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COMPARED BEHAVIORAL RESPONSES OF MALE *Drosophila melanogaster* (Canton S) TO NATURAL AND SYNTHETIC APHRODISIACS

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Abstract—Cuticular aphrodisiacs from *D. melanogaster* females were further characterized and the male response specificity towards such natural and synthetic unsaturated hydrocarbons was investigated. The behavioral activity seems to be correlated with some chain-length requirement and double-bond position; at least one double bond in position 7 seems necessary. This position is more abundant among natural monoenes, and among dienes which also bear a second double bond in position 11, whatever the chain length. Bioassays of the synthetic (Z,Z)-7,11-heptacosadiene yielded a dose–response curve close to that of the natural mixture of heptacosadienes in which the 7-11 isomer is predominant. This female specific 7,11 heptacosadiene appears to be the most potent aphrodisiac for males of the species. Its threshold is lower than that of both 7,11-nonacosadiene and 7-pentacosene which might also play a role in sex and species recognition.

Key Words—*Drosophila melanogaster*, *Diptera*, *Drosophilidae*, aphrodisiac pheromone, recognition pattern, cuticular hydrocarbons, *heptacosadienes*, mass spectrometry.

INTRODUCTION

Chemical communication is a common means among insects to control sequences of precopulatory behavior and orient them towards a partner of the appropriate sex and species. In this respect, hydrocarbons from the cuticle of many insects have been found to be behaviorally active (Howard and Blomquist,

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1982). In *Drosophila melanogaster*, the existence of behaviorally active substances has been suggested for some years by various authors (for a review, see Ehrman, 1978). In recent work, female compounds were shown to induce male precopulatory behavior in the strain Canton S (Venard and Jallon, 1980; Tompkins et al., 1980).

The isolation of female active compounds required a quantitative behavioral test. For this, Jallon and Hotta (1979) supposed that the intensity of male courtship reflected the intensity of stimuli produced by females. They chose to measure the average value of cumulative time of characteristic wing vibrations in a number of standardized males. This measurement was used by Antony and Jallon (1982) in bioassays designed to test candidate compounds from fly extracts obtained by cuticular washes of mature females (3–4 days) with hexane. Bioassays demonstrated that the induction of male wing vibrations was associated with female cuticular compounds but not with male ones. Such extracts were then analyzed with gas–liquid chromatography coupled with mass spectrometry (GC-MS) after partial purification by a combination of thin-layer chromatography and preparative gas chromatography. Active compounds were found to be long-chain unsaturated hydrocarbons (25–29 carbons), either dienes or monoenes. Sexual dimorphism was clear, as only female *D. melanogaster* produced dienes. It was shown that for a dose equivalent to that borne by a single female, only the heptacosadiene fraction was behaviorally active (Antony and Jallon, 1982).

The data reported here deal with the characterization of unsaturated hydrocarbons from females and an analysis of the male response towards natural and synthetic compounds.

MATERIALS AND METHODS

Wild-type Canton S strain flies were bred at 25°C with a 12/12 dark–light cycle. Sexes were separated a few hours after eclosion under a light ether anesthesia. Males and females were held separately until maturity.

Cuticular hydrocarbons were obtained from extracts resulting from cuticular washes of flies of given sexes and ages. Macroextracts were obtained with 250 flies bathed in 1.5 ml hexane for ca. 2 min and shaken for 1 min in a vortex. Microextracts were obtained by submerging a single fly in 50 μl hexane for 2 min and shaking for 1 min. Both extracts were the same as those described by Antony and Jallon (1982). Purified fractions were quantified using standard commercial alkanes.

For bioassays, a given amount of hydrocarbon was deposited with a microcap on a "dummy" male consisting of a freshly killed male thoroughly washed with hexane. Hexane was evaporated for 2 min, and the dummy was introduced into an observation chamber together with a test male. Cumulated wing vibration times of test males ($\overline{\Sigma T}_N$) were measured for 5 min as described by Jallon and Hotta (1979).

For ozonolysis, ozone was allowed to flow 1 min into hexane solutions of unsaturated dienes or monoenes separated according to their chain lengths. The ozonides were injected directly into the GC to release aldehydes. The chain lengths of the resulting aldehydes were determined according to their comigration in gas chromatrography (GC) with standard aldehydes or by coupled gas chromatography-mass spectrometry (GC-MS).

For methoxymercuration–demercuration, the procedure used was the same as that one previously described by Pechine et al. (1985).

