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Genetic control of male cuticular hydrocarbons in *Drosophila melanogaster*

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Summary

7-tricosene (7-T) and 7-pentacosene (7-P) are the two main hydrocarbons on the cuticle of male *Drosophila melanogaster*. These two substances might play a pheromonal role during courtship behaviour. We investigated the genetic basis of the quantitative polymorphism observed in the production of 7-T and 7-P. Strains of different geographic origin, with males producing either predominantly 7-T or predominantly 7-P, were hybridized with strains carrying genetic markers. We found that chromosome II changes the balance between 7-T and 7-P while chromosome III regulates the overall quantity of both 7-monoenes. We have also characterized and roughly mapped *sept* and *smoq*, two genetic factors on chromosome II that act additively on the production of both cuticular hydrocarbons. The genetic control of the variation in 7-T and 7-P varies between *D. melanogaster* strains and between *D. melanogaster* and its sibling species *D. simulans*. The possible evolutionary and physiological causes of this variation as well as its functional implication for courtship behaviour are discussed.

1. Introduction

Insect cuticular hydrocarbons have become a subject of increasing interest over recent years because of their role in speciation and evolution (Coyne *et al.* 1994; Scott, 1994; Cobb & Ferveur 1996*a*). As well as playing a fundamental role in limiting desiccation (Edney, 1977), many of these substances act as pheromones, being species- or sex-specific (Shorey & Bartell, 1970; Carlson *et al.* 1971; Howard & Blomquist, 1982).

In *Drosophila melanogaster* there is a qualitative sexual dimorphism, with females of most wild-type strains producing mainly 7,11-heptacosadiene (7,11-HD, 27C) which is the principal female pheromone, inducing male courtship in this species (Antony & Jallon, 1982; Ferveur *et al.* 1996). Male-predominant cuticular hydrocarbons show a strong clinal polymorphism, with 7-tricosene (7-T, 23C), predominant in males from temperate regions, being gradually replaced by 7-pentacosene (7-P, 25C) in males from tropical and equatorial strains (J.-M. Jallon, unpublished data). The biosynthesis of 7-T and 7-P is thought to be closely linked (Jallon, 1984; Ferveur *et al.* 1989; Ferveur, 1991), perhaps indicating a relatively simple genetic control.

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Scott & Richmond (1988) carried out a set of crosses between two geographical strains of *D. melanogaster* and suggested that quantitative variation of male 7-T and 7-P levels is controlled by loci from all chromosomes. However, because they did not use markers or balancer chromosomes, they were not able to provide any more detailed information. Using EMS mutagenesis, we have discovered a locus on chromosome III, *nerd*, which substantially decreases the production of 7-T without altering that of 7-P (Ferveur & Jallon, 1993*b*). This mutation also affects male mating success, perhaps indicating that 7-T plays a pheromonal role in stimulating *D. melanogaster* females, as suggested by Jallon (1984). Further evidence for this hypothesis has been provided by Scott (1994), although this interpretation is controversial (Cobb & Ferveur, 1996*b*). 7-P, which is present in both sexes, seems to elicit male courtship behaviour, at least in the Canton-S strain (Antony *et al.* 1985).

More detailed data on the genetic control of 7-T and 7-P levels exist for the sibling species *D. simulans*. In this species there is no qualitative cuticular hydrocarbon sexual dimorphism, and flies of both sexes from most strains carry high levels of 7-T and relatively low levels of 7-P (Jallon, 1984). 7-T acts as a sex pheromone in this species, despite the absence of any sexual dimorphism (Jallon, 1984). 7-P may also be

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a sex pheromone in *D. simulans*, although the evidence is weak (Cobb & Jallon, 1990). A few *D. simulans* strains from around the Gulf of Benin in Africa show low levels of 7-T and high levels of 7-P (Luyten, 1982). We used the existence of *D. simulans* strains showing different levels of 7-T and 7-P to characterize a gene, *Ngbo* (II, 65.3), which exerts a major control over the ratio of 7-T to 7-P in both males and females of this species (Ferveur, 1991). EMS mutagenesis also revealed the existence of another gene, *kété* (I, 18.5), which decreases the production of 7-T by 70% in *D. simulans* flies of both sexes (Ferveur & Jallon, 1993*a*).

