A Genetic Analysis of Male-Predominant Pheromones in *Drosophila melanogaster*

David Scott[1] and Rollin C. Richmond

Department of Biology, Indiana University, Bloomington, Indiana 47405

Manuscript received December 7, 1987
Revised copy accepted March 24, 1988

ABSTRACT

Chemical signals from males play an important role in stimulating *Drosophila melanogaster* females to mate, and male-predominant pheromones may influence a female's choice of mates. Male-predominant pheromones also inhibit courtship, thereby functioning as antiaphrodisiacs. Interstrain variation in the ratio of two male-predominant pheromones (7-tricosene and 7-pentacosene) has been reported, but the genetic basis for this potentially important variation has not been examined. In a series of crosses between strains that differ radically in the amounts of 7-tricosene and 7-pentacosene, we have identified both *X*-linked and autosomal contributions to interstrain variation in the amounts of these compounds. The *X*-linked loci act as enhancers for production of the compound predominant in the strain from which the *X* chromosome originated. Autosomal factors for each of the two compounds appear to segregate as high *vs.* low, with incomplete dominance of high 7-tricosene over low, and low 7-pentacosene over high. A significant negative correlation between the quantities of 7-pentacosene and 7-tricosene in the F_2 and backcross progeny, but not in the F_1s or parentals, indicates linkage between autosomal loci regulating the expression of each compound. However, the phenotypic distributions of the backcross progeny indicate that additional unlinked loci are also directly involved in the production of these two hydrocarbons.

SEXUAL dimorphism for cuticular hydrocarbons is ubiquitous in *Drosophila melanogaster*. On males, the predominant cuticular hydrocarbons are those with 23- or 25-carbon chains and one double bond (ANTONY and JALLON 1982; JALLON 1984; ANTONY *et al.* 1985). The most common of these male-predominant compounds are 7-tricosene (23 carbons) and 7-pentacosene (25 carbons). Either or both of these compounds may be present in relatively large quantities on males, depending on the strain, but very little of either compound is present on virgin females (ANTONY and JALLON 1982; JALLON 1984; VAN DEN BERG *et al.* 1984; SCOTT 1986).

Male-predominant compounds may be important in several types of inter- and intrasexual behavioral interactions. In the Canton-S strain, 7-tricosene, the male-predominant cuticular hydrocarbon (ANTONY and JALLON 1982), is acquired by females during mating as a result of contact with the male (VAN DEN BERG *et al.* 1984; SCOTT 1986). Both 7-tricosene acquired from males and topically applied synthetic 7-tricosene decrease the amount of courtship directed toward virgin females, so the compound does function as an antiaphrodisiac (SCOTT 1986; SCOTT, RICHMOND and CARLSON 1988). Its predominance on the male cuticle suggests that 7-tricosene also inhibits courtship between males (SCOTT and RICHMOND 1987). Similarly, 7-pentacosene functions as an antiaphrodisiac for males from strains in which it is the male-predominant hydrocarbon (SCOTT and JACKSON 1988).

Chemical cues from the male also play a role in stimulating females to mate. As females are courted they become less active, which increases the probability of a successful copulation attempt (MARKOW and HANSON 1981). However, females homozygous for either of two independently isolated, recessive olfactory mutations, smellblind (*sbl*: ACEVES-PINA and QUINN 1979) and olfactory D (*olfD*: RODRIGUES and SIDDIQI 1978), continue to be active in the presence of courting males and thus do not mate as quickly (TOMPKINS *et al.* 1982; GAILEY, LACAILLADE and HALL 1986). The difference in mating speed between wild-type and olfactory-deficient females persists when both are paired with wingless (silent) males (GAILEY, LACAILLADE and HALL 1986), so this effect is not due to disruptions in the perception of courtship song, an important component of the male's courtship display (VON SCHILCHER 1976a, b; KYRIACOU and HALL 1980, 1982, 1984).

The male olfactory cue(s) provided to females has not been identified, but male-predominant pheromones may be involved (JALLON 1984). AVERHOFF and RICHARDSON (1976) reported that females of inbred lines could be induced to mate more readily with siblings when exposed to airflow containing pheromones from other genotypes, suggesting po-

[1] Present address: Biochemistry Group, Department of Chemistry, Montana State University, Bozeman, Montana 59717.

lymorphism for male pheromones. Extensive interstrain variation in the ratios of two male-predominant cuticular hydrocarbons, 7-tricosene and 7-pentacosene, has since been documented (JALLON 1984; VAN DEN BERG *et al.* 1984). Furthermore, differences have been noted in female preference between males from strains in which one or the other of these two compounds predominates. For example, Canton-S males, for which 7-tricosene is the predominant hydrocarbon (ANTONY and JALLON 1982; VAN DEN BERG *et al* 1984; SCOTT 1986), are able to mate more quickly with Canton-S females than are males from a strain in which 7-tricosene is virtually absent and 7-pentacosene predominates (JALLON 1984). JALLON (1984) also reported a negative correlation between latency to mating and 7-tricosene levels when males from other strains were tested with Canton-S females.

