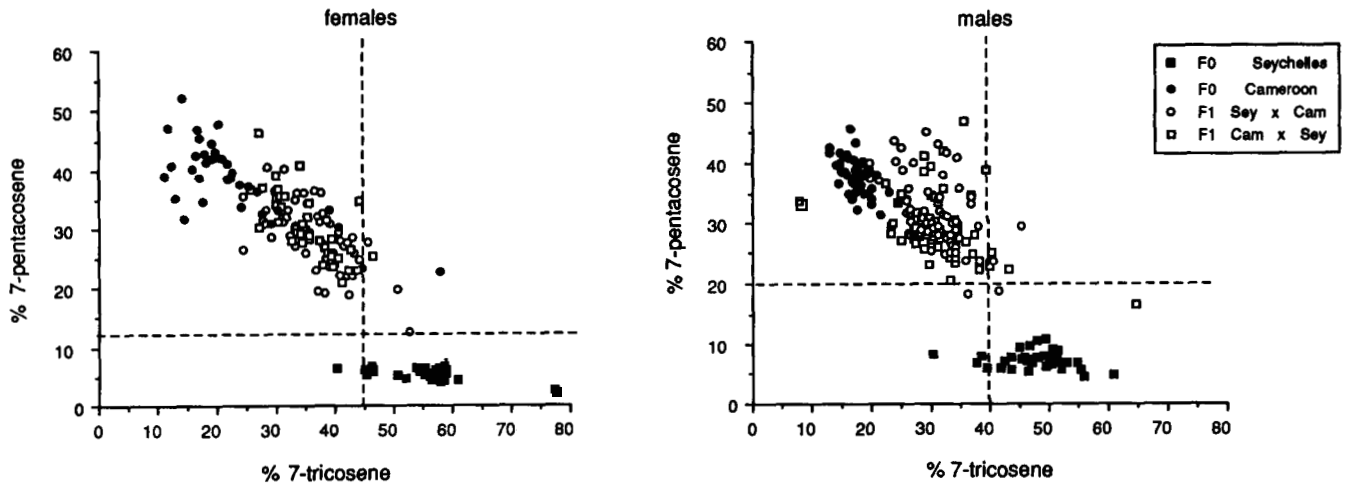

FIGURE 1.—Distribution of individual male and female flies from Seychelles (Sey) and Cameroon (Cam) strains and their reciprocal hybrids (F_1 = female × male), according to their percentage of 7-tricosene (% 7-T) and 7-pentacosene (% 7-P). % 7-T and % 7-P are expressed as a percentage of total hydrocarbons. Each fly was given a pair of *x-y* coordinates on the basis of its % 7-T and % 7-P. Dotted lines represent the cut-off points used to define the two hydrocarbon phenotype groups, S and non-S, and were empirically set at 40% 7-T and 20% 7-P for males, 45% 7-T and 12.5% 7-P for females. The two leading diagonal cells contain either Seychelles or Cameroon plus F_1 flies and represent more than 95% of the available data (Cameroon and F_1 distributions overlap and could not be discriminated by this method). When a point fell into one of the other two diagonal cells, it was integrated into one of the two more populated cells on the basis of immediate proximity. In this way, data were classified into two different hydrocarbon phenotypes, Seychelles (S) and non-Seychelles (non-S) with Cameroon plus F_1 flies. With this binary system, the misclassification probability for S and non-S cells was 0.015 for males and 0.005 for females.

distributions of the Cameroon and Seychelles strains appeared unimodal, suggesting low intrastrain variation for genes controlling 7-monoene ratio (Figure 2). There is no full dominance effect: pooled reciprocal F_1 flies were normally distributed and their distribution partially overlapped but significantly differed from that of Cameroon flies ($t = 14.5$, d.f. = 173, $P < 0.001$ for males and $t = 11.2$, d.f. = 125, $P < 0.001$ for females). Backcrosses to Seychelles (Bc Sey) yielded a bimodal distribution with roughly equal peaks (49.7%:50.3% for males; 48.7%:51.3% for females). Distributions for both sexes, tested with a χ^2 goodness of fit, were not significantly different from a theoretical 1:1 ratio. Backcrosses to the Cameroon strain (Bc Cam) appeared unimodal. Their distribution overlapped those of both Cameroon and F_1 flies. Mean values (−0.60 and −0.17 for males and females, respectively) were roughly intermediate between those of their Cameroon (−0.76 and −0.64) and F_1 (+ 0.02 and + 0.21) same sex parents. The distribution of F2 flies appeared bimodal with two unequal peaks (81.4%:18.6% for males and 73.1%:26.9% for females). The mean value of the major peak was located between those of Bc Cam and the F_1 (−0.29 for males and −0.01 for females). Distributions for both sexes were tested with a χ^2 goodness of fit test. Males, but not females, were significantly different from a theoretical 3:1 ratio distribution ($\chi^2 = 4.53$, d.f. = 1, $P < 0.05$). This effect could be explained by the same sampling effect described previously.

The overall results strongly suggest that the ratio of 7-T : 7-P is controlled by a Mendelian character carried on a single pair of autosomes, each parental genotype being partially dominant, with flies having *Cameroon* chromosomes showing a stronger effect.

Further one-factor ANOVA comparisons of 7-T:7-P, carried out between S class flies, showed a significant effect of autosomal background: parental Seychelles differed from both Backcross to Seychelles and F_2, for males ($F = 22.4$; d.f. = 2, 165; $P < 0.001$) and females ($F = 11.7$; d.f. = 2, 189; $P < 0.001$).

Mapping the major gene(s) involved in 7-T:7-P variation: *Chromosomal localization:* The marked G strain, which carries *garnet* (*X* chromosome), *net* and *cinnabar* (second chromosome), shows the same type of hydrocarbon pattern as the Seychelles strain. Cameroon and G strains produce very different percentages of 7-T and 7-P; they also show differences in the absolute quantity of both substances, although the sums of both 7 monoenes (Σ 7-M) and the total hydrocarbon amount (Σ Hc) are similar. The reciprocal F1s, which showed no significant difference in their 7-T and 7-P absolute production (intermediate between those of their same-sex parents), were pooled for further analysis. Their Σ7-M and ΣHc values were close to those of both parental strain values. Results are shown in Table 2.

The backcross progeny was clearly divided into two hydrocarbon phenotype classes. Flies expressing the morphological marker cinnabar often showed a S hydrocarbon phenotype like that of the G strain. Flies which did not express the cinnabar marker often showed a hydrocarbon pattern similar to that of the F_1 (non-S) flies.

Recombinations between the S hydrocarbon and cinnabar phenotypes, only scored in F_1 female × G

TABLE 1

Results of gas chromatographic analysis of 7-tricosene (7-T) and 7-pentacosene (7-P) for male and female offspring of 16 strains

		Males							Females						
			Phenotype distribution		%7-T		%7-P			Phenotype distribution		%7-T		%7-P	
Cross No.	Female × Male	*n*	Non-S	S	Non-S	S	Non-S	S	*n*	Non-S	S	Non-S	S	Non-S	S
Parental stains															
	S × S	35	0	100		47.6 ± 5.8		7.3 ± 1.5	30	0	100		55.0 ± 6.7		5.4 ± 1.0
	C × C	43	100	0	17.7 ± 2.8		37.7 ± 3.4		35	100	0	22.7 ± 8.7		38.5 ± 6.2	
Reciprocal F_1 hybrids															
	S × C	60	98.2	1.8	31.4 ± 6.5		29.4 ± 5.3		40	100	0	35.2 ± 5.2		30.5 ± 5.6	
	C × S	72	98.6	1.4	30.1 ± 5.1		31.4 ± 5.9		52	98.6	1.4	36.7 ± 6.0		28.7 ± 5.8	
Backcrosses to Cameroon															
2	C × (C × S)	33	96.7	3.3	22.5 ± 8.9		32.7 ± 5.3		47	95.7	1.3	30.5 ± 11.3		31.2 ± 8.7	
3	(C × S) × C	32	100	0	16.3 ± 5.5		35.3 ± 4.3		45	100	0	26.9 ± 8.6		34.2 ± 5.5	
6	C × (S × C)	37	97.3	2.7	20.7 ± 6.0		34.4 ± 5.1		40	100	0	27.9 ± 8.2		33.3 ± 7.1	
7	(S × C) × C	25	100	0	20.0 ± 4.3		36.1 ± 5.5		38	100	0	28.3 ± 6.7		32.7 ± 5.9	
Backcrosses to Seychelles															
1	S × (C × S)	41	29.3	70.7	29.6 ± 5.4	46.9 ± 6.2	27.9 ± 3.5	9.8 ± 3.2	42	47.6	52.4	32.9 ± 6.4	55.0 ± 5.2	27.9 ± 5.0	6.6 ± 1.3
4	(C × S) × S	60	45.0	55.0	28.8 ± 4.3	48.3 ± 4.3	29.2 ± 3.4	10.3 ± 2.8	57	38.6	61.4	37.6 ± 5.6	55.6 ± 4.2	24.6 ± 5.8	8.0 ± 2.3
5	S × (S × C)	41	70.7	29.3	27.1 ± 3.5	45.6 ± 7.8	30.4 ± 3.7	9.8 ± 1.8	35	60.0	40.0	35.1 ± 7.3	55.3 ± 4.8	25.6 ± 7.7	7.7 ± 2.6
8	(S × C) × S	41	51.2	48.8	30.7 ± 3.7	45.2 ± 3.7	26.5 ± 5.0	12.3 ± 3.4	55	52.7	47.3	34.5 ± 8.2	56.0 ± 3.9	27.7 ± 6.0	6.7 ± 2.1
Reciprocal F_2 hybrids															
9	(S × C) × (C × S)	55	78.2	21.8	24.4 ± 6.3	46.9 ± 5.4	31.7 ± 6.0	11.5 ± 2.2	57	71.9	28.1	30.3 ± 7.4	54.1 ± 4.3	30.2 ± 6.5	7.7 ± 2.2
10	(C × S) × (S × C)	54	81.5	18.5	25.3 ± 5.5	46.0 ± 3.5	32.3 ± 5.7	11.9 ± 2.1	58	69.0	31.0	31.0 ± 6.7	54.2 ± 4.2	31.6 ± 6.4	8.0 ± 2.0
11	(C × S) × (C × S)	44	84.1	15.9	24.5 ± 5.0	47.2 ± 3.2	32.7 ± 3.3	11.1 ± 2.0	60	78.3	21.7	31.0 ± 6.5	57.6 ± 3.3	31.0 ± 5.7	7.0 ± 1.6
12	(S × C) × (S × C)	46	78.3	21.7	24.4 ± 5.1	47.4 ± 2.9	34.0 ± 5.6	12.1 ± 2.1	59	69.5	30.5	31.0 ± 7.9	55.0 ± 3.5	31.2 ± 7.0	7.9 ± 2.2
One-factor ANOVA *F*					29.48***	0.76	11.70***	8.56***				10.23***	0.75	9.46***	5.26***
	d.f.				(14,550)	(8,167)	(14,550)	(8,167)				(14,557)	(8,191)	(14,557)	(8,191)

Flies of Seychelles and Cameroon strains were reciprocally crossed, their F_1 reciprocally backcrossed to each parental strain, and crossed in four differents ways (F_2). Distribution of %7-T and %7-P (±1 SEM) for each hydrocarbon phenotype, S and non-S, for males and females of each strain, was estimated with the method described for Figure 1. To determine the mode of hydrocarbon phenotype inheritance (autosomal, sex chromosomal and cytoplasm factors), an exhaustive set of pair-wise strain comparisons was carried out differing by only one hereditary factor in each case (WAHLSTEN 1979; BAUER and SOKOLOWSKI 1988). S/non-S distributions were compared between strains with a chi-square homogeneity test using Yates' correction. The mean percentages of 7-T and 7-P within the S and non-S classes were analyzed with a one-factor ANOVA, and pair-wise comparisons of strains were tested using Fisher's PLSD test.

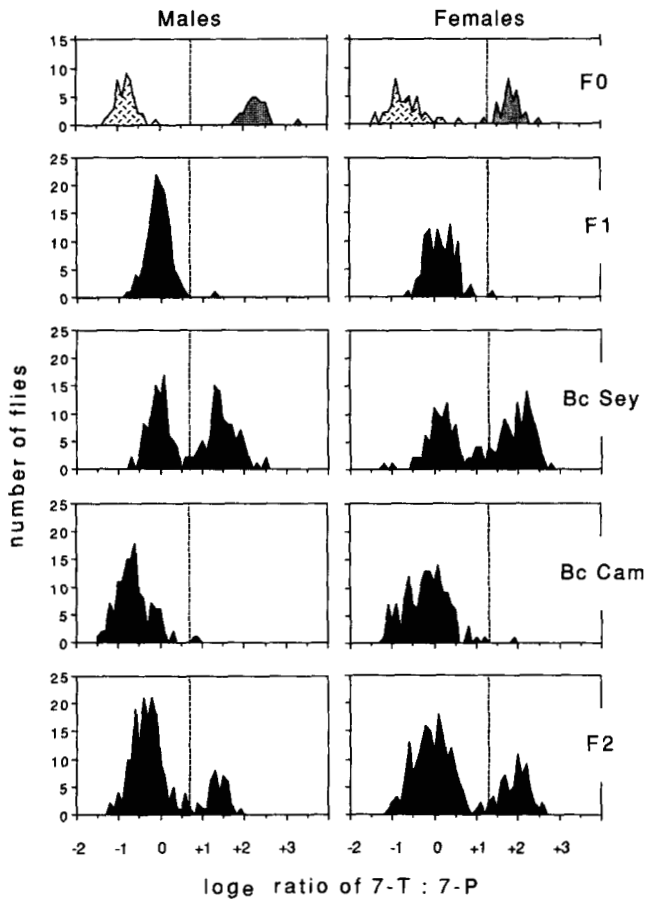

FIGURE 2.—Frequency distribution of $\log_e$ 7-T:7-P values (in groups of $\log_e$ 0.1) for offspring of various crosses from Cameroon (Cam) and Seychelles (Sey) parental strains. Dotted F_0 values represent the Seychelles (S) phenotype, hatched values the Cameroon (non-S) phenotype. Dotted lines indicate the empirical cut-off point between S and non-S (+0.69 for males, +1.28 for females). Reciprocal F_1 flies were pooled. Bc Sey and Bc Cam represent the offspring of male or female F_1 flies backcrossed to parental strain flies (Sey or Cam). F_2 flies are the offspring of the four possible crosses between the two reciprocal F_1 hybrids.

male progeny, were rare. Only 8% of cinnabar flies expressed the non-S hydrocarbon phenotype while 4.2% of non-cinnabar flies expressed the S hydrocarbon phenotype. This suggests that the major locus responsible for the 7-T:7-P ratio variation is relatively close to the *cinnabar* locus, on the second chromosome.

Precise mapping on the second chromosome: The multiply marked second chromosome (*nt b py sd pm*) is carried by the NBPSP strain, which shows a similar hydrocarbon pattern to the Seychelles strain. NBPSP and F_1 flies, which were, respectively, homozygous and heterozygous for the multiple marker second chromosome, showed clear differences in their 7-T:7-P ratio distribution: all NBPSP flies ($n = 13$) belonged to the S phenotype, while all F_1 flies ($n = 35$) expressed non-S phenotype. Most backcross flies (57 out of 58) homozygous for the *black-pearly* segment had a S hydrocarbon phenotype while most backcross flies (23 out of 25) heterozygous for this segment expressed a non-S hydrocarbon phenotype like the F_1 hybrids. An empirical cut-off value, discriminating both hydrocarbon phenotypes, was derived from these distributions (Figure 3).

Males were divided into those expressing either black or pearly. Out of 170 "black" males, 116 were non-S, thus the probability for a black (non-pearly) fly being non-S was ≈0.685. Out of 166 "pearly" males, 47 were non-S, thus the probability for a pearly (non-black) fly being non-S was ≈ 0.283. Note that the sum of these probabilities (0.97) is very close to 1, because flies from both these groups inherit complementary recombinant segments of their second chromosome from the same F_1 female parents. The distance between *black* (II,45) and *pearly* (II, 74) in *D. simulans* is 29 recombination units (J. S. BARKER, personal communication). The locus modifying the 7-T:7-P ratio is thus located on the second chromosome at 65.3 ± 1.0 (the error value was calculated from the misclassification probability), while the *cinnabar* locus is located at II, 57.5. We call this hydrocarbon locus *Ngbo* (the word means "forest" in the dialect of the people living in the West African equatorial forest where 7-P rich flies are found).

The absolute production of 7-T and 7-P was also studied. The production of 7-P, which differs between S flies (116 ng) and non-S flies (335 ng), showed no significant differences within S or non-S classes. S flies produced more 7-T (730 ng) than non-S flies (430 ng). Within the S phenotype group, a significant difference for 7-T production was found between pearly flies varying either for black ($t = 5.04$, d.f. = 174, $P < 0.001$) or for spread ($t = 5.36$, d.f. = 175, $P < 0.001$) irrespective of other markers. Thus, 7-P variation is mainly dependent upon *Ngbo,* while 7-T varies with *Ngbo* and other genes on the second chromosome.

Dosage effect of the second chromosome on 7-T and 7-P levels: Flies of the W parental strain, which bear *Curly,* a second chromosome dominant marker, and express a Seychelles-like hydrocarbon phenotype, were reciprocally crossed with Cameroon flies (Table 3). Cameroon males showed a significant increase in the absolute amount of 7-monoenes over a six-month period ($t = 2.38$, d.f. = 34, $P < 0.025$), while there was no significant change in the $\log_e$ ratio of 7-T : 7-P ($t = 0.653$, d.f. = 34, $P =$ NS). F_1 males with a Curly phenotype and a *Cameroon X* chromosome backcrossed to Cameroon females produce offspring whose males share all hereditary components except their second chromosome. Comparisons between groups of wild phenotype males showed that the number of *Cameroon* second chromosomes was responsible for the main variation in the absolute level of 7-P ($F = 26.8$; d.f. = 3, 109; $P < 0.001$) and 7-T ($F = 21.6$;

TABLE 2

Chromosomal localization of factors controlling the production of 7-monoenes in males and females

Generation	Cross (Female × Male)	Phenotype	*n*	log$_e$ 7-T:7-P	%7-T	%7-P	Q 7-T	Q 7-P	Σ 7-M	Σ Hc
Males										
F_0	Cam × Cam	+	18	−0.76 ± 0.06	17.2 ± 0.8	36.5 ± 0.9	227 ± 17	475 ± 28	702 ± 43	1309 ± 76
	G × G	g, nt cn	31	+2.56 ± 0.10	50.3 ± 1.1	4.6 ± 0.9	679 ± 29	53 ± 4	730 ± 31	1366 ± 65
F_1	Cam × G G × Cam	g or +	32	+0.52 ± 0.07	37.0 ± 1.0	22.3 ± 0.9	515 ± 19	320 ± 22	935 ± 33	1406 ± 51
Backcross	F1 × G	(g/nt) cn	66	+2.33 ± 0.08	52.1 ± 0.9	6.0 ± 0.6	631 ± 24	74 ± 9	705 ± 26	1204 ± 38
	+	(g/nt) +								
	G × F_1		115	+0.39 ± 0.06	35.2 ± 0.8	24.7 ± 0.7	458 ± 14	327 ± 12	786 ± 18	1307 ± 28
Females										
F_0	Cam × Cam	+	10	−0.61 ± 0.10	20.5 ± 1.8	36.5 ± 1.1	414 ± 47	770 ± 110	1185 ± 144	2113 ± 293
	G × G	g, nt cn	8	+2.85 ± 0.07	59.9 ± 1.0	3.5 ± 0.2	1152 ± 52	68 ± 6	1220 ± 55	1926 ± 84
F_1	Cam × G G × Cam	+	42	+0.59 ± 0.07	39.3 ± 1.2	22.2 ± 0.9	783 ± 32	445 ± 23	1228 ± 38	1986 ± 52
Backcross	F_1 × G	(g/nt) cn	48	+2.59 ± 0.07	58.3 ± 0.7	4.9 ± 0.5	1071 ± 37	92 ± 11	1162 ± 38	1827 ± 54
	+									
	G × F_1	(g/nt) +	69	+0.54 ± 0.07	38.8 ± 1.0	23.5 ± 0.8	755 ± 24	478 ± 27	1234 ± 39	1975 ± 60

Data shown are mean log$_e$ 7-T:7-P ratio, mean %7-T, mean %7-P, mean 7-T and 7-P quantities (Q 7-T, Q 7-P), mean of their sum (Σ 7-M) and mean total hydrocarbon (Σ Hc) production. All quantities are given in ng. Parental strains Cameroon (Cam) and G, a marked strain carrying *garnet* (g) on the *X* chromosome, *net* (nt) and *cinnabar* (cn) on the second chromosome, were reciprocally crossed. Reciprocal backcrosses were performed between F_1 and G flies. Backcross data were pooled according to the presence/absence of cinnabar.

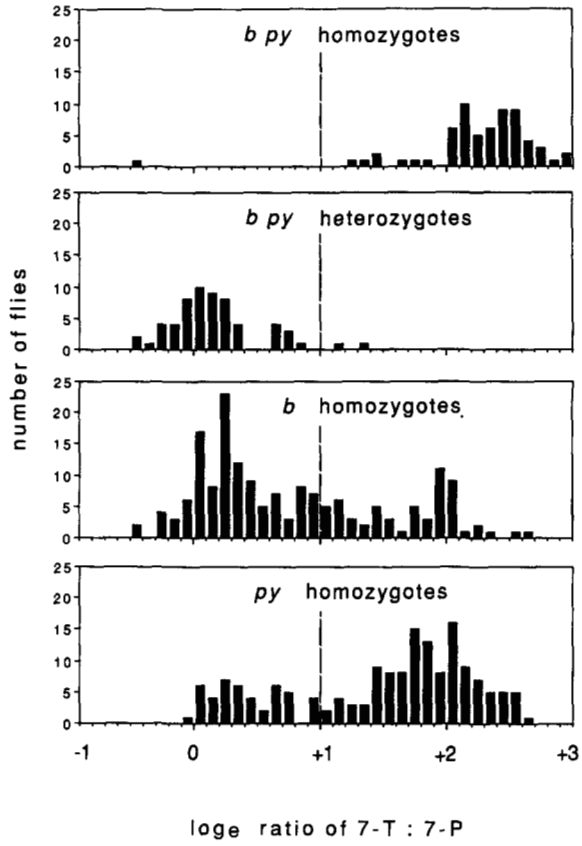

FIGURE 3.—Frequency distribution of log$_i$ 7-T:7-P for backcross male flies showing combinations of the black (b) and pearly (py) markers on their second chromosomes. Cameroon females were crossed with NBPSP males carrying all five markers. F_1 females were backcrossed to NBPSP males. Parental NBPSP males were pooled with the backcross "*b py*" homozygotes. F_1 males were pooled with the "*b py*" heterozygotes. Dotted lines represent the empirical cut-off point (+ 1.00) between the S (to the right) and non-S (to the left) hydrocarbon phenotypes.

d.f. = 3, 109; $P < 0.001$). F_1 males, whatever the cross, were significantly different from both F_0 and Bc flies (pair-wise comparison with Fisher PLSD, $P < 0.05$), which in turn were not significantly different. Comparisons of Curly males with either one or two *W* second chromosomes confirmed that the second chromosome was responsible for the variation of the absolute amount of 7-P ($F = 109.7$; d.f. = 3, 105; $P < 0.001$), absolute amount of 7-T ($F = 4.55$; d.f. = 3, 105; $P < 0.01$), and also for Σ7-M ($F = 12.1$; d.f. = 3, 105; $P < 0.001$) and ΣHc ($F = 11.2$; d.f. = 3, 105; $P < 0.001$). There were significant differences between F_0 and F_1 or Bc flies (pair-wise comparisons) but not between Bc and F_1 flies. Finally, the production of 7-P showed a clear dosage effect: male flies with none, one or two *Cameroon* second chromosome(s), respectively, produced a mean of 53, 345 or 616 ng of 7-P.

DISCUSSION

We have previously implicated one pair of autosomes in the control of the ratio of 7-T:7-P in *D. simulans* (FERVEUR, COBB and JALLON 1989). In that study we evaluated the effects of autosomes and sex chromosomes by using the C(I)RM strain with attached *X* chromosome females; however, we were not able to exclude the effect of other hereditary factors. In this study we have shown that the major variation in the ratio of 7-monoene hydrocarbons (7-T:7-P) in *D. simulans* is controlled by *Ngbo*, a single locus (or a group of very tightly linked loci) on the second chromosome at position 65.3. The two known alleles of *Ngbo* (*Seychelles* and *Cameroon*) act codominantly on the ratio of 7-T:7-P. *Ngbo* seems to be the major locus controlling variation in absolute levels of 7-P, while interacting with other autosomal genes to affect 7-T production.

We studied both percentage measures (% 7-T and % 7-P) and the ratio of 7-T:7-P, all of which show low interindividual variability and are relatively stable over time, which is not the case with absolute 7-monoene levels. For example, only small nonsignificant changes were detected in hydrocarbon percentages of references Seychelles and Cameroon strains, as compared to the data published by JALLON (1984). These differences may be due to slightly different methods of analysis, and/or to genetic drift. However, these parameters also present certain difficulties. For example, we could not distinguish the distributions of Cameroon and of the reciprocal F_1 flies because of the overlap of their % 7-T and % 7-P values. Moreover, the variation in the percentage of either one of the two monoenes can be produced by a change in the absolute production of other substances.

Given that the sum of both compounds (as a proportion of total hydrocarbons) is constant for each sex regardless of strain, we used the 7-T:7-P ratio, a parameter which provides a way of avoiding external variations and enhances phenotypic differences when the variations in the two compounds are negatively correlated. Such a ratio analysis of pheromone blends has proved useful in the study of other insects (KLUN and MAINI 1979; COLLINS and CARDÉ 1985). Changes in the ratio of 7-T:7-P were shown to be mainly due to a reciprocally linked variation in the absolute production of 7-T and 7-P. Using the NBPSP multimarked strain, S flies showed 300 ng more 7-T than non-S F_1 flies, while 7-P levels were 219 ng higher in non-S flies. The dose-dependent increase of 7-P level (280 ng for each *Cameroon* second chromosome), and the fact that 7-P variation on the *Cameroon* second chromosome is exclusively linked with *Ngbo*, strongly suggest that *Ngbo* codominantly controls 7-P synthesis. The two known alleles of *Ngbo*, *Seychelles* and *Cameroon*, are respectively hypomorphic and hypermorphic with regard to 7-P production.

Although the main variation of the ratio of 7-T:7-P is due to the second chromosome's control of 7-P

TABLE 3

Dosage effect of the second chromosome on the absolute production of 7-pentacosene (7-P) and 7-tricosene (7-T)

Generation	Cross (Female × Male)	Phenotype	*n*	log$_e$ 7-T:7-P	Q 7-T	Q 7-P	Σ 7-M	Σ Hc
F_0	Cam × Cam	+	18	−0.83 ± 0.09	275 ± 27	604 ± 44	879 ± 61	1466 ± 78
	W × W	Cy	33	+2.37 ± 0.05	545 ± 19	53 ± 3	599 ± 21	984 ± 34
F_1	Cam × W	+	22	+0.72 ± 0.06	570 ± 27	279 ± 15	850 ± 34	1400 ± 58
		Cy	31	+0.31 ± 0.06	456 ± 24	334 ± 17	790 ± 34	1296 ± 56
	W × Cam	+	30	+0.31 ± 0.08	513 ± 32	363 ± 21	876 ± 39	1405 ± 65
		Cy	18	+0.23 ± 0.10	457 ± 31	364 ± 26	821 ± 42	1359 ± 70
Backcross	Cam × (Cam × not compressed	+	43	−0.63 ± 0.07	342 ± 22	621 ± 35	962 ± 46	1518 ± 77
		Cy	27	+0.13 ± 0.06	437 ± 25	379 ± 16	817 ± 34	1298 ± 58

For hydrocarbon parameters, refer to Table 2. Strains used were Cameroon (Cam) and W, a marked strain with the dominant marker Curly (Cy; II, 6.8). Parental strains were reciprocally crossed and [Cy] males from the (Cam females × W males) cross were backcrossed to Cam females.

production, variation in 7-T levels also plays a role. Unlike 7-P production, 7-T levels seem to be influenced by multiple genetic factors. Independently of the main effect caused by *Ngbo*, factors modifying the absolute production of 7-T (but not that of 7-P) were detected on the second chromosome. The percentage of 7-T was also affected by factors segregating in the autosomal background, probably located on the third chromosome.