Given the fact that *D. melanogaster* and *D. simulans* are relatively closely related, and that certain loci appear to be shared, it is at least possible that the similar cuticular hydrocarbon phenotypes in the two species, in particular 7-T and 7-P levels, are under similar genetic control. In order to investigate this possibility, we hybridized five *D. melanogaster* strains showing different male cuticular hydrocarbon profiles and carried out a preliminary localization of genetic factors implicated in the levels of 7-T and 7-P in males of this species.

2. Methods

(i) Drosophila melanogaster *strains and crosses*

Taï: collected from the Ivory Coast in 1981 (Jallon & David, 1987); 7-P rich males.

Brazzaville: collected from the Congo in 1983 (Ferveur *et al.* 1996); 7-P rich males.

B15: collected from Canada in 1982 (Sokolowski, 1985); 7-T rich males.

AS: a multi-marked strain carrying six recessive markers on chromosome II: *aristaless* (*al*, 0.1), *nubbin* (*nub*, 47.0), *light* (*lt*, 55.0), *straw*^{2} (*stw*, 55.1), *scabrous*^{2} (*sca*, 66.7) and *speck*^{2} (*sp*, 107.0) (Lindsley & Zimm, 1992); 7-T rich males.

L: a double-balancer strain (SM1,*Cy*/*Pm*;TM2, *Ubx*/*Sb*) (Lindsley & Zimm, 1992); 7-T rich males.

Substitution of chromosomes II and III was carried out according to the protocol of Bauer & Sokolowski (1985): flies from each parental strain, either Taï (T) or B15 (B), were crossed to flies from the L (double-balancer) strain. F_1 flies were backcrossed to L flies. Sibling F_2 flies carrying the same single parental autosome (from either strain, and on either chromosome II or III) were bred. F_3 flies with isogenic chromosome II from B strain were crossed with F_3 flies carrying an isogenic chromosome III from T strain, and reciprocally. After two generations (F_5) flies have isogenic autosomes from both parental strains (B/T or T/B).

All strains were reared on yeast–cornmeal agar *Drosophila* medium and kept at 25 °C on a 12:12 h light:dark cycle. In each cross, we took four or five pairs of flies.

(ii) *Extraction and analysis of cuticular hydrocarbons*

Cuticular hydrocarbons were extracted from 4-day-old individual male flies according to the method described by Ferveur (1991) and analysed by gas chromatography. Thirteen hydrocarbons were systematically detected in male flies. Each compound was measured in terms of its absolute quantity, relative to an internal hexacosane standard, and in terms of its proportion of the sum of all 13 hydrocarbons. The percentages of 7-tricosene (% 7-T) and 7-pentacosene (% 7-P) were used to characterize each strain. The absolute quantities of both 7-monoenes (Q7-T and Q7-P) showed more instrastrain variation, over time, than the hydrocarbon percentages. Absolute quantities were therefore measured only in flies which were bred simultaneously.

3. Results

(i) *Autosomal control of the production of 7-monoenes*

Males from the Taï and B15 strains show large differences in their percentages of 7-tricosene (% 7-T) and of 7-pentacosene (% 7-P) (Table 1). No significant difference was found between the reciprocal F_1 males, suggesting that the X-chromosome has no significant effect on % 7-T ($t = 0{\cdot}24$; D.F. = 47; $P > 0{\cdot}05$) and % 7-P ($t = 1{\cdot}21$; D.F. = 47; $P > 0{\cdot}05$) in this cross. The values of % 7-T and % 7-P for F_1 flies, intermediate between those of the parental strains, also suggest that there is no major dominant autosomal effect of either strain. F_5 flies with substituted chromosomes II and III show that the percentage of both 7-monoenes depend mainly on the origin of chromosome II. Males homozygous for the chromosome II B (from the B15 strain) produced much more % 7-T ($t = 73{\cdot}59$, D.F. = 56; $P < 0{\cdot}001$) and less % 7-P ($t = 19{\cdot}67$, D.F. = 56; $P < 0{\cdot}001$) than males homozygous for chromosome II T (from the Taï strain). The rest of the genetic background, notably chromosomes X and III, also seem to have a slight effect on the percentages of both 7-monoenes.