Male-predominant hydrocarbons may be important both as antiaphrodisiacs and as olfactory cues that enhance female receptivity; they therefore could play a role in interstrain differences in female preference. The genetic basis for variation in the absolute and relative amounts of male-predominant hydrocarbons has received little attention, but JALLON (1984) suggested that the genetic bases for interstrain variation might be relatively simple. Here we describe a genetic analysis of male-predominant pheromones based on crosses between strains in which the predominant male hydrocarbon is either 7-tricosene or 7-pentacosene. Our results indicate that the expression of both compounds is more complex than previously supposed, because it appears to be controlled by *X*-linked loci and by at least two different groups of autosomal loci.

MATERIALS AND METHODS

Drosophila stocks: All flies were from either the Canton-S (CS) or Tai-Y (TY) strains. Both strains are wild type with respect to visible mutations and are highly inbred. The TY strain is an isofemale line originally from the Ivory Coast of Africa (JALLON 1984). CS males have about 425 ng of 7-tricosene (ANTONY *et al.* 1985; SCOTT 1986) but only about 80 ng of 7-pentacosene (ANTONY *et al.* 1985). Conversely, TY males have only about 40 ng of 7-tricosene but about 700 ng of 7-pentacosene (JALLON 1984, and see results below). Several hydrocarbons are present on the cuticle of males, but the majority of the variation in male cuticular hydrocarbons between these two strains is due to differences in the amounts of 7-tricosene and 7-pentacosene (JALLON 1984). Virgin females of both strains possess very little 7-tricosene or 7-pentacosene (ANTONY and JALLON 1982; JALLON 1984; ANTONY *et al.* 1985; SCOTT 1986).

Crosses: All flies were reared and maintained at room temperature (18–22°) on standard cornmeal-molasses-agar medium inoculated with live yeast. Both reciprocal crosses (TY males by CS females; CS males by TY females) were used to generate F_1s, resulting in two sets of progeny that differed in maternal effects and the sex chromosomes of males. For each reciprocal cross, 12 males were single-pair mated to females of the other strain. After mating, four females were transferred to each of three 60-ml food vials and allowed to lay eggs for 2–3 days. The females were then discarded.

The F_1 were collected at daily intervals over the first 3 days after eclosion began. Males and females were separated under ether anesthesia and maintained on yeasted food medium for 3 days. When they were 3–4 days old, 15 randomly chosen male progeny from each of the two reciprocal crosses were backcrossed either to virgin TY females or to virgin CS females, producing all four possible backcrosses. Backcrossing the male F_1s from different reciprocal crosses to females of the same parental strain allowed us to vary the origin of the *Y* chromosome without changing that of the *X* or the autosomes, because male progeny of the backcrosses would carry their grandfather's *Y* and their mother's *X*.

After they had been mated to females from one or the other parental strain, the F_1 males were returned to their storage vials for 1 day to allow them to replace any hydrocarbons that had been transferred to the females during mating (SCOTT 1986). After 1 day, they were anesthetized with ether and washed in hexane for analysis of their cuticular hydrocarbons (see below). Thus the F_1 males that were analyzed for cuticular hydrocarbons were also the males that fathered the progeny of the backcrosses.

Females to which the F_1 males had been mated were individually placed in 30 ml vials containing yeasted food medium and allowed to lay eggs for 2 days. This resulted in 15 vials for each of the four types of backcross matings. We began with a surplus of vials because, at the time the crosses were made, we did not know how fertile the matings would be. However, of the 60 total vials, progeny eclosed from all but four, which were distributed over three of the backcross groups. After progeny began to eclose, five vials from each of the backcross groups were chosen at random. The rest were discarded. Three males were randomly selected for analysis from each of the five vials in each group, for a total of 15 males from each backcross.

In a second group of matings, 25 F_1 males from each of the initial reciprocal crosses were single-pair mated with females from the same rearing vials. These were not necessarily sib matings, because the F_1 progeny in each vial could have been from any of four different females. The 25 mated F_1 females from each of the initial crosses were then placed in 0.25-liter culture bottles to lay eggs. From each set of F_2 progeny, 30 males were chosen at random for analysis. Again the progeny were separated by the direction of the initial cross to determine whether the *Y* chromosome influenced hydrocarbon phenotypes. In each set of F_2, both CS and TY *X* chromosomes (and recombinants) were segregating, but only the grandfather's *Y* was present. Separating the F_2 in this way also allowed a determination of whether maternal factors affected hydrocarbon phenotype, since these would also differ according to the direction of the initial cross.

Analysis of cuticular hydrocarbons: Hydrocarbons were removed from individual males by washing them for 1 min in 100 μl of hexane (Baker). This procedure removes about 95% of the hydrocarbons from adult males (SCOTT 1986). All males used were 4–5 days old. During the wash, 100 ng of 11-eicosenyl acetate (Sigma) were added to each sample as an internal standard. The samples were stored in glass vials at −20° until they were analyzed.

For analysis, individual hexane washes were evaporated to 2–3 μl under a gentle stream of nitrogen. The sample was then injected into a Varian 3300 gas chromatograph equipped with a flame ionization detector, a split/splitless injector and a 25 m × 0.32 mm i.d. DB-1 fused silica capillary column (J & W Scientific). The column tempera-

TABLE 1

Mean amounts (ng) of 7-tricosene, 7-pentacosene and total (7-tricosene + 7-pentacosene), ± 1 SE, on males from TY and CS strains and on male hybrids produced from a series of crosses

	Tricosene	Pentacosene	Total	*n*
TY males	41 ± 3.1	707 ± 36.9	748 ± 38.7	15
CS males	432 ± 13.0	84 ± 7.1	516 ± 14.9	25
F_1 males (CS *X*)	316 ± 12.2	219 ± 11.0	535 ± 19.3	30
F_1 males (TY *X*)	243 ± 9.0	312 ± 11.8	555 ± 17.4	30
F_2 males (CS *Y*)	290 ± 10.9	229 ± 13.5	519 ± 12.9	30
F_2 males (TY *Y*)	278 ± 11.4	207 ± 17.8	485 ± 14.3	30
F_2 males, total	283 ± 7.8	218 ± 11.2	501 ± 9.8	60
Male progeny of backcross to CS female				
(CS *X*, TY *Y*)	398 ± 25.3	232 ± 17.8	630 ± 23.1	15
(CS *X*, CS *Y*)	394 ± 20.2	135 ± 24.0	529 ± 32.7	15
Total	396 ± 15.9	183 ± 17.2	579 ± 21.8	30
Male progeny of backcross to TY female				
(TY *X*, CS *Y*)	223 ± 24.1	455 ± 44.1	678 ± 36.8	15
(TY *X*, TY *Y*)	247 ± 24.9	408 ± 28.3	655 ± 35.7	15
Total	235 ± 17.2	431 ± 26.1	666 ± 25.3	30

The origins of the *X* and *Y* chromosomes are designated by (CS *X*) or (TY *X*) and (CS *Y*) or (TY *Y*), respectively.

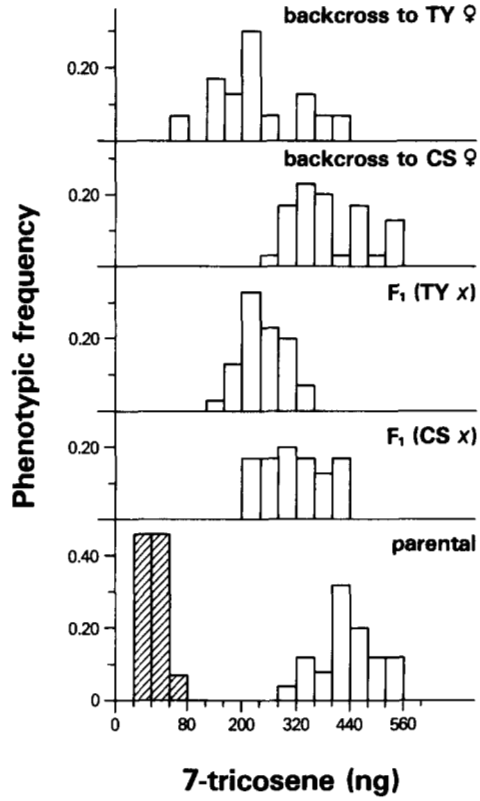

FIGURE 1.—The phenotypic distribution of 7-tricosene on TY and CS males and the male progeny from a series of hybrid crosses. The shaded parental histogram represents TY males, and the unshaded histogram represents CS males. The 7-tricosene is given in 40-ng intervals, except for amounts less than 80 ng, which are given in 20-ng intervals to illustrate the low recovery of TY phenotypes. F_1 (CS *X*) represents heterozygous males with a CS *X* chromosome and F_1 (TY *X*) represents heterozygotes with a TY *X* chromosome.

ture was programmed from 190° to 275° at 2.5°/min. The split vent (150:1 ratio) was opened automatically 0.10 min after injection. 7-Tricosene and 7-pentacosene were identified on the basis of their predominance on males of one or the other strains, by comparison with synthetic standards and by comparison with previously published results (ANTONY and JALLON 1982; JALLON 1984). Peak areas were calculated by a Varian 4270 integrator.

Variation between wild-type strains includes differences in the absolute amounts of these compounds and in their ratios (VAN DEN BERG *et al.* 1984; JALLON 1984), so for each individual we measured both the absolute amount of each compound and the 7-pentacosene/7-tricosene ratio. The ratio provides a simultaneous measure of each hydrocarbon that is independent of general increases or decreases in hydrocarbon quantity. We also analyzed the total of the two compounds. Statistical methods were taken from SOKAL and ROHLF (1981) and SNEDECOR and COCHRAN (1980).

RESULTS

CS and TY males: For both 7-tricosene and 7-pentacosene the CS and TY means differed by about a factor of 10 (Table 1). Between strains, there was no overlap in ranges for either compound (Figures 1 and 2). For 7-tricosene, the highest TY value was 64 ng and the lowest CS value was 296 ng. For 7-pentacosene, the quantities were approximately reversed, with the highest CS value being 192 ng and the lowest TY value 547 ng. These phenotypes are consistent with JALLON's (1984) description. At least some of each compound is always produced by males of both strains, so the variation between strains is due to differences in quantitative expression.

The total male-predominant hydrocarbons (7-tricosene + 7-pentacosene) also differed between strains, with TY totals significantly higher than CS ($t = 5.4$, $P < 0.01$, Table 1). The ranges for CS and TY males were 365–717 ng and 585–1015 ng, respectively, so there was some phenotypic overlap between the two strains. Of the TY males, 7 of 15 had totals less than the CS maximum, and 4 of 25 CS males had totals greater than the TY minimum.