Ngbo is not the only locus involved in pheromone synthesis: we have also isolated *kete*, a locus on the *X* chromosome, which mainly affects 7-tricosene levels (J.-F. FERVEUR, in preparation). *X* chromosome effects have previously been implicated in 7-T production in *D. simulans* (BENAMAR and JALLON 1983; FERVEUR, COBB and JALLON 1989), and in the total production of 7-monoenes in *D. melanogaster* (SCOTT and RICHMOND 1988). Because we used the protocol of WAHLSTEN (1979) in this study, we could not test the hemizygous effect of this chromosome between different male strains. A further difficulty was that most parental strains, especially Cameroon and Seychelles, produced equivalent amounts of total 7-monoenes (Q 7-T + Q 7-P) and no significant differences were observed between their reciprocal hybrids which could be linked to the *X* chromosome.

Nevertheless, there are clear quantitative differences between the sexes. Females produce more hydrocarbons (Σ7-M and ΣHC) and have higher % 7-T values and lower % 7-P values, and higher absolute 7-T levels than homotypic males. There is an additive effect on 7-monoene synthesis due to an *X* chromosome dosage effect or to the sexual determination these chromosomes control.

SCOTT and RICHMOND (1988) investigated the absolute production of 7-T and 7-P in *D. melanogaster*, hybridizing the 7-T-rich CS strain with the 7-P-rich Taï strain. They concluded that there was a polygenic control of these characters in this species, based on the autosomes and the X chromosome. We have obtained similar but not identical results for *D. melanogaster*; it appears that there are differences in the genetic control of 7-monoene polymorphism between *D. melanogaster* and *D. simulans*.

Hydrocarbon phenotype is only indirectly linked to gene expression. JALLON (1984) proposed a scheme for *Drosophila* cuticular hydrocarbon biosynthesis in which it was suggested that *D. melanogaster* and *D. simulans* share most enzymatic steps. This scheme agreed with most of the known data about animal fatty acid synthesis, especially with the biosynthesis of Z-9-tricosene by *Musca domestica* (HOWARD and BLOMQUIST 1982; BLOMQUIST, DILLWITH and ADAMS 1988), and involved a small number of enzymes and thus a small number of structural genes. A series of studies involving the injection of precursors and the use of mutants has supported this model (CHAN YONG and JALLON 1986; JALLON *et al.* 1986, 1988; FERVEUR, COBB and JALLON 1989).

According to this scheme, 7-T and 7-P should result respectively from the decarboxylation of 17-tetracosenoic acid (24 C) and 19-hexacosenoic acid (26 C), the 26 carbon fatty acid resulting from the 24 carbon molecule by the addition of two carbons through an elongation process. Given that *Ngbo* is involved in the 7-T:7-P ratio variation, it could act on either the elongation or decarboxylation steps in JALLON's (1984) scheme. In either case, the result would be an alteration in the relationship between the speeds of the two reactions.

In Seychelles-like flies, the decarboxylation step must be faster than elongation, leading to an accumulation of 7-T. The decrease in the ratio of 7-T:7-P shown by non-S flies could thus be due either to a decrease in the speed of decarboxylation or to an increase in the speed of elongation, resulting in an

increase in the production of longer-chain molecules. The interpretation by which *Ngbo* modifies enzymatic specificity is supported by the fact that 7-P-rich strains like Cameroon synthesize a significant amount (3–5%) of 7-heptacosene (27 C) which cannot be detected in 7-T-rich strains.

The behavioral and evolutionary implications of hydrocarbon polymorphisms in both *D. simulans* and *D. melanogaster* are still unclear. We do not know why certain geographical strains show different hydrocarbon profiles, nor what happens if and when flies from the different strains meet in the wild, although there is no isolation between geographical strains in the laboratory (COBB and JALLON 1990). Any possible behavioral role of the 7-monoene blend in *D. simulans* will have to be studied using bioassays combining different ratios and amounts of both compounds. Although nonpheromonal cues are also important in mate choice in both *D. simulans* and *D. melanogaster*, the different hydrocarbon profiles of the two species probably play a role in isolation.

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