The role of the autosomes from another 7-P rich strain, Brazzaville, was tested by substituting one or two homologous chromosome(s) of this strain in flies from the double-balancer chromosome strain L, which has a 7-T rich phenotype (Table 2). Each chromosome II from Brazzaville strain (II^{BZ}) increases, in a dose-dependent manner, the % 7-P ($\approx +11\%$ for each BZ chromosome). However, the corresponding reduction of % 7-T was only significant in homozygous II^{BZ} flies ($\approx -18\%$; $t = 7.33$; D.F. = 86; $P < 0{\cdot}001$). This variation is similar to the summed variations of absolute quantities of 7-pentacosene (Q7-P) and 7-tricosene (Q7-T). Q7-P increases with the number of II^{BZ} chromosomes, whereas Q7-T is significantly reduced only in homozygous II^{BZ} flies ($t = 4{\cdot}01$;

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Table 1. *Mean ± standard error of percentages of 7-tricosene (% 7-T) and 7-pentacosene (% 7-P) in male flies of various genotypes*

Generation	Strain	Chromosome genotype X	II	III	(*n*)	% 7-T	% 7-P
F_0	Taï	T	T	T	(30)	1·3 ± 0·3	59·6 ± 1·5
F_0	B15	B	B	B	(29)	46·9 ± 1·2	10·6 ± 0·8
F_1	B15 × Taï	B	B/T	B/T	(30)	23·1 ± 1·0	27·7 ± 1·1
F_1	Taï × B15	T	B/T	B/T	(19)	22·2 ± 2·1	24·0 ± 2·1
F_0	L	L	SM1/Pm	TM2/Sb	(11)	51·5 ± 1·6	17·9 ± 1·6
F_5	TB	L	T	B	(29)	17·8 ± 0·7	41·8 ± 0·9
F_5	BT	L	B	T	(27)	46·5 ± 0·8	17·8 ± 0·7

Reciprocal F_1 flies result from female × male crosses. The L strain carried two balancer chromosomes, SM1 and TM2. Chromosomes II and III from parental strains B15 (B) and Taï (T) were substituted, after five generations, in the background of the L strain. Percentages are given relative to the sum of the 13 hydrocarbons detected on the male cuticle (for more details, see Materials and Methods).

Table 2. *Role of one and two chromosome(s) II and chromosome(s) III from the Brazzaville (BZ) strain on production of various hydrocarbons in male flies*

Generation	Strain	Chromosome genotype X	II	III	(*n*)	% 7-T	% 7-P	Q7-T (ng)	Q7-P (ng)	ΣHc (ng)
F_0	BZ	BZ	BZ/BZ	BZ/BZ	(26)	4·2 ± 0·2	52·4 ± 0·8	50 ± 3	639 ± 31	1217 ± 55
F_0	L	L	SM1/Pm	Sb/TM2	(10)	42·9 ± 1.2	15·1 ± 0·7	778 ± 54	268 ± 54	1802 ± 9
F_2	II^{BZ}/SM1	L	SM1/BZ	Sb/TM2	(16)	38·5 ± 0·9	26·5 ± 0·6	699 ± 24	486 ± 25	1823 ± 63
F_3	II^{BZ}/II^{BZ}	L	BZ/BZ	Sb/TM2	(19)	25·1 ± 0·4	37·7 ± 0·6	405 ± 17	608 ± 25	1616 ± 64
F_2	III^{BZ}/TM2	L	SM1/Pm	BZ/TM2	(15)	43·1 ± 0·9	17·6 ± 0·5	828 ± 32	337 ± 13	1917 ± 62
F_3	III^{BZ}/III^{BZ}	L	SM1/Pm	BZ/BZ	(20)	29·1 ± 0·9	21·3 ± 0·6	294 ± 18	212 ± 11	995 ± 43

Data shown are mean ± S.E. of the percentages of 7-tricosene (% 7-T) and 7-pentacosene (% 7-P) and also absolute amounts (in ng) of 7-tricosene (Q7-T), 7-pentacosene (Q7-P) and the sum of all hydrocarbons (ΣHc). The L strain carries two balancer chromosomes, SM1 and TM2, homologous to chromosomes II and III respectively (for more details see Materials and Methods). F_1 males resulting from the hybridization of L strain flies with BZ strain flies, were backcrossed to L strain females to produce flies heterozygous for one chromosome BZ (F_2). F_2 flies with the same chromosome balancer were crossed to produce progeny with two chromosomes BZ (F_3).

D.F. = 86; $P < 0{\cdot}001$). Males homozygous for II^{BZ} show a slight reduction in total hydrocarbon-production, ΣHc (−210 ng) as compared with heterozygous II^{BZ} males. This value corresponds to the sum of the variations between Q7-T (−290 ng) and Q7-P (+120 ng).

These data suggest that the II^{BZ} chromosome carries at least two different loci. A first co-dominant factor mainly increases the production of 7-P and slightly reduces that of 7-T; its effect is particularly visible in the dose-dependent variation of % 7-P. A second partially recessive factor appears to decrease the production of 7-T and thus affects ΣHc.

The Brazzaville chromosome III (III^{BZ}) has a significant effect on ΣHc and in particular on Q7-T. Males homozygous for the III^{BZ} chromosome showed extremely low levels of ΣHc (−45%) and an even greater proportional decrease in Q7-T (−62%) compared with III^{BZ} heterozygous males. This recessive factor is probably responsible for the low level of Q7-T and ΣHc observed in Brazzaville males (Table 2). The III^{BZ} chromosome also seems to segregate with a slight dose-dependent increase in % 7-P (+3% with each BZ chromosome), which might be controlled by another co-dominant factor.

(ii) *Loci on chromosome II controlling 7-T and 7-P levels*

The reciprocal genetic control of % 7-P and % 7-T was investigated by crossing the BZ strain with flies from the AS multi-marked strain. AS males show a 7-T rich hydrocarbon phenotype (48·5 ± 1·6% 7-T, 12·4 ± 0·6% 7-P), with a total quantity of hydrocarbons which is very close to that of the 7-T rich L strain (2116 ± 143 ng).

The effect of one II^{BZ} chromosome on % 7-P was clear: flies with one or no copies of the II^{BZ} chromosome were distributed around two different

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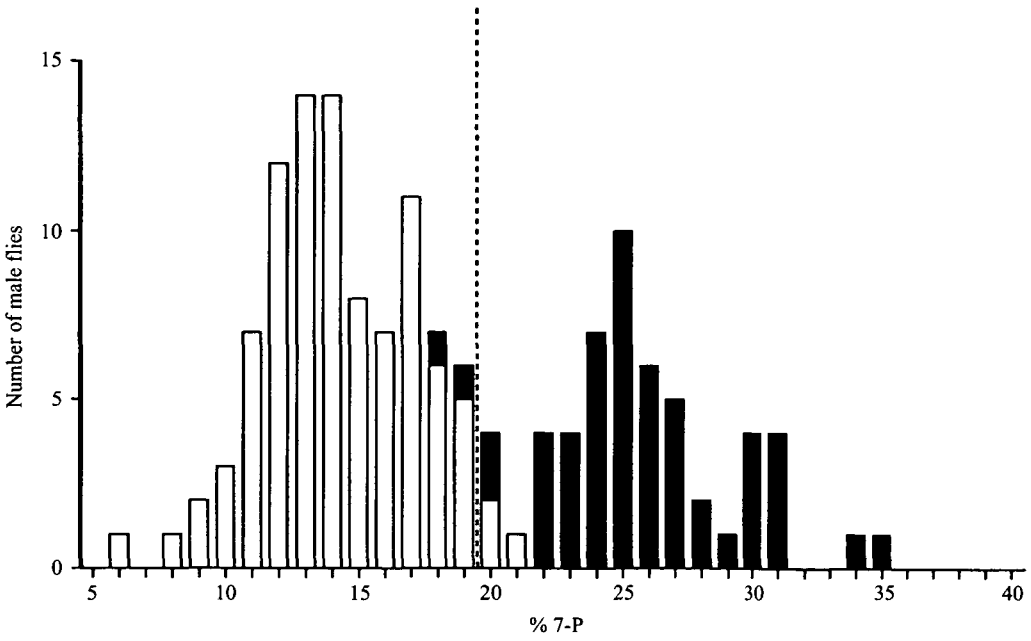

Fig. 1. Distribution of percentage 7-pentacosene (% 7-P) for males flies of various genotypes. Open columns, male flies with no chromosome II^{BZ} (F_0 *AS*+F_2 *Cy/AS;Sb/Ubx*+F_2 *Cy/Pm;AS/Ubx*+F_2 *Cy/PM;BZ/Ubx*+F_3 *Cy/Pm;AS/AS*). Filled columns, males with one chromosome II^{BZ} (F_1 *AS/BZ*+F_2 *Cy/BZ;SB/Ubx*). These flies were taken from the experiment where the chromosome(s) II from the BZ and AS strains were substituted in the L strain (see Table 2). The dashed line represents the empirical cut-off point for % 7-P between flies without (< 19%) or with one (> 19%) chromosome II^{BZ}. The misclassification probability was lower than 0·015 (2/137 flies).

modes and were therefore distinguished by a heuristic cut-off point of 19% 7-P (Fig. 1). This value gives a low misclassification probability of 0·015 and was used to classify males with a potentially recombinant chromosome II (II$^{BZ/AS}$) into two phenotypes: low and high % 7-P (low < 19% < high). Chromosome II was divided into four segments, each segment delimited by the presence of adjacent markers from the AS chromosome, and the frequency of the high % 7-P phenotype was measured in recombinant males (Table 3). The BZ-like high % 7-P phenotype tended to segregate with *nub*$^+$ (from BZ strain), suggesting that the two characters are closely linked. We have called this genetic factor *sept* (*seven pentacosene*).

This character has complex effects on the absolute quantities of 7-T and 7-P. Heterozygous *sept*^{BZ}/*sept*^{AS} males produced more 7-P than homozygous *sept*^{AS}/*sept*^{AS} males (494 ± 13 ng and 395 ± 9 ng, respectively; $t = 6{\cdot}26$, D.F. = 324, $P < 0{\cdot}001$). Conversely, Q7-T was significantly higher in homozygous males than in heterozygous males (1256 ± 27 ng and 866 ± 26 ng, respectively; $t = 10{\cdot}01$, D.F. = 324, $P < 0{\cdot}001$) as was ΣHc (2654 ± 51 ng and 2120 ± 61 ng, respectively; $t = 6{\cdot}66$, D.F. = 324, $P < 0{\cdot}001$). When both *sept*$^{AS/AS}$ and *sept*$^{AS/BZ}$ genotypes were analysed separately, ΣHc was highly positively correlated with both Q7-T ($r = 0{\cdot}908$, $n = 195$ and $r = 0{\cdot}919$, $n = 131$, respectively) and Q7-P ($r = 0{\cdot}792$ and $r = 0{\cdot}906$, respectively). There was also a significant correlation between Q7-T and Q7-P, although not so high ($r = -0{\cdot}644$ and $r = -0{\cdot}780$, for *sept*$^{AS/AS}$ and *sept*$^{AS/BZ}$, respectively). This reinforces the hypothesis that other loci on chromosome II also affect the variation of Q7-T and Q7-P.

To investigate the possible existence of a second locus, the absolute levels of 7-T, 7-P and ΣHc were separately measured in homozygous *sept*^{AS} flies and in heterozygous *sept*^{AS}/*sept*^{BZ} flies. Hydrocarbon levels were compared for the four segments of chromosome II which were either homozygous marked (−/−) or heterozygous (+/−) (Table 4). In both *sept*^{AS} homozygotes and *sept*^{AS}/*sept*^{BZ} heterozygotes, higher variation of levels of Q7-T, Q7-P and ΣHc tended to segregate with *straw* (*stw*; II, 55.1). Heterozygous *stw*$^-$/*stw*$^+$ flies produce less 7-T (400–500 ng) and more 7-P (80–240 ng) than homozygous *stw*$^-$ flies. The factor associated with the *straw* marker on the II^{BZ} chromosome substantially decreased the 7-monoene production by 30% in homozygous *sept*^{AS} males and 40% in heterozygous *sept*^{AS}/*sept*^{BZ} males. We have called this factor *smoq* (small monoene quantities) because of the low quantities of hydrocarbons it produces. *sept* and *smoq* show different effects and segregate with different markers (*nub* and *stw*, respectively) but neither can yet confidently be described as a single gene.

When both factors come from the same strain (BZ or AS), they exert an additive effect on Q7-T and ΣHc and an antagonistic effect on Q7-P. This is shown by the comparison of males with all four possible combined genotypes of *nub* and *stw* (Table 5). Homozygous *nub*$^-$*stw*$^-$ males showed much higher levels of Q7-T and ΣHc than *nub*$^+$*stw*$^+$ flies, whilst the effect on Q7-P was at the limit of significance.