(*Z*,*Z*)-7,11-heptacosadiene and a few monoenes [(*Z*)-7-tricosene, (*Z*)-7-pentacosene, (*Z*)-9-tricosene, (*Z*) 9-pentacosene, and (*Z*)-9-heptacosene] were synthesized (Davis and Carlson, manuscript in preparation; Carlson et. al., 1974). The synthetic (*Z*,*Z*)-7,11-heptacosadiene contained 2.5% of the geometric isomers *Z*,*E* and *E*,*Z*, as measured by gas chromatography.

RESULTS

Further Characterization of Female Cuticular Unsaturated Hydrocarbons. Antony and Jallon (1982) described hydrocarbons present in the cuticles of fe-

TABLE 1. AMOUNTS OF UNSATURATED CUTICULAR HYDROCARBONS BORNE BY MATURE FEMALE AND MALE *D. melanogaster* CANTON S STRAIN INDIVIDUALS (4 DAYS OLD, IN ng/fly)[a]

			Females (30)	Males (30)
23C	diene			
	monoenes	A	±	±
		B	52.5 (±7.5)	423 (±7.8)
		C		24.8 (±2.6)
25 C	diene		36 (±4.5)	
	monoenes	A	106.5 (±12)	±
		B	108 (±9)	96 (±11.5)
		C	7.5 (±1.5)	
27 C	diene		420 (±25.5)	
	monoenes	A		
		B	70.5 (±9)	±
		C		
29 C	diene		192 (±19.5)	
	monoenes	A		
		B		±
		C		

[a] The sign ± represents trace.

male (26 components) and male (15 components) *D. melanogaster* Canton S. They also presented quantification of extracts of large pools of flies 3–5 days old. Jallon (1984) recently compared the unsaturated components of individual mature 4-day-old flies of both sexes (Table 1). Although a few small quantitative differences concerning major compounds in either sex were apparent in individuals as compared with pools, the clear sexual dimorphism of cuticular hydrocarbons was confirmed. It appeared that interindividual variability is relatively low, always less then 15%. Some of the minor components evidenced in pools were not seen in these microextracts, but the major component of female cuticles, absent in male cuticles, was the same heptacosadiene; its GC-MS spectrum is shown in Figure 1A.

Several isomers for female monoenes and dienes were shown by GC-MS analysis. The set of individual chromatograms clearly showed several peaks for tricosene, pentacosene, and heptacosene and one peak for pentacosadiene, heptacosadiene, and nonacosadiene. It was then necessary to try to determine the positions of double bonds in these isomers. These positions were determined using two chemical methods, ozonolysis and methoxymercuration–demercuration, followed by the analysis of resulting products with GC and GC-MS. Using the latter method to study the monoenes of *D. melanogaster* males and *D. simulans* males and females, Pechine et al. (1985) have shown that it is not necessary to separate the various monoenes and dienes.

For female monoenes, methoxymercuration–demercuration of total extracts followed by a GC-MS analysis with chemical ionization showed clearly that at least two positional isomers were present at each chain length of 23, 25, and 27 carbons. A quantitative estimate for isomers 7-8 (B) and 9-10 (A) deduced from ratios of mass fragmentation peak heights is shown in Table 2 and is compared with data calculated directly from GC peak areas. The two pentacosenes are present in similar amounts, whereas 7-tricosene and 7-heptacosene are very dominant. The ozonolysis of isolated mixtures of monoenes with either 23, 25, and 27 carbons yielded fragments which comigrated by GC with aldehydes comprising chain lengths with 14/16, 16/18, and 18/20 carbons, respectively. The main aldehydes always corresponded to position 7 (isomer B), but position 9 was also present (isomer A) in lower amounts, as already described in male hydrocarbons. Both methods suggest the existence of small amounts of other positional isomers, probably at carbons 5–11.

The determination of double bonds in dienes was performed with similar chemical methods. Methoxymercuration–demercuration led to dimethoxy dienes which, when analyzed by GC-MS, could be interpreted more easily than those of monomethoxy derivatives. An example is shown for heptacosadienes (Figure 1B). Four possible dimethoxy derivatives may be formed, depending on the position of the methoxy groups. These can be on either carbon supporting the double bond, leading to 8 expected cleavages (aa′ to dd′, Figure 1B). Di-

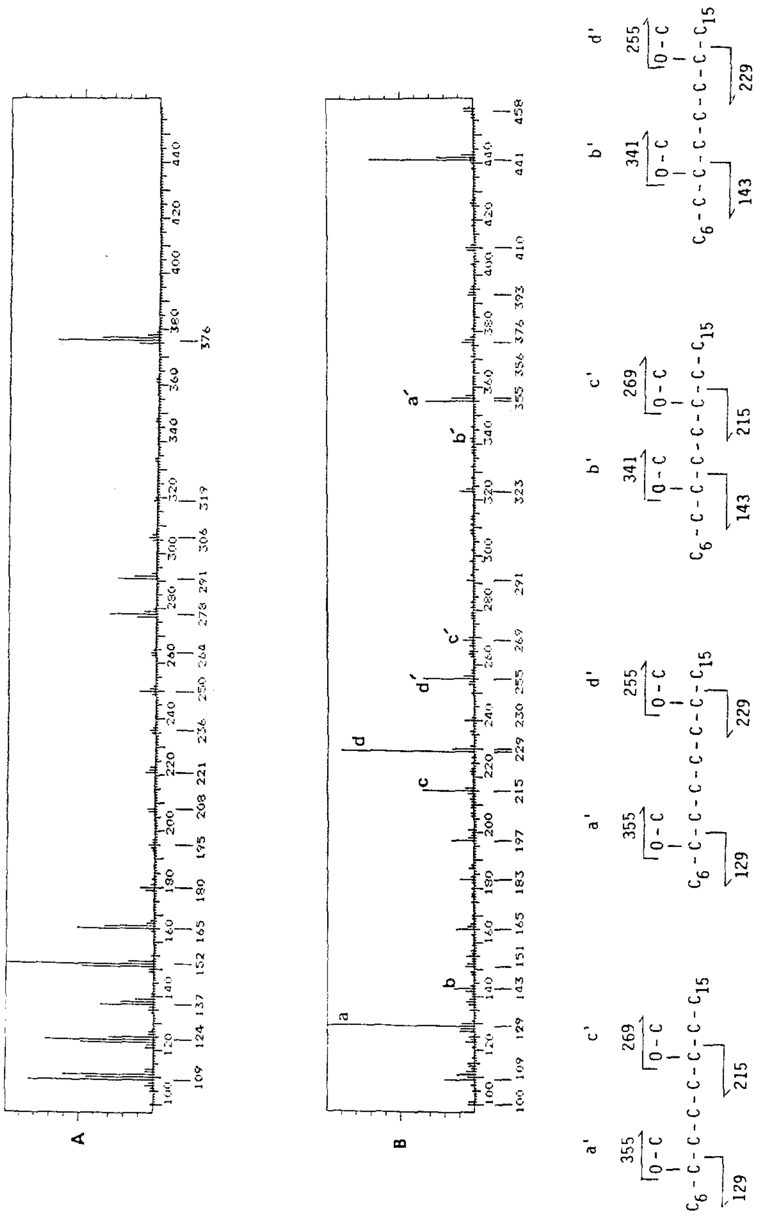

FIG. 1: Mass spectra of the heptacosadiene fraction of female *D. melanogaster*. (A) Natural, chemical ionization with methane. (B) Dimethoxy derivatives, chemical ionization with ammonia.

TABLE 2. DOUBLE-BOND POSITIONS FOR MONOENE ISOMERS (A, B) AND DIENES DETERMINED BY METHOXYMERCURATION–DEMERCURATION AND OZONOLYSIS[a]

		Position of double bonds	GC areas (%)	Methoxymercuration–demercuration (%)	Ozonolysis
Monoenes					
23 C	A	9	10	10	minor
	B	7	90	90	major
25 C	A	9	45	40	minor
	B	7	55	60	major
27 C	A	9	26	15	minor
	B	7	74	85	major
Dienes					
25 C		7,11			
27 C		7,11			
29 C		7,11			

[a] The relative abundances of the various positional isomers are indicated either as percentages or levels (number of crosses).

methoxy derivatives had OCH_3 groups preferentially located on the C-27 diene backbone as far from each other as possible. The fragmentation pattern peaks aa′ and bb′ are clearly associated with fragmentations adjacent to carbons 7 and 8, respectively; peaks cc′ and dd′ correspond to fragmentation adjacent to carbons 11 and 12. Ozonolysis of isolated dienes with 25, 27, and 29 carbons always led to the following major fragments: a-aldehydes with 14, 16, and 18 carbons were found for hydrocarbons with, respectively, 25, 27, and 29 carbons, indicating a common position 11-12 for one of the double bonds; b-heptanal was obtained in all chain lengths, suggesting that the other double bond was always present in position 7-8. In summary, position 7 for double bonds predominated in monoenes whereas the 7,11 isomers predominated in dienes. Both methods suggest that other positional isomers of dienes are present, although in much smaller amounts which might affect carbons 5/13, mainly 5,9 and 9,13. In the GC-MS spectrum of nonmodified heptacosadienes shown in Figure 1A, peaks 165 and 291 correspond to breakings of the bonds adjacent to double bonds. The major peaks 278 and 152 were probably obtained from a complex rearrangement, although its result is equivalent to a simple breaking of the double bonds.

Quantitative Variations of Male Behavioral Responses as Function of Female Hydrocarbon Quantities. Earlier work has shown that the specificity of male responses appeared rather broad: although for a dose equivalent to that borne by a single female only heptacosadiene yielded long responses, important responses could be induced by higher doses of various female unsaturates but not by female saturates. It was thus necessary to consider this problem in more detail. To start such a study, we used the natural compounds present in female cuticle. We isolated in large amounts four diene fractions and four monoene fractions of known chain lengths (23–29 C) using a combination of thin-layer chromatographies and preparative GC. To test the specificity of male responses, each fraction was bioassayed using the procedure described above, for a range of doses close to those in vivo between 0 and 500 ng, and for higher doses to 2000 ng. The variations of the average cumulative vibration times induced in test males ($\overline{\Sigma T_N}$) with the amounts of hydrocarbons used in the bioassay (q) are shown in Figure 2.

A few obvious conclusions can be drawn which are valid, even if one takes into account the variability observed in measured behavioral responses. As far as dienes are concerned (Figure 2A), whatever the value of q, tricosadienes produce only very low responses. The three other dienes with chain lengths of 25/29 carbons yield classical dose–response curves with evidence for (1) a

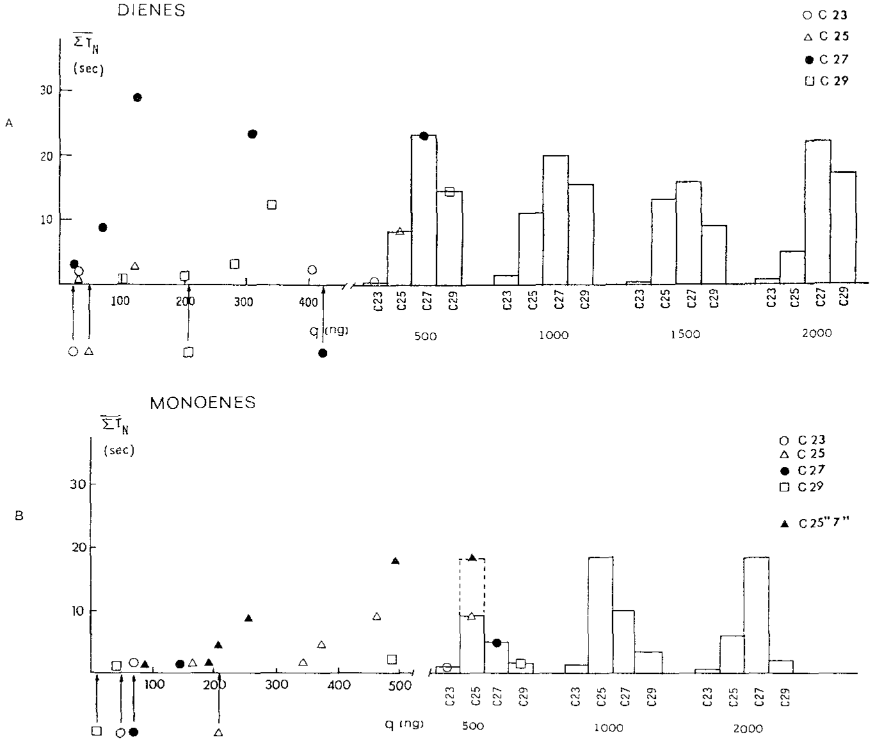

FIG. 2: Variations of induced vibration responses ($\overline{\Sigma T_N}$) of Canton S tester males with various amounts of natural hydrocarbon fractions (q). (A) Dienes; (B) monoenes: for pentacosenes, the curve is also represented for 7-pentacosene alone (▲). Arrows indicate doses borne by single females for each compound.

threshold, (2) an increase of $\overline{\Sigma T_N}$ values with increasing doses, and (3) a plateau which shows a saturation effect. For 100 ng of heptacosadienes, the plateau value is already reached ($\overline{\Sigma T_N} = 21 \pm 3$ sec), while values induced by comparable amounts of either pentacosadienes and nonacosadienes remain very low. It thus seems that the response threshold for heptacosadienes is much lower than those for both other dienes; for these dienes the corresponding plateau values $\overline{\Sigma T_N}$ seem also smaller: 10 ± 4 for pentacosadienes and 14 ± 3 for nonacosadienes. Figure 2B shows dose–response curves observed for monoenes; whatever the quantity, tricosenes and nonacosenes only produce low responses. Pentacosenes and heptacosenes lead to higher responses but the threshold for pentacosenes seems lower than that of heptacosenes and higher than that of heptacosadienes.

The study of the specificity of male responses shows that considering the in vivo quantities for each type of compound from the female cuticle (also shown in Figure 2A and B), only the heptacosadiene fraction is present at a level (420 ± 25 ng) above the threshold and at a level for which saturation is reached. This fraction seems to correspond to the most efficient chemical signal which triggers male courtship.

The diene and monoene fractions which have a high activity consist of more or less complex mixtures of isomers (see Tables 1 and 2). For C-25 to C-29 dienes, the isomer with double bonds in positions 7 and 11 is by far the most abundant. For monoenes, isomer 7 is predominant among heptacosenes but only slightly more abundant than isomer 9 in the pentacosene fraction. As the preparative isolation of such isomers is difficult, it was easier to use synthetic isomers.

Comparative Study of Natural and Synthetic 7,11-heptacosadienes. Synthetic (Z,Z)-7,11-heptacosadiene was used in dose–response bioassays as previously to measure induced vibrations of tester males. Up to 100 ng, no sustained vibration was observed. Above 100 ng, the vibration increased towards a plateau ($\overline{\Sigma T_N} = 16 \pm 3$ sec) (Figure 3A). Synthetic (Z,Z)-7,11-heptacosadiene which comigrates in GC with the main natural heptacosadiene isomer is thus behaviorally active. If one compares dose–response curves of both natural and synthetic heptacosadienes consisting of a predominant molecular species with small amounts of isomers, one observes that the synthetic mixture has an efficiency slightly less than the natural one between 300 and 2000 ng, although this difference is not significant. For tested amounts above 500 ng, responses obtained with the natural (A) or synthetic (B) hydrocarbons (Figure 3) show no significant differences [$P(t) > 0.05$]. For smaller doses, the difference is more important: it looks as if the response threshold for the synthetic compound might be higher than that of the natural compound.

We also tested several synthetic monoenes: (Z)-7- and (Z)-9-tricosene, (Z)-7- and (Z)-9-pentacosene, and (Z)-9-heptacosene. No synthetic tricosene induced sustained vibrations of tester males even for a dose of 2000 ng. This is

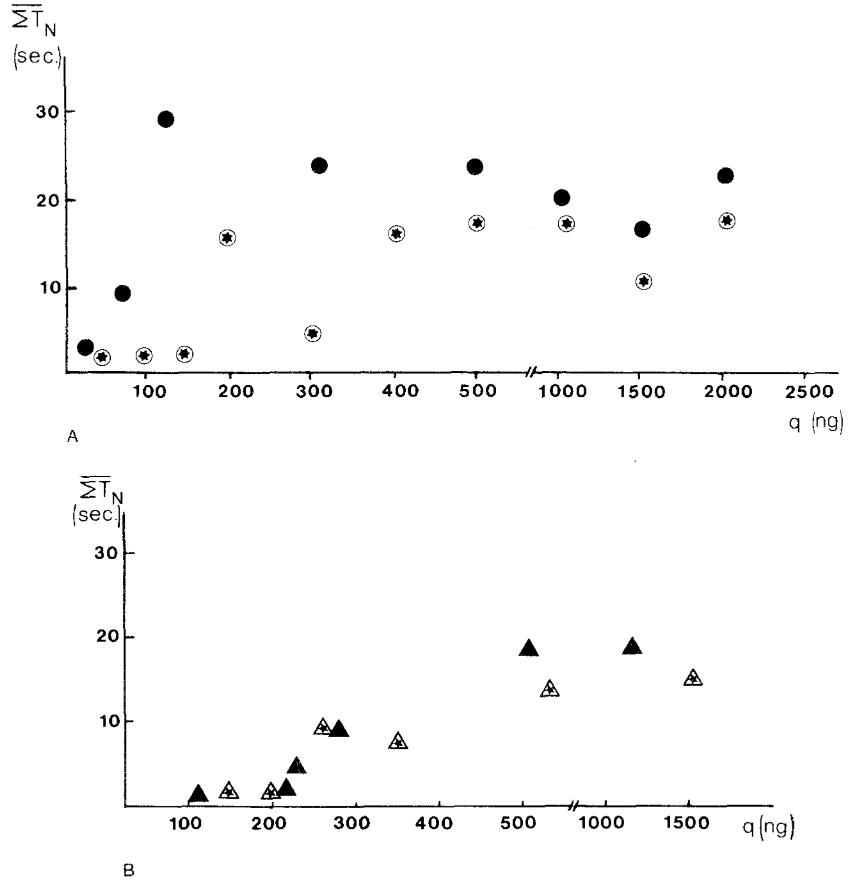

FIG. 3: Dose–response curves of the induced vibration responses ($\overline{\Sigma T_N}$) of Canton S tester males to natural and synthetic compounds as a function of the tested amount (A) heptacosadienes: ● natural 7,11-heptacosadiene; ⊛ synthetic (*Z,Z*)-7,11-heptacosadiene. (B) 7-Pentacosene: ▲ natural; ⟁ synthetic (*Z*)-7-pentacosene.

in agreement with the weak activity of the natural mixture of tricosenes. None of the (*Z*)-9-monoenes exhibited significant activity, but synthetic (*Z*)-7-pentacosene induced the dose-dependent responses shown in Figure 3B in comparison with those of the mixture of natural pentacosenes. If the latter curve is corrected for doses of 7-pentacosene present in the mixture, the obtained curve appears very similar to that corresponding to the synthetic (*Z*)-7-pentacosene.

DISCUSSION

Cuticles of female *D. melanogaster* Canton S include several unsaturated long-chain hydrocarbons which have been shown to be potentially aphrodisiac

(Antony and Jallon, 1982). These compounds were fractionated according to their chain length and degree of unsaturation, and dose–response variations of induced wing vibrations of tester males were established for each. Only the heptacosadiene fraction was present in the cuticle at a dose higher than its estimated aphrodisiac threshold. 7,11-heptacosadiene was the main constituent, but it could not be separated from small amounts (ca. 3%) of other positional isomers. Synthetic 7,11-heptacosadienes had a behavioral activity not very different from that of the natural mixture of heptacosadienes but a small difference in estimated thresholds was observed. The synthetic product contained ca. 97.5% of (*Z*,*Z*)-7,11-heptacosadiene and 2.5% of two geometric isomers. It is thus clear that 7,11-heptacosadiene is a potent aphrodisiac for *D. melanogaster* Canton S males. It is suggested that 7,11-heptacosadiene with a *Z*,*Z* configuration is both the most active compound for males and the most abundant compound in female cuticle, but it is also possible that positional isomers also present in the natural fraction might synergize its activity. Such an hypothesis is supported by the use of a number of *D. melanogaster* mutant lines whose females have different amounts of the same 7,11-heptacosadiene isomer measured by GC. When presented to Canton S tester males, such living females induce varying levels of wing vibrations. Figure 4 shows that the intensity of male responses is positively correlated with the average dose of heptacosadiene borne by each type of female. The level of those responses are higher than those obtained in bioassays. The organization of the reconstituted hydrocarbon layer may not be identical in the bioassay situation with that of the native cuticle. Moreover, moving females are known to be more stimulatory than immobilized females (Tompkins et al., 1982) and, consequently, more than our inanimate dummies.

Other unsaturated hydrocarbons which were shown to be potential aphrodisiacs when used at high doses were present in Canton S female cuticles at doses lower than the estimated thresholds for their aphrodisiac effects. However, it is necessary to state that absolute threshold values might have been overestimated because of the bioassay itself and that aphrodisiac effects of each isolated compound might be additive or synergistic. Nevertheless, the specificity of *D. melanogaster* Canton S male responses for two structural parameters could be partially characterized.

In all behaviorally active dienes, the 7,11 isomer was predominant. Large amounts of 9-alkenes were present with the major 7 isomers in behaviorally active monoene fractions. As synthetic 9-monoenes with 23/27 carbons were not shown to induce much response, it is suggested that the more active natural monoenes have a double bond in position 7 as do the active dienes. It appears that the presence of a second double bond in position 11 enhances the biological activity of the compound.

Dienes (7,11) with 25/29 carbons and monoenes (7) with 25/27 carbons were found to have significant behavioral activity. Among dienes, heptacosadienes were optimum; among monoenes, pentacosenes were optimum. This

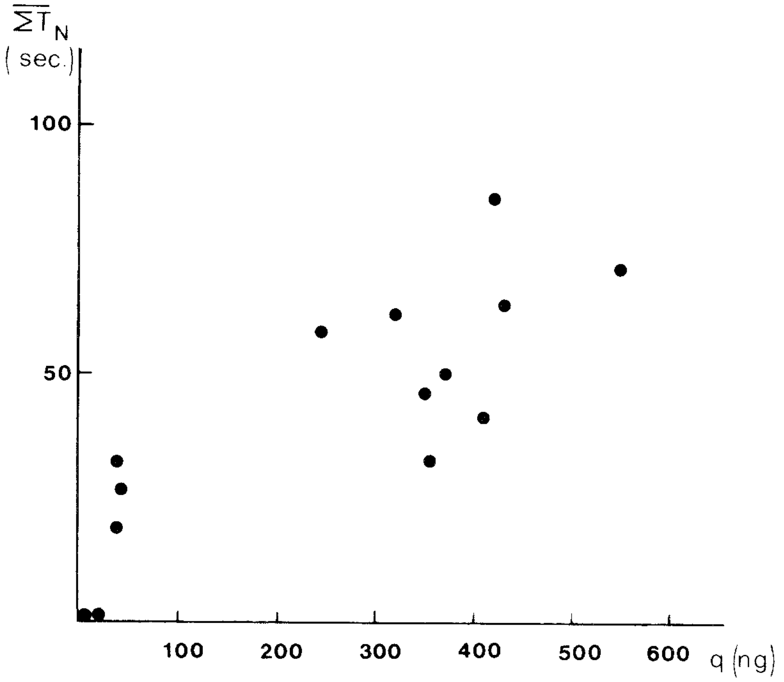

FIG. 4: Vibration responses ($\overline{\Sigma T_N}$) of tester males induced by various amounts of 7,11-heptacosadiene borne by various female individuals of different wild-type strains and mutants. The tested flies were live females from the following strains: wild types, Canton S and Tai Y. The mutant strains were either female sterile mutants: Fs(1) ovo^{D1}, or agametic mutant par ecd^{1ts}; or alleles of transformer 2: tra 2^{ts} bred at 16°C (tra/tra*, tra/tra^{+}*), bred at 18°C (tra/tra*, tra/tra**), or bred at 29°C (tra/tra**, tra/tra**); tra 2 non^{ts} allele, tra/tra**, tra/tra***. * Corresponds to a female phenotype and ** to a male phenotype, but with a chromosonal female genotype.

probably corresponds to some structural requirement. Obviously there is some size constraint as tricosadiene and tricosene are inefficient. The upper size limit cannot be defined: although 29-carbon compounds are active, higher homologs were not available for testing. However, the high aphrodisiac activity of young flies of both sexes is puzzling as they have only small amounts of monoenes and dienes of 25/29 carbons, but bear complex mixtures of monoenes and dienes with 31/37 carbons (Antony, Pechine, and Jallon, unpublished results). This suggests that compounds of higher molecular weight may also be behaviorally active.

The fact that Canton S males responded to a rather broad range of unsaturated hydrocarbons suggests that the usefulness of these chemicals for sex and species recognition by flies may be limited.

Discrimination of D. melanogaster *Females and Males by Canton S Males.* Dienes were completely absent in Canton S males, but 7-pentacosene was present at a dose of about 100 ng/male, which is less than the threshold level and thus should not induce much courtship from conspecific males. This is exactly what has been experimentally observed (Hall, 1978; Jallon and Hotta, 1979). Thus the aphrodisiac molecules are sufficient to allow Canton S males to distinguish Canton S males from Canton S females. However, we have found males of the same species but belonging to other strains (Oregon K and Tai Y) which are much richer in 7-pentacosene: males of either strain have, respectively, ca. 380 and 560 ng of 7-pentacosene. Such males are courted by Canton S males when they are decapitated or etherized but not when they are active (Jallon, 1984). This strongly suggests that these males produce both an aphrodisiac and an inhibitory signal which is behaviorally controlled. It might be the release of the antiaphrodisiac vaccenylacetate which is produced by the ejaculatory bulb (Butterworth, 1969; Jallon et al., 1981) or a possible acoustic rejection wing display (Jallon and Ikeda, unpublished results).

Discrimination of D. melanogaster *Females and* D. simulans *Females by Canton S Males.* Females of all *D. melanogaster* strains which have been examined up to now contain 7,11-heptacosadiene in their cuticle, although in different amounts (Luyten, 1983). For example, Tai Y females have only 10% of the amount borne by a Canton S female; they are courted much less than Canton S females by Canton S males and mated with less. It is suggested that 7,11-heptacosadiene is a common aphrodisiac for all strains of *D. melanogaster*, although there is variability in both its production level by females and its detection characteristics by males (Jallon, 1984).

7,11-heptacosadiene and other dienes are present in only trace quantities in males and females of the sibling and sympatric species *D. simulans* (Jallon, 1984); a number of strains have been studied in this respect (Luyten, 1982). While 7-pentacosene, another potential aphrodisiac for *D. melanogaster* Canton S males, is not abundant in *D. simulans* females of most strains (e.g., Seychelles 100 ng/female), it is very abundant in a few. For example, *D. simulans* Yaounde females contain 780 ng, which is higher than the saturation level for male *D. melanogaster* Canton S. Indeed Canton S males actively court them (Jallon et al., 1982). However, neither any *D. simulans* Yaounde female nor any *D. simulans* Seychelles female copulated with a Canton S male in 30 min (Jallon, 1984). Although Canton S males displayed vigorous wing vibrations towards *D. simulans* Yaounde females, they were unable to achieve copulation: either 7-pentacosene is not an inducer for copulation, or they are rejected by the *D. simulans* females, which do not recognize aspects of their species-specific courtship song and behavior.

Such interactions are forced in the laboratory and may never take place in the field. However, they reveal that 7,11-heptacosadiene is a sex-specific and species-specific chemical signal in all strains of *D. melanogaster*. However, chemical signals are part of a complex communication system also involving acoustic and visual signals which might be complementary and thus produce a nonambiguous sex and species recognition.

REFERENCES

ANTONY, C. and JALLON, J.-M. 1982. The chemical basis for sex recognition in *Drosophila melanogaster. J. Insect Physiol.* 28:873–880.

BUTTERWORTH, F.M. 1969. Lipids of *Drosophila*: A newly detected lipid in the male. *Science* 163:1356–1357.

CARLSON, D.A., DOOLITTLE, R.E., BEROZA, M., ROGOFF, W.W., and GRETZ, G. 1974. Muscalure and related compounds. I Response of house flies in olfactometer and pseudo-fly tests. *J. Agric. Food. Chem.* 22:194–197.

EHRMAN, L. 1978. Sexual behavior, pp. 127–180, *in* M. Ashburner & T.R.F. Wright, (eds.). The Genetics and Biology of *Drosophila*, vol. 2b. Academic Press, New York.

HALL, J.C. 1978. Courtship among males due to male sterile mutation in *Drosophila melanogaster. Behav. Genet.* 8:125–141.

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

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

JALLON, J.-M., and HOTTA, Y. 1979. Genetic and behavioral studies of female sex-appeal in *Drosophila. Behav. Genet.* 8:487–502.

JALLON, J.-M., ANTONY, C., and BENAMAR, O. 1981. Un anti-aphrodisiaque produit par les mâles de Drosophile et transféré aux femelles lors de la copulation. *C.R. Acad. Sci. Paris* 292:1147–1149.

JALLON, J.-M., BENAMAR, O., LUYTEN, I., and ANTONY, C. 1982. Modulations de la production des hydrocarbures cuticulaires aphrodisiaques des Drosophiles résultant de perturbations génétiques et physiologiques. *Colloq.* INRA 7:297–302.

LUYTEN, I. 1982. Variations intraspécifiques et interspécifiques des hydrocarbures cuticulaires chez *Drosophila simulans. C.R. Acad. Sci. Paris* 295:723–736.

LUYTEN, I. 1983. Variations intra- et interspécifiques des hydrocarbures cuticulaires et des interactions comportementales chez quatre espèces affines du sous-groupe melanogaster. Thèse de spécialité, Université de Paris VII.

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

TOMPKINS, L., HALL, J.C., and HALL, L. 1980. Courtship-stimulating volatile compounds from normal and mutant *Drosophila. J. Insect Physiol.* 26:689–697.

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

VENARD, R., and JALLON, J.-M. 1980. Evidence for an aphrodisiac pheromone of female *Drosophila. Experientia* 36:211–212.