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Control of female pheromones in *Drosophila melanogaster* by homeotic genes

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Summary

We have investigated the role of the Antennapedia and Bithorax complexes (ANT-C and BX-C) on the production of cuticular hydrocarbons in *Drosophila melanogaster*. In males, there is little, if any, influence of these complexes on the hydrocarbon pattern. In females, there are large and opposite effects of these complexes on diene production: two ANT-C mutations cause an increase in diene production and a reduction of monoenes, whereas most BX-C mutations result in a decrease in dienes and an increase in monoenes, although their sum remains constant. The effect is the highest in *Mcp* and *iab6* females. It is suggested that a factor originating from the prothorax might activate the conversion of monoenes to dienes in females. The abdomen seems to have a crucial role in the production or control of pheromones: abdominal segments four to seven have the main effects, with a most dramatic effect for segments four and five.

1. Introduction

In *Drosophila melanogaster*, there is a marked sexual dimorphism among cuticular hydrocarbons. In many wild-type populations, 7,11-heptacosadiene (7,11-HD) is the main female hydrocarbon, or 5,9-heptacosadiene in African populations (Jallon & Péchiné, 1989). By contrast, 7-tricosene (7T) and 7-pentacosene (7P) are most abundant in male cuticles, as well as in both sexes of the sibling species *Drosophila simulans* (Jallon, 1984). These long-chain unsaturated hydrocarbons play a protective role against dessication (Gibbs, 1995) and also pheromonal roles in courtship (Jallon, 1984; Antony *et al*., 1985; Scott, 1994; Ferveur & Sureau, 1996).

In Diptera, the abdomen has been implicated in hydrocarbon production. Housefly studies with isolated body segments have shown that the major site of hydrocarbon synthesis lies in abdominal segments two to seven (Dillwith *et al*., 1981). Nissani (1977) and Jallon and Hotta (1979), using gynandromorphs (sexual mosaics) of *D. melanogaster*, showed that the sex appeal focus was principally located in the abdomen and suggested that the abdomen might therefore be the main site of pheromone synthesis. The hydrocarbon composition of mosaic flies confirmed that the sex specificity of hydrocarbons was linked to the abdomen and showed that both anterior and posterior tergites seemed to contribute independently and additively to the hydrocarbon dimorphism (Coyne & Oyama, 1995). Oenocytes, which are subcuticular abdominal cells, have been implicated in pheromone biosynthesis or regulation. In *Drosophila*, an ectopic feminisation of these cells by the *tra* gene leads to the feminisation of cuticle hydrocarbons in the males (Ferveur *et al*., 1997). Ferveur *et al*. have also shown that a transient expression (of two hours) of the *tra* gene between 12 hours and 48 hours of adult life is also sufficient to enable the synthesis of female pheromones up to 4 days later. This long-lasting effect of *tra* recalls the effect of the pheromone factor, released from the head during the few hours after emergence, which is required for the female pheromone biosynthesis (Wicker & Jallon, 1995a).

These different studies strongly suggest that the abdomen is indeed the location of pheromone production, but little is known about their precise site(s) of synthesis or regulation. They may be multiple: on the one hand, factors required for the regulation may come from different body parts (head, abdominal oenocytes); on the other hand, enzymes necessary for pheromone biosynthesis might be located in different cell types (fat body, oenocytes, abdominal integu-

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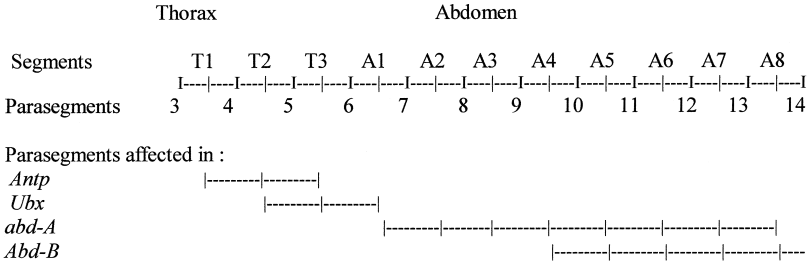

I----|----I delimits one segment. T1, T2, T3: thoracic segments 1, 2, 3. A1 to A8: abdominal segments 1 to 8.
|----I----| delimits one parasegment (PS).