Effects of *Y* chromosome and maternal factors: The two sets of F_2 male progeny differed genetically only in the origin of their maternal cytoplasm and *Y* chromosomes, which provided a test for the effects of maternal cytoplasm and *Y* chromosome on hydrocarbon phenotype. Neither 7-tricosene ($t = 0.8$, $P > 0.40$) nor 7-pentacosene ($t = 1.0$, $P > 0.20$) differed significantly between the two sets of F_2 progeny (Table 1), so neither the maternal cytoplasm nor the *Y* chromosome contributed significantly to variation in hydrocarbon phenotypes.

The backcross progeny provided a second test of *Y* chromosome effects, because the origin of the *Y* chromosome could be changed independently of the other chromosomes. For example, by backcrossing both F_1 (TY *Y*) and F_1 (CS *Y*) males to females from

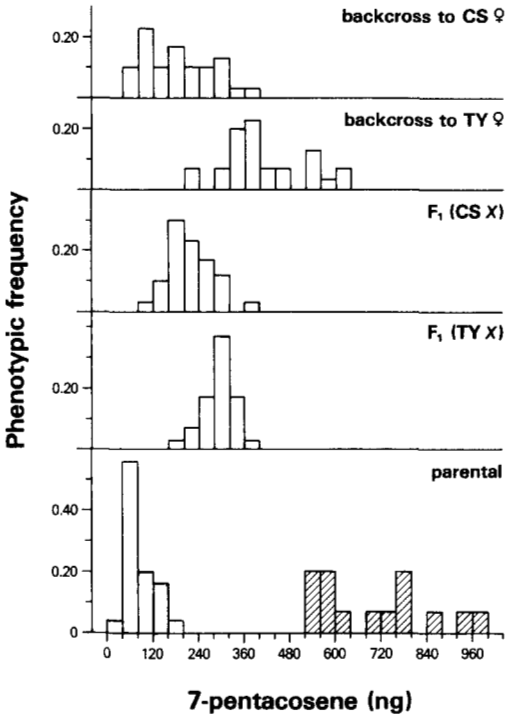

FIGURE 2.—The phenotypic frequency distribution of 7-pentacosene on CS and TY males and the male progeny from a series of hybrid crosses. The shaded parental histogram represents the TY males, the unshaded histogram represents CS males. The amounts of 7-pentacosene are given in 40-ng intervals. For definitions of histogram headings, see Figure 1.

the same strain, two sets of progeny would be generated that differed only in their *Y* chromosomes. The results of this cross are shown at the bottom of Table 1.

Regardless of the maternal strain, the difference in 7-tricosene between the progeny with TY *Y* chromosomes and CS *Y* chromosomes was not significant ($t < 0.7$, $P > 0.40$, Table 1), so the origin of the *Y* chromosome did not affect the quantity of 7-tricosene. For 7-pentacosene, the difference between progeny with CS and TY *Y* chromosomes was not significant in one of the backcrosses (to TY females, $t = 0.9$, $P > 0.20$), but was in the other ($t = 3.2$, $P < 0.01$). This difference did not occur in the F_2 and was absent in one set of backcross progeny (Table 1), so it was not caused directly by the *Y* chromosome.

F_1 males: The phenotypic distributions of the F_1 indicate that production of both compounds is under the control of *X*-linked loci (Figures 1 and 2). The amount of 7-tricosene is significantly higher ($t = 4.8$, $P < 0.01$) in F_1 males carrying a CS *X* chromosome than in those with a TY *X* (Table 1, Figure 1), but F_1 males with a TY *X* have significantly higher quantities of 7-pentacosene ($t = 5.8$, $P < 0.01$, Table 1, Figure 2). Thus TY and CS *X* chromosomes are associated with increased amounts of 7-pentacosene and 7-tricosene, respectively. The totals did not vary significantly between the two F_1s, indicating no *X*-linked effects on total male-predominant hydrocarbons.

If only *X*-linked factors controlled the amounts of the two hydrocarbons, F_1 males would express phenotypes characteristic of males from the maternal strain. However, for each compound F_1 males differed from maternal strain males, indicating effects from autosomal loci. F_1 (CS *X*) males have significantly less 7-tricosene than CS males ($t = 6.4$, $P < 0.01$), and F_1 (TY *X*) males had about six times as much 7-tricosene as TY males (Table 1). A similar pattern occurred for 7-pentacosene (Table 1, Figure 2). Using other strains differing with respect to 7-tricosene, JALLON (1984) also found that both *X*-linked and autosomal loci influence the regulation of that compound.

The F_1s were generally intermediate between CS and TY, so there were no strong dominance relationships between CS and TY alleles. However, if autosomal effects were completely additive the F_1 (TY *X*) means would be closer to those for TY males, due to the influence of the *X* chromosome. This was not the case. For both 7-tricosene (Table 1, Figure 1) and 7-pentacosene (Table 1, Figure 1), the F_1 (TY *X*) means were nearer to those of CS males than to those of TY males, indicating partial dominance of the CS phenotype (high 7-tricosene, low 7-pentacosene) over the TY phenotype (low 7-tricosene, high 7-pentacosene). For the total (Table 1), a stronger dominance effect was apparent. Both F_1 means were close to the low means characteristic of CS males, so total appears to segregate as high *vs.* low, with incomplete dominance.

Between F_1 males from different reciprocal crosses, higher mean quantities of 7-tricosene were accompanied by lower mean quantities of 7-pentacosene, and *vice versa* (Table 1). However, there was no direct negative correlation between the two compounds when all F_1 males were combined ($r = -0.07$, $P > 0.60$, 58 d.f.). Analysis of covariance between 7-tricosene and 7-pentacosene, with the *X* chromosome as a treatment factor, showed significant *X* chromosome effects ($F(1,57) = 14.5$, $P < 0.01$), but no covariance between the two hydrocarbons ($F(1,57) < 0.01$, $P > 0.90$). Thus the differences between males from the two reciprocal crosses were due to differences in the origin of the *X* chromosome, rather than to any general covariance between the two hydrocarbons.

The phenotypic distributions for the 7-pentacosene/7-tricosene ratios (Figure 3) followed the same general trends as the distributions for the absolute amounts of the compounds (Figures 1 and 2). The ratio for F_1 (TY *X*) males was significantly higher than the ratio for F_1 (CS *X*) males ($t = 9.6$, $P < 0.01$, see Table 2), indicating an *X*-linked effect on the ratios. An autosomal effect is also evident, because

TABLE 2

The 7-pentacosene/7-tricosene ratios (mean ± 1 SE) for CS and TY males and the male progeny of different crosses

	Ratio	*n*
CS males	0.20 ± 0.016	25
TY males	18.56 ± 1.440	15
F_1 (CS *X*) males	0.71 ± 0.037	30
F_1 (TY *X*) males	1.32 ± 0.051	30
F_2 males	0.87 ± 0.073	60
Male progeny of backcross to:		
CS females	0.49 ± 0.053	30
TY females	2.51 ± 0.485	30

the ratio for F_1 (CS *X*) males was higher than that of CS males ($t = 12.6$, $P < 0.01$, Table 2), while the ratio for F_1 (TY *X*) males was lower than that of TY males (Figure 3, Table 2).

F_2 males: The hydrocarbon phenotypes of the F_2 males were approximately intermediate between CS and TY, but tended to be nearer those for CS males (Table 1). This indicates partial dominance by the CS phenotype, consistent with that observed in the F_1. For total male-predominant hydrocarbons, dominance appeared to be virtually complete, because the F_2 means were actually somewhat lower than the CS mean (Table 1). A relatively strong dominance by the CS phenotype was also evident when the compounds were measured as a ratio (Table 2).

Although the individual quantities of 7-pentacosene and 7-tricosene were not negatively correlated in the F_1, a negative correlation was present in both sets of F_2 males. The correlation was -0.46 ($P = 0.01$) for F_2 (CS *Y*) males; for F_2 (TY *Y*) males it was -0.60 ($P < 0.01$, 28 d.f. for each). The correlation for the total F_2 was -0.52 ($P < 0.01$, 58 d.f.). The negative correlation was not present in either TY males ($r = 0.53$, $n = 15$, $P < 0.05$), or CS males ($r = 0.02$, $n = 25$, $P \gg 0.50$), so it was not due to "nongenetic" factors, such as environmental or metabolic constraints on hydrocarbon phenotype.

Both TY and CS *X* chromosomes, and recombinants, were segregating in the F_2, so males carrying these chromosomes were, by necessity, combined for the correlation analysis. However, combining individuals with different *X*'s would not in itself cause a negative correlation, because no correlation was present in the F_1 when males with different *X* chromosomes were combined. The negative correlation in the F_2 indicates autosomal loci regulating 7-tricosene that do not segregate independently of those regulating 7-pentacosene.

Backcross progeny: For both 7-tricosene and 7-pentacosene, the phenotypic distribution of the progeny from backcrosses to CS females covered almost the entire range of both F_1 (CS *X*) and CS males

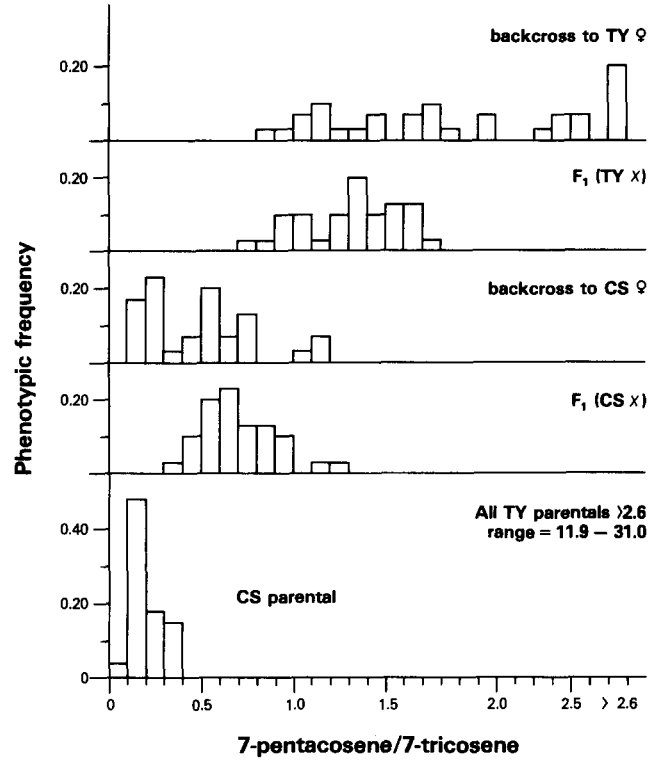

FIGURE 3.—Phenotypic distribution for 7-pentacosene/7-tricosene ratios in CS males and male progeny from a series of crosses between CS and TY strains. The distribution for TY males is summarized, but not shown due to the extreme difference between it and the other distributions. For definitions of histogram headings, see Figure 1.

(Figures 1 and 2). The mean quantity of 7-tricosene on the backcross progeny was significantly greater than that on F_1 (CS *X*) males ($t = 4.0$, $P < 0.01$), so an increase in the quantity of 7-tricosene was associated with the increased frequency of CS autosomes, indicating additive autosomal effects. This was also the case for the 7-pentacosene/7-tricosene ratios, which were intermediate between the F_1 (CS *X*) and CS means (Table 2, Figure 3). The amount of 7-pentacosene tended to be less in the backcross progeny than in the F_1 (CS *X*) (Figure 2), but the difference was not significant (Table 1).

The progeny of backcrosses to TY females had significantly more 7-pentacosene than F_1 (TY *X*) males ($t = 4.2$, $P < 0.01$), but were not intermediate between F_1 (TY *X*) and TY males (Table 1). This was also the case for the 7-pentacosene/7-tricosene ratio (Table 2, Figure 3). The amount of 7-tricosene did not differ significantly between the backcross progeny and the F_1s (Table 1). In general, the TY phenotype was less strongly represented in progeny from backcrosses to TY females than the CS phenotype was in the backcrosses to CS females (Figures 1–3). This is consistent with the F_1 and F_2 results, which both indicated partial dominance by the CS phenotype.

In the total backcross progeny, there was a signif-

icant negative correlation between the quantities of 7-tricosene and 7-pentacosene ($r = -0.63, P < 0.01$, 58 d.f.), as there had been in the F_2. The origin of the *X* chromosome was known for each individual in the backcross progeny, so we analyzed the covariance between 7-tricosene and 7-pentacosene for the total backcross progeny, with the origin of the *X* chromosome included as a treatment factor. In the backcrosses, differences in the *X* would be coupled with differences in autosomal frequency. For example, progeny from backcrosses to CS females would have both a CS *X* and a greater frequency of CS autosomes. A significant "*X*-linked" effect was evident ($F(1,57) = 11.0, P < 0.01$; see Table 1 for means). The covariance between 7-tricosene and 7-pentacosene was also significant ($F(1,57) = 5.0, P < 0.03$), with a negative regression coefficient (-0.22 ± 0.10). These results support those obtained from the F_2, and indicate autosomal factor(s) regulating one compound that do not segregate independently from factor(s) regulating the other.

Number of autosomes contributing to hydrocarbon phenotype: To estimate whether one or more autosomes were involved in the regulation of 7-tricosene and 7-pentacosene we compared the absolute amounts of each compound among the TY-derived lines (TY, F_1 (TY *X*), and progeny from backcrosses to TY females), because the TY phenotype acted as a partial recessive to CS. The TY and F_1 (TY *X*) phenotypes did not overlap (Figures 1 and 2), so we were able to determine with little ambiguity whether individual backcross progeny belonged to TY or non-TY phenotypic categories. In the CS-derived lines, the CS and F_1 (CS *X*) phenotypes overlapped considerably (Figures 1 and 2), so we were not able to assign individual backcross progeny to specific phenotypic categories.

If factors on one autosome were involved in the regulation of 7-tricosene and 7-pentacosene, a 1:1 ratio of TY:non-TY phenotypes would be expected in the backcross to TY females. A 1:3 ratio would be expected from loci on two autosomes. The observed ratios clearly favor the latter. For 7-pentacosene, 7 of 30 of the backcross progeny fell within the TY range, very close to the 7.5 of 30 that would be expected from a 1:3 ratio, and significantly different from a 1:1 expectation (binomial probability = 0.003). For 7-tricosene, 2 of 30 of the backcross progeny were TY. This is significantly fewer than would be expected from even a 1:3 ratio (binomial probability = 0.01), indicating that at least two autosomes are involved in 7-tricosene regulation.

For total male-predominant hydrocarbons, neither the phenotypes of CS and TY males, nor the phenotypes of the progeny from backcrosses to TY females, fell into distinct categories. Thus we were unable to determine whether the phenotypic ratios in the progeny of backcrosses to TY females were consistent with contributions from loci on one autosome, or more than one. However, the means for the F_2s were very near the CS mean, so there is little effect, if any, from "high total" alleles segregating in the F_2. This result suggests that total is regulated by loci on at least two autosomes.

We attempted to determine whether loci controlling either 7-pentacosene or 7-tricosene were genetically linked to total loci by calculating the correlation coefficients between total and the two hydrocarbons. However, the quantities of the two compounds were not independent of the total in the TY or CS strains, or the F_1s. For the CS strain, the correlations between total and 7-pentacosene or 7-tricosene were 0.49 ($P < 0.02$), and 0.88 ($P < 0.001$), respectively, 23 d.f. for each. For the TY strain, they were 0.99 ($P < 0.001$), and 0.58 ($P < 0.03$), 13 d.f. each. In each case, the correlation was greatest for the hydrocarbon in the greatest quantity, presumably because variation in the quantity of that hydrocarbon would make the greatest contribution to variation in the total. In the F_1s, the respective correlations were 0.73 and 0.63 ($P < 0.001$, 58 d.f. for each).

In the F_2 and backcrosses, a different pattern emerged. In both cases, 7-pentacosene was significantly correlated with total: $r = 0.73$ and 0.72 for the F_2 and backcross, respectively ($P < 0.001$, 58 d.f. for each). However, 7-tricosene was not: $r = 0.21$ ($P > 0.10$) for the F_2 and 0.10 ($P > 0.50$) for the backcross. These results suggest that loci controlling 7-pentacosene could be linked to those controlling total, but indicate that there is no linkage between loci controlling 7-tricosene and total.

DISCUSSION

Male pheromones play an important role in stimulating females to mate (TOMPKINS *et al.* 1982; GAILEY, LACAILLADE and HALL 1986). The ratio of 7-tricosene to 7-pentacosene, both predominant male pheromones, may vary considerably between strains (VAN DEN BERG *et al.* 1984), and JALLON (1984) has suggested that the quantity of 7-tricosene on a male influences female mating speed. Additionally, male-predominant compounds function as antiaphrodisiacs (SCOTT 1986; SCOTT, RICHMOND and CARLSON 1988). Understanding the genetics of 7-tricosene and 7-pentacosene regulation would provide a basis for predictions about the complexity of biochemical and genetic changes required for pheromonal shifts between populations.

Both *X*-linked and autosomal loci appear to be involved in the regulation of 7-tricosene and 7-pentacosene. The *X*-linked loci for the CS and TY strains bias hydrocarbon production toward 7-tricosene and 7-pentacosene, respectively. Autosomal loci involved in the regulation of 7-tricosene and 7-pentacosene segregate as high *vs.* low, and dominance

is incomplete. There is a significant negative correlation between the quantities of the two compounds in the F_2 and backcross progeny, which indicates that autosomal factors regulating one compound are not independent of those regulating the other. A negative correlation could result from biochemical constraints between the two compounds, such that an increase in one caused a decrease in the other. In this case, a negative correlation should also be present in CS, TY and F_1 males, but it was not. It is therefore likely that the negative correlations in the F_2 and backcrosses were due to genetic linkage between loci regulating the two hydrocarbons.

Two lines of evidence indicate that unlinked autosomal loci also contribute to the regulation of each compound. First, an increase in one of these compounds was not necessarily accompanied by a decrease in the other. For example, the amount of 7-pentacosene was greater in the progeny of backcrosses to TY females, compared to F_1 (TY *X*) males, but the quantity of 7-tricosene did not decrease significantly (Table 1). A similar situation occurred in the backcrosses to CS females: these progeny had more 7-tricosene than the F_1 (CS *X*) males did, but not significantly less 7-pentacosene (Table 1). This shows that the quantities of the two compounds can be independent. Second, for the absolute amounts of each compound, the phenotypic frequencies in the progeny from backcrosses to TY females are consistent with effects from loci on at least two autosomes, but significantly different from an expectation based on effects from just one autosome.

These two sets of results are not mutually exclusive, and could be accounted for by either of two models. In the first, alleles for high pentacosene could be linked to those for low tricosene and *vice versa*, and these groups could be duplicated on each of at least two autosomes. Thus high levels of one hydrocarbon would tend to be accompanied by low levels of the other, and both would be controlled by loci on two or more autosomes. In the second, unlinked loci could code for "structural" enzymes, which were directly involved in the synthesis of 7-tricosene and 7-pentacosene, while linked autosomal loci could control production of "regulatory" factors that influenced the relative quantities of the two compounds. The effects of the linked autosomal loci might be similar to those of the *X*-linked loci affecting hydrocarbon production.

In general, expression of the maternal strain phenotype was weaker in hybrids with a TY *X* than in hybrids with a CS *X*. This was particularly evident when hydrocarbons were measured as ratios. Partial dominance by CS alleles would have shifted the phenotypes of hybrid males toward the CS phenotype. Additionally, TY *X*-linked loci could exert a weaker effect on hydrocarbon phenotype. However, neither of these causes can explain the low recovery of TY phenotypes in the backcrosses to TY females. Even if loci on two autosomes contributed to variation in hydrocarbon phenotype, the recovery of TY phenotypes was significantly lower than would be expected.

Loci regulating the total male-predominant hydrocarbons could have contributed to the apparent loss of TY phenotypes. Low total appears to be more or less dominant over high, and the F_2 results suggest that loci regulating total are present on at least two autosomes. TY alleles regulating 7-tricosene and 7-pentacosene act as partial recessives, and loci on at least two autosomes appear to be involved in the production of the two hydrocarbons. Under these conditions, a complete TY genome might be required for full expression of the TY phenotype.

Previous research has indicated that insect hydrocarbons are derived from relatively short chain fatty acids by elongation and decarboxylation (BLOMQUIST and JACKSON 1979; DILLWITH, BLOMQUIST and NELSON 1981; HOWARD and BLOMQUIST 1982). Elongation seems to occur by addition of two carbon units (HOWARD and BLOMQUIST 1982). On this basis, JALLON (1984) proposed that, in male Drosophila, elongation of a 16 carbon precursor to 24 carbons and subsequent decarboxylation produces 7-tricosene, whereas elongation of the same precursor to 26 carbons with subsequent decarboxylation produces 7-pentacosene. The enzyme responsible for elongation has been termed an elongase (JALLON 1984).

Presumably, a 24 carbon elongase would be more active in CS males, and a 26 carbon elongase in TY males. A relatively simple genetic change could then be responsible for the rather dramatic difference in hydrocarbon phenotypes. However, our results suggest that the genetic basis for the differences in hydrocarbon phenotype is complex. Linked autosomal factors appear to control the relative quantities of the two compounds, while unlinked loci affect the production of each compound independently. Other loci, which seem to be present on at least two autosomes, may regulate the total hydrocarbon production and thereby indirectly influence the amounts of both 7-tricosene and 7-pentacosene. Finally, *X*-linked loci also play a significant role in the production of these hydrocarbons.

We thank JEAN-MARC JALLON (Laboratoire de Biologie et Genetique Evolutives du CNRS, Gif-sur-Yvette, France) for providing stocks from the Tai-Y strain of *D. melanogaster*. Software for the statistical analyses was developed by R. E. LUND (Montana State University, Bozeman). This research was funded by the National Science Foundation (BSR 84-15518 to R.C.R.) and the National Institutes of Health (Training Grant GM 7757 to D.S.).

LITERATURE CITED

ACEVES-PINA, E. O., and W. G. QUINN, 1979 Learning in normal and mutant *Drosophila* larvae. Science **206:** 93–96.

ANTONY, C., and J.-M. JALLON, 1982 The chemical basis for sex

recognition in *Drosophila melanogaster*. J. Insect Physiol. **28:** 873–880.

ANTONY, C., T. L. DAVIS, D. A. CARLSON, J.-M. PECHINE, and J.-M. JALLON, 1985 Compared behavioral responses of male *Drosophila melanogaster* (Canton-S) to natural and synthetic aphrodisiacs. J. Chem. Ecol. **11:** 1617–1629.

AVERHOFF, W., and R. RICHARDSON, 1976 Multiple pheromone system controlling mating in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. *USA* **73:** 591–593.

BLOMQUIST, G., and L. JACKSON, 1979 Chemistry and biochemistry of insect waxes. Prog. Lipid Res. **17:** 319–345.

DILLWITH, J. W., G. J. BLOMQUIST and D. R. NELSON, 1981 Biosynthesis of hydrocarbon components of the sex pheromone of the housefly, *Musca domestica*. Insect Biochem. **11:** 187–197.

GAILEY, D. A., R. C. LACAILLADE and J. C. HALL, 1986 Chemosensory elements of courtship in normal and mutant, olfaction deficient *Drosophila melanogaster*. Behav. Genet. **16:** 375–405.

HOWARD, R. W., and G. J. BLOMQUIST, 1982 Chemical ecology and biochemistry of insect hydrocarbons. Annu. Rev. Entomol. **27:** 149–172.

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

KYRIACOU, C. P., and J. C. HALL, 1980 Circadian rhythm mutations in *Drosophila melanogaster* affect short-term fluctuations in the male's courtship song. Proc. Natl. Acad. Sci. *USA* **77:** 6729–6733.

KYRIACOU, C. P., and J. C. HALL, 1982 The function of courtship song rhythms in *Drosophila melanogaster*. Anim. Behav. **30:** 794–801.

KYRIACOU, C. P., and J. C. HALL, 1984 Learning and memory mutations impair acoustic priming of mating behaviour in *Drosophila*. Nature **308:** 62–64.

MARKOW, T. A., and S. J. HANSON, 1981 Multivariate analysis of *Drosophila* courtship. Proc. Natl. Acad. Sci. **78:** 430–434.

RODRIGUES, V., and O. SIDDIQI, 1978 Genetic analysis of chemosensory pathways. Proc. Indian Acad. Sci. **87B:** 147–160.

SCOTT, D., 1986 Sexual mimcry regulates the attractiveness of mated *Drosophila melanogaster* females. Proc. Natl. Acad. Sci. *USA* **83:** 8429–8433.

SCOTT, D., and L. L. JACKSON, 1988 Interstrain comparison of male-predominant antiaphrodisiacs in *Drosophila* melanogaster. J. Insect Physiol. In press.

SCOTT, D., and R. C. RICHMOND, 1987 Evidence against an antiaphrodisiac role for cis-vaccenyl acetate in *Drosophila melanogaster*. J. Insect Physiol. **33:** 363–369.

SCOTT, D., R. C. RICHMOND and D. A. CARLSON, 1988 Pheromones exchanged during mating: a mechanism for mate assessment in *Drosophila*. Anim. Behav. **36:** 1164–1173.

SNEDECOR, C. W., and W. G. COCHRAN, 1980 *Statistical Methods*, Ed. 7. Iowa State Press, Ames, Iowa.

SOKAL, R. R., and F. J. ROHLF, 1981 *Biometry*, Ed. 2. Freeman, San Francisco.

TOMPKINS, L., A. GROSS, J. C. HALL, D. A. GAILEY and R. W. SIEGEL, 1982 The role of female movement in the sexual behavior of *Drosophila melanogaster*. Behav. Genet. **12:** 295–307.

VAN DEN BERG, M. J., G. THOMAS, M. HENDRICKS and W. VAN DELDEN, 1984 A reexamination of the negative assortative mating phenomenon and its underlying mechanisms in *Drosophila melanogaster*. Behav. Genet. **14:** 45–61.

VON SCHILCHER, F., 1976a The role of auditory stimuli in the courtship of *Drosophila melanogaster*. Anim. Behav. **24:** 18–26.

VON SCHILCHER, F., 1976b The function of sine song and pulse song in the courtship of *Drosophila melanogaster*. Anim. Behav. **24:** 622–625.

Communicating editor: A. T. C. CARPENTER