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Table 3. *Mapping of the* sept *locus on chromosome II in male flies*

	Markers of chromosome II					Probability of high % 7-P
n	*al*	*nub*	*stw*	*sca*	*sp*	phenotype
33	+	+	.	.	.	0·85
19	−	+	.	.	.	0·79
19	+	−	.	.	.	0·46
45	−	−	.	.	.	0·29
114	.	+	+	.	.	0·73
27	.	+	−	.	.	0·63
31	−	−	+	.	.	0·23
157	.	−	−	.	.	0·26
103	.	.	+	+	.	0·64
42	.	.	+	−	.	0·67
39	.	.	−	+	.	0·31
145	.	.	−	−	.	0·28
74	.	.	.	+	−	0·62
60	.	.	.	+	+	0·35
109	.	.	.	−	+	0·30
62	.	.	.	−	−	0·39

Recombinant flies were issued from backcrosses between F1 (AS females × BZ males) females and AS males. All chromosomal segments, delimited by two adjacent markers, were considered for all possible genotypes: +, heterozygote *BZ/AS*; −, homozygote *AS/AS*. For each genotype, the probability of segregation of the high '% 7-P phenotype' (> 19% = $sept^{AS}/sept^{BZ}$) was determined. The $sept^{BZ}$ allele (from Brazzaville strain) appears to segregate most likely with the nub$^+$ phenotype (in flies heterozygous nub^-/nub^+). The relatively high probability of a 'false positive' ($0{\cdot}23 < P < 0{\cdot}31$), e.g. nub$^-$ flies with high % 7-P, could be due to other factors of the genetic background. The comparison of probabilities of high % 7-P phenotype between the markers al$^+$ and stw$^+$ suggests that the $sept^{BZ}$ allele segregates most likely with the former marker.

Table 5. *Role of the chromosomal segment* nub–stw, *of various genotypes, in the production of male hydrocarbons*

Genotype			Q7-T	Q7-P	ΣHc
nub	*stw*	N	(ng)	(ng)	(ng)
+	+	(177)	614±25	415±12	1626±37
−	−	(176)	1323±28	457±13	2874±51
t-test			***	*	***
+	−	(27)	1115±51	488±33	2426±94
−	+	(31)	1029±47	353±18	2213±88
t-test			ns	***	ns

Flies showing different genotypes, recombining or not within the *nub–stw* chromosomal segment, were compared with a *t*-test for their production of predominant hydrocarbons. +, homozygous *AS/AS*; −, heterozygous *AS/BZ*. The significance level of the test: *** $P < 0{\cdot}001$, * $P < 0{\cdot}05$; ns, not significant. For hydrocarbon parameters, refer to Table 2.

Conversely, males recombinant within this segment of the chromosome showed highly significant differences for Q7-P and no significant differences for Q7-T and ΣHc. This result also explains why the variations between Q7-T and Q7-P are not highly correlated in recombinant flies.

4. Discussion

These results show that there is a complex genetic control of the cuticular hydrocarbon polymorphism found in male *Drosophila melanogaster*. We have discovered at least two loci on chromosome II (*sept* and *smoq*) and two unidentified loci on chromosome

Table 4. *Role of each segment of chromosome II on production of various hydrocarbons in male flies*

Segment of chromosome II						$sept^{AS}/sept^{AS}$				$sept^{AS}/sept^{BZ}$		
al	*nub*	*stw*	*sca*	*sp*	(*n*)	Q7-T	Q7-P	ΣHc	(*n*)	Q7-T	Q7-P	ΣHc
−	−	.	.	.	(53)	1392±467	421±166	2987±807	(11)	983±404	554±244	2440±941
Mean difference						334	32	733		412	206	1011
+	+	.	.	.	(10)	1058±390	389±109	2254±661	(59)	571±191	348±96	1429±409
.	−	−	.	.	(141)	1373±367	411±133	2892±671	(34)	1127±314	631±150	2800±709
Mean difference						470	89	956		475	236	1175
.	+	+	.	.	(38)	903±262	322±100	1936±507	(111)	652±220	395±120	1625±462
.	.	−	−	.	(123)	1389±373	414±135	2910±683	(39)	1132±288	613±147	2738±674
Mean difference						411	82	825		517	238	1195
.	.	+	+	.	(48)	978±260	332±97	2085±497	(91)	615±211	375±115	1543±458
.	.	.	−	−	(57)	1279±364	351±113	2574±612	(24)	845±207	486±126	2054±430
Mean difference						84	−19	87		223	108	464
.	.	.	+	+	(42)	1195±323	370±132	2487±615	(54)	622±254	378±145	1590±618

Q, quantity, in nanograms.
Flies with no or one copy of the $sept^{BZ}$ allele (Brazzaville strain) were analysed separately. For each chromosomal segment, delimited by two adjacent markers, the differences (in ng) in hydrocarbon production between the two genotypes were analysed ([homozygote for the markers]−[heterozygote for the same markers]; ex: $[al^-nub^-]-[al^+nub^+]$); +, homozygous *AS/AS*; −, heterozygous *AS/BZ*. For each measure, the remaining part of the chromosome was not controlled. For hydrocarbon parameters, refer to Table 2.

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III. Unlike Scott & Richmond (1988), we did not find an X-chromosome effect, probably because we did not use the same hydrocarbon parameters, or because our strains did not differ for these parameters, or both. In the strains tested here, the two principal autosomes exert different effects on the production of 7-tricosene (7-T) and 7-pentacosene (7-P). Chromosome II affects the balance between 7-T and 7-P while chromosome III affects the level of production of all cuticular hydrocarbons, in particular that of 7-T. The ratio of 7-T to 7-P, which enables us to discover a single genetic factor controlling this phenotype in *D. simulans* (Ferveur, 1991), gave less informative results in *D. melanogaster*, presumably because of the multiple genetic control in this species.

The detailed effects of the characters on each autosome are particularly striking. Although chromosome II clearly affects the balance between 7-T and 7-P, the phenotype is in fact relatively complex. *sept*, which segregates with *nubbin* (II, 47.0), was characterized by its effect on % 7-P. The allele from the Brazzaville strain ($sept^{BZ}$) increases Q7-P and decreases both Q7-T and the sum of all hydrocarbons (ΣHc). The second factor, *smoq*, which segregates with *straw* (II, 55.1), controls the production of 7-T, of 7-P and probably of other hydrocarbons. One copy of the Brazzaville character ($smoq^{BZ}$) reduces the production of all hydrocarbons by 30–40%. The additive effects of $sept^{BZ}$ and $smoq^{BZ}$ represent a large part of the 7-monoene variation segregating with the II^{BZ} chromosome. *sept* and *smoq* have additive effects on Q7-T and on ΣHc, but they have antagonistic effects on Q7-P.

The main effect observed for chromosome III was the control of Q7-T. Substantial inter-strain differences were found. Two copies of III^{BZ} significantly reduce % 7-T (29·1 %) and only slightly increase % 7-P (21·3 %), whereas two copies of $III^{Taï}$ do not affect % 7-T (46·5 %) and barely increase % 7-P (17·8 %)

These results suggest that the similar high 7-P : low 7-T hydrocarbon profiles found in Taï and Brazzaville males have very different genetic bases. Schematically speaking, in Brazzaville males the phenotype is produced by a decrease in % 7-T (chromosome III), an increase in % 7-P (chromosome II) and, no doubt, by a series of interactions and background effects. In Taï males, however, the principal effect (a decrease in % 7-T correlated with an increase in % 7-P) seems to segregate with chromosome II. Chromosome III, in Taï strain, seems to have a minor effect on percentages of 7-monoenes.

Similar phenotypic effects have been found in *D. simulans*, where a single gene, *Ngbo* (II, 65.3), controls the natural 7-T : 7-P polymorphism that exists between strains from around the Gulf of Benin and from elsewhere in the world. One copy of $Ngbo^{Cam}$ reduces % 7-T by 16% and increased % 7-P by 14%. This result is similar to the combined effects of *sept* and *smoq*. The effect of the Brazzaville chromosome III is similar to the hydrocarbon phenotype shown by the mutant *nerd* (Ferveur & Jallon, 1993*b*). This mutant decreases Q7-T by 48%, without significantly changing Q7-P. Another mutation, *fruitless* (III, 62.0), markedly decreases 7-T and 7-P levels (Cobb & Ferveur, 1996*a*).

One of the problems involved in comparing results from different studies is the use of different measures. For example, Scott & Richmond (1988) exclusively present data for absolute quantities of cuticular hydrocarbons, whereas we have presented both quantitative and proportional data. There are a series of reasons for this. Firstly, it should be noted that absolute levels can show substantial variations from one generation to another, primarily because they are inevitably linked to the size of the fly. Toolson & Kuper-Simbron (1989) found changes in the hydrocarbon profile of *D. pseudoobscura* flies over several generations. We have found important variations in absolute quantities, but not in composition, over time (Ferveur, 1991) and have adopted the procedure of only using absolute quantities in genetic studies where lines are bred and tested simultaneously. Secondly, although those main cuticular hydrocarbons that act as sex pheromones have clear dose-related effects, as measured in nanograms (Antony *et al.* 1985), it seems extremely likely that the overall complex of cuticular hydrocarbons takes the form of a bouquet, with different components either acting synergistically or inducing specific behavioural acts (Adams, 1986; Oguma *et al.* 1992; J.-F. Ferveur & G. Sureau, unpublished data). This concept implies that data on both proportions and absolute quantities will be necessary in order to understand functional aspects of cuticular hydrocarbon production. Finally, the two measures clearly interact. Although our data show that 7-T and 7-P are not simply inversely proportional, increasing the absolute level of one of these substances without proportionally increasing the other will inevitably alter the relative proportions of the two substances. Given that the total quantity of hydrocarbons on a given fly is finite, we need to take account of both the absolute and the relative levels of cuticular hydrocarbons, whilst remembering that the two measures are inter-related.

The phenotypic similarities and genetic dissimilarities between *D. simulans* and *D. melanogaster* and between strains of *D. melanogaster* have two important implications. Firstly, they suggest that cuticular hydrocarbon profiles are extremely rich phenotypes, and that apparently simple changes (e.g. mainly 7-T → mainly 7-P) may have complex genetic and biochemical bases. Whilst the biosynthetic schema put forward by Jallon (1984) has been supported by biochemical studies in our laboratory (Chan-Yong & Jallon, 1986; M. Pennanec'h *et al.* unpublished data), the data presented here and the results from other genetic studies (for a review, see Cobb & Ferveur 1966*a*) suggest that the corresponding genetic schema will be much more complex, with several genes

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intervening to affect the observed phenotype and the underlying biosynthetic pathways. The types of hydrocarbons produced must depend upon the structure of specific enzymes, controlled by essential gene(s), while the final amount of these hydrocarbons would depend upon both the quantity of each enzyme and the availability of biochemical precursors. For example, the *Ngbo* gene, characterized for its dose-dependent effect on the production of 7-P in *D. simulans*, is thought to be a structural gene that changes enzyme specificity (Ferveur, 1991). Other genes, like *kété* in *D. simulans*, might be physiologically dependent and therefore might regulate the 'fine tuning' of the hydrocarbon profile (Ferveur & Jallon, 1993*a*). The cuticular hydrocarbon phenotype can thus be affected by both quantitative and qualitative factors that can be detected through proportional and absolute measures. Pleiotropic effects will also play a role; genes controlling body size or non-hydrocarbon biosynthesis pathways may have an effect on the absolute quantities of certain hydrocarbons, thus also altering the hydrocarbon profile of the fly.

Secondly, these data raise the question of the evolution of cuticular hydrocarbon phenotypes. Both *D. melanogaster* and *D. simulans* show geographical variation for 7-T and 7-P levels (Luyten, 1982; Ferveur *et al.* 1996; J.-M. Jallon, unpublished data). Both species have a world-wide distribution, and in both cases strains from central Africa show high levels of 7-P and low levels of 7-T (in both males and females in the case of *D. simulans*, in males only in *D. melanogaster*), whereas flies from elsewhere have high 7-T levels. Given the probable African origin of both species (Lemeunier *et al.* 1986), it is possible that 7-P is the ancestral form in both species. However, whilst *D. melanogaster* shows a very clear latitudinal cline between 7-T rich and 7-P rich strains, with geographically intermediate strains showing an intermediate profile (J.-M. Jallon *et al.* unpublished data), in *D. simulans* the effect is apparently more clear-cut, with the 7-P rich strains being limited to the Gulf of Benin, and no intermediate morphs ever having been found. This is probably due to the different genetic bases of the control of the 7-T:7-P in these species. This character is controlled by a single gene (*Ngbo*) in *D. simulans* (Ferveur, 1991), whereas we have shown here that at least two genetic factors are involved in *D. melanogaster*. There does not appear to be a homologue of *Ngbo* in *D. melanogaster*. This difference also suggests that we are probably dealing with an example of convergent evolution of 7-P levels in these two species.

The evolution and genetics of *Drosophila* cuticular hydrocarbons are subjects that are only beginning to be properly understood. In the future both geographical strains and mutants will have to be used in order to grasp not only the effects of particular genes on hydrocarbon profiles, but also the evolution of naturally occurring polymorphisms.

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