Complex	Alleles used	strength of the allele	effect
ANT-C			
	Antp 17	+	T2 → T1
	Antp D42	+++	
	Antp 73b (gain of function)	+++	ectopic expression of *Antp* in the head
BX-C			
	Ubx1	+++	ventral :A1,T3 → T2
	Ubx bx-34e	+	dorsal :T2,T3 → T1
	abd-A		in the adult cuticle : A2, A3, A4 → A1 A5, A6, A7 ⇢ A1 to A4
	Df P10	+++	disrupts *Ubx* and *abd-A* A2 especially transformed
	abd-AC1 Abd-B M1	+	A1-A8 → anteriorA1+ posterior T3
	Abd-BMc: (*Microcephalus*)	+++	affects eye, scutellar bristle
	Mcp1 (revertant)	+++	A4 → A5
	iab6	+++	A5,A6 → A4
	iab7	+++	A5-A7 → A4

Fig. 1. Summary of the defects in the identity of body segments resulting from mutations in ANT-C or BX-C. These transformations are described by Lindsley and Zimm (1992) and in this article.

ment). These hydrocarbons, once synthesised, are transported to target tissues by a lipoprotein, the lipophorin (Pho *et al*., 1996).

To define further the role of the different parts of the body in pheromone production, we have analysed the cuticular hydrocarbons of flies mutant for different homeotic genes of the Antp and bithorax complexes (ANT-C and BX-C). These genes are involved in the specification of cephalic, thoracic and abdominal segments (Lewis, 1978). They are conveniently organised according to the body region they affect: ANT-C genes control segmental development in the posterior head and first and second thoracic segments. Genes of the bithorax complex are also arranged in the order of their action along the body, proceeding from the third thoracic segment (at the 5′ transcriptional end) to the most posterior abdominal segments (at the 3′ transcriptional end) (Sanchez-Herrero & Akam, 1989). Mutations that eliminate one gene generally result in the transformation of specific segments into more anterior ones. Studies of different ANT-C and BX-C mutants confirm the main role of the abdomen in dieneic hydrocarbon production or control and especially of the fourth, fifth and sixth abdominal segments. By contrast, the effects of an amorphic *Antp* allele, which led to higher amounts of female dienes and diminution of monoenes owing to a higher conversion of monoenes to dienes, suggests the possible existence of an activating factor originating from the prothorax on pheromone synthesis.

2. Materials and methods

(i) *Fly stocks and genetics*

All strains were reared on yeast–cornmeal agar *Drosophila* medium and kept at 25 °C in a 12 h dark–light cycle.

Mutant strains used are described below. They are derived from stocks containing various balancer third chromosomes. Some of them contained weak mutant alleles of *Ubx* and other punctual mutations, which might have limited effects on segment differentiation

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Table 1. *Percentages of monoenes in mature males and of monoenes and* 7,11*-dienes in mature females heterozygous for one mutant allele of* Antp

Mutant allele (crosses)	Genotypes[a]	Male monoenes[b] (%)	Female monoenes[b] (%)	Female 7,11-HD +7,11-ND[b] (%)
Antp 73b	*Antp 73b*/+	69·6±0·8	26·3±0·7	33·5±1·5
(F0: M *Antp*/*TM3*)	+/*TM3*	67·2±1·8	24·5±1·6	34·0±1·0
		(NS)	(NS)	(NS)
Antp 73b	*Antp 73b*/+	71·4±0·7	22·7±2·1	30·3±1·0
(F0: F *Antp*/*TM3*)	+/*TM3*	71·0±0·6	22·0±1/9	28·2±1·8
		(NS)	(NS)	(NS)
Antp 17	*Antp 17*/+	69·4±0·9	21·2±0·7	31·5±2·5
(F0: M *Antp*/*TM3*)	+/*TM3*	70·7±0·4	24·4±0·3	27·5±1·2
		(NS)	($p < 0·05$)	(NS)
Antp 17	*Antp 17*/+	69·4±0·7	20·4±1·0	31·3±0·9
(F0: F *Antp*/*TM3*)	+/*TM3*	70·9±0·9	24·9±1·6	27·7±2·3
		(NS)	($p < 0·01$)	(NS)
Antp D42	*Antp D42*/+	69·0±1·5	15·8±0·9	43·9±1·3
(F0: M *Antp*/*TM3*)	+/*TM3*	66·8±1·9	22·9±1·8	29·6±1·0
		(NS)	($p < 0·01$)	($p < 0·01$)
Antp D42	*Antp D42*/+	73·3±0·5	17·9±3·1	44·2±4·0
(F0: F *Antp*/*TM3*)	+/*TM3*	70·5±0·4	27·7±1·0	30·2±0·9
		($p < 0·05$)	($p < 0·01$)	($p < 0·01$)

[a] The genotypes *Antp*/+ were obtained by crossing *Antp*/*TM3* males with wild-type Canton-S females (F0: M *Antp*/*TM3*) or vice versa (F0: F *Antp*/*TM3*).
[b] Values are means of four groups of five flies±SEM. The probability values given are for comparison of control (+/*TM3*) with experimental flies.
Abbreviations: 7,11-HD, 7,11-heptacosadiene; 7,11-ND, 7,11-nonacosadiene; NS, non-significant at the 0·05 probability level.

and thus might modify pheromone production. To avoid these possible effects, stable mutant lines were established, carrying the same balancer chromosome TM3, Sb. The genotype of each stock and the cytological map positions are listed after the abbreviations used in the data tables and figures. A more detailed description of the mutations can be found in Lindsley and Zimm (1992). A summary of their effects on the identity of body segments is also given in Fig. 1.

Antp 73b: *In(3R) Antp*^{73b} *red*^{1}*e*^{1}*/TM3, Sb*; localization: 84B1-2
Antp 17: *Df(3R)Antp*^{17}*/TM3, Sb*; localization: 84B1-2
Antp D42: *Antp*^{D42}*/TM3, Sb*; localization: 84B1-2
Ubx 1: *Ubx*^{1}*e*^{4}*/TM3, Sb*; localization: 89E1-2
Ubx bx-34e: *Ubx*$^{bx\text{-}34e}$*Abd-B*^{Mc}*/TM3, Sb*; localization: 89E1-2
DfP10: Df(3R) *P10, abd-A*^{P10}*/TM3, Sb*; localization: 89C1; 89E1
abd-A Abd-B M1: *Df(3R)abd-A*$^{-C1}$, *Abd-B*^{M1}*e*^{11}*ro*^{1}*ca*^{1}*/TM3, Sb*; localization: 89E3-4
Mcp1: Abd-B$^{Mcp\text{-}1}$*/TM3, Sb*; localization: 89E7-8
iab6: Tp(3;3)*Abd-B*$^{iab6\text{-}Vn0}$, *Abd-B*$^{iab6\text{-}Vn0}$, *Vn0*^{1}*/TM3, Sb*; localization: 89E7-8
iab7: *Abd-B*$^{iab7\text{-}Mx2}$*/Dp(3;3)P5*, *Sb*^{1}; localization: 89E7-8
DfP9/DpP5: *Df(3R)P9/Dp(3;3)P5; Df(3R)*89E1; 89E5/*Dp(3;3)* 89E1; 90A

The last two stocks carry a duplication of BX-C, *Dp(3;3)P5*. Without this duplication, the mutant flies would be sterile.

(ii) *Crosses*

Males from each strain were crossed to Canton-S (wild-type) females and the hydrocarbon composition of each of the two genotypes was analysed (mutation/+and+/*TM3* or mutation/+and+/Dp*P5*). The reciprocal crosses (mutant females × Canton-S males) were also performed and their progenies analysed. Flies were isolated just after imaginal eclosion and sexes were held separately until 5 days of age.

(iii) *Hydrocarbon analyses*

Cuticular hydrocarbons were extracted from groups of five flies and gas chromatography analysis of the extracts was performed as described previously (Wicker & Jallon, 1995*a*). Flies were analysed in four groups of five individuals and all data are quantified as percentages of hydrocarbons. For each sex and each genotype, chemical analysis concerned mainly 7,11-dienes (7,11-HD and 7,11-nonacosadiene (7,11-ND)), which are characteristic of mature wild-type females, and 7-monoenes (7 tricosene (7T) and 7 pentacosene (7P)), which are abundant in wild-type males.

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(iv) *Statistics*

All hydrocarbons between 23 and 29 carbons were measured in each extract. Among individuals within a strain, absolute quantities of each hydrocarbon are much more variable than their relative percentages. These later parameters were selected for the statistical analysis. As all values were found to be normally distributed, comparisons were made between experimental and control flies from the same cross using a ANOVA/MANOVA test and Statistica software.

3. Results

(i) *Influence of ANT-C*

In males, the different *Antp* mutants did not induce any change in the hydrocarbon pattern, significant at the 0·05 probability level, except for one cross leading to the *Antp D42*/+ genotype, in which a small increase (4%) in monoenes was observed (Table 1).

In females the dominant gain-of-function allele *Antp 73b* did not lead to any significant modification of the monoene or 7,11-diene levels (Table 1). Flies heterozygous for the hypomorphic allele *Antp 17* showed significant monoene decreases in both crosses (−13% and −18%, respectively) but no significant diene level changes for both crosses. The effect of *Antp* on hydrocarbon production was more sensitive for the dominant amorphic allele *Antp D42*: a high diene increase occurred (+47%), paralleled by a monoene decrease (−31%). Variations of diene and monoene percentages in *Antp* mutant females are included in Fig. 2.

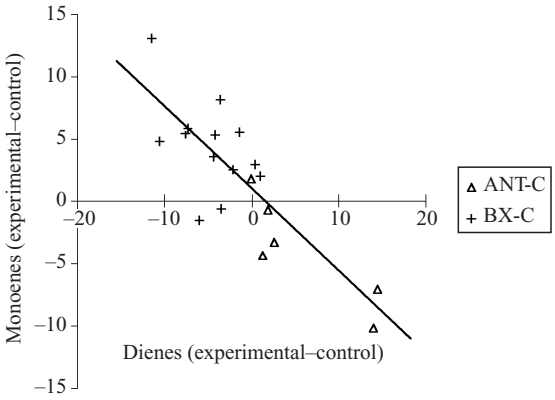

Fig. 2. The relationship between the mean variations in the proportions of monoenes and dienes in experimental female flies heterozygous for various alleles of ANT-C or BX-C, compared with control flies. Values are the difference between experimental and control mean values for the same allele and the same cross. A linear regression from these 20 points yields a straight line, which is shown in the figure, with a correlation coefficient of −0·83.

(ii) *Influence of a total deletion or duplication of BX-C*

The comparison of males bearing deficiency *DfP9* with those with the duplication *DpP5* showed a small but significant decrease in total monoenes for only one cross (Table 2). In females, the deficiency resulted in a decrease in total unsaturated hydrocarbons and in 7,11-HD + 7,11-ND that was significant for both crosses.

(iii) *Influence of mutations in the BX-C*

Males heterozygous for various BX-C alleles did not show much change in their hydrocarbon pattern (data not shown). However, lower amounts of both total monoenes and 7T + 7P were observed (−13%) in *Mcp1* males resulting from the cross (F0: M *Mcp1*/*TM3* × F CS).

In females heterozygous for all mutated BX-C alleles except *iab7*, the hydrocarbon pattern was significantly modified with a significant increase in monoene levels and/or a significant decrease in 7,11-

Table 2. *Percentages of monoenes in mature males and of monoenes and* 7,11*-dienes in mature females carrying one dose* (DfP9) *or three doses* (DpP5) *of BX-C*

	M *DfP9*/*DpP5* × F CS		M CS × F *DfP9*/*DpP5*	
Crosses Genotypes[a,b]	*DfP9*/+(%)	*DpP5*/+(%)	*DfP9*/+(%)	*DpP5*/+(%)
Male monoenes	73·4 ± 0·3	71·3 ± 0·9	65·8 ± 1·0*	70·0 ± 0·8
Female dienes + monoenes	60·7 ± 1·8**	66·1 ± 1·0	63·4 ± 0·8*	66·2 ± 0·6
Female 7,11-HD + 7,11-ND	36·5 ± 1·5*	39·2 ± 1·9	28·8 ± 0·6**	39·9 ± 1·7

[a] The genotypes *DfP9* and *DpP5* were obtained by crossing males from the strain *DfP9*/*DpP5* with wild-type females or vice versa.

[b] Values are means of four groups of five flies ± SEM. For each cross values are compared between *DfP9 vs. DpP5*.

Abbreviations: 7,11-HD, 7,11-heptacosadiene; 7,11-ND, 7,11-nonacosadiene; *, significant at the 0·05 probability level; **, significant at the 0·01 probability level; CS, Canton-S strain (wild type).

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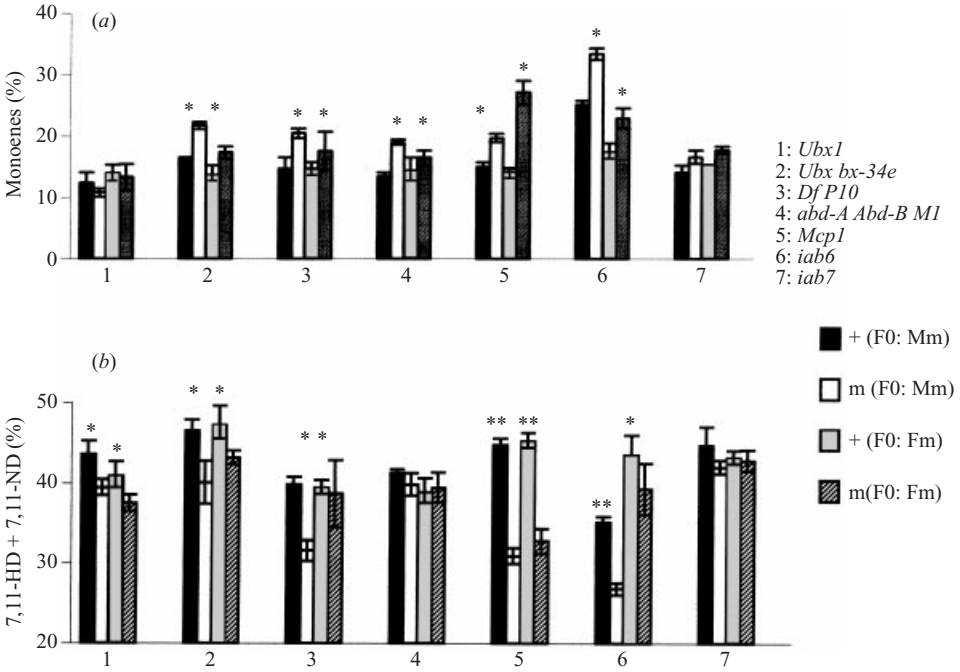

Fig. 3. Percentages of monoenes (a) and 7,11-dienes (b) in mature females heterozygous for mutant alleles of BX-C. Flies carrying one allele of the mutation m or wild type for m (m and +, respectively) were obtained by crossing males heterozygous for m with wild-type (Canton-S) females (F0: M m) or vice versa (F0: F m). Abbreviations: 7,11-HD, 7,11-heptacosadiene; 7,11-ND,7,11-nonacosadiene; *, values from m and + flies produced by the same cross that are different at the 0·05 probability level; **, values from m and + flies produced by the same cross that are different at the 0·01 probability level.

diene levels, with no change of their sums (Figs 2, 3). However, the effect on monoenes was not significant for *Ubx1*, and nor was that on dienes for *abd-A Abd-B M1*. The effect was particularly marked for *Mcp1* (−70% for dienes) and *iab6* (−23% for dienes) whichever the cross.

If one takes into account all female mutant data, an inverse linear correlation is observed between diene and monoene percentages (Fig. 2), with a correlation coefficient of −0·83.

4. Discussion

(i) *Pheromone biosynthesis in Drosophila*

The biosynthesis of *D. melanogaster* female-specific dienes requires two desaturation steps: the first, common to males and females of most populations, introduces a double bond in position ω7 in linear saturated fatty acids, probably linked to a Δ9 desaturase acting on palmitic acid (Wicker-Thomas *et al*., 1997; Dallerac *et al*., 2000). Another desaturation step is required to introduce a second double bond in position ω11, leading to the ω7,11 fatty acids. These ω7 and ω7,11 fatty acids would then be elongated by a small number of elongases and decarboxylated by decarboxylase(s) to produce cuticular hydrocarbons (Pennanec'h *et al*., 1997). Previous experiments have shown that, in females, if the second desaturation does not take place, ω7 fatty acids are still elongated, usually to produce hydrocarbons with only one double bond at position 7. The result is an increase in 7-heptacosene and 7-nonacosene as well as a decrease in 7,11-heptacodadiene and nonacosadiene (Wicker & Jallon, 1995*a*, 1995*b*).

The biosynthesis of monoenic fatty acids could be ubiquitous because several ESTs corresponding to the first desaturase have been found in various tissues such as fat body, brain, ovaries (data in flybase). However, the tissue expression of the second desaturase (and the proper elongases) might be more restricted. Genetical studies by Ferveur *et al*. (1997) using the GAL4-UAS *tra* strain suggested that oenocytes might be candidates.

(ii) *Implication of the ANT-C in pheromone biosynthesis*

The *Antp* locus is required in imagos for the proper development of the dorsal pro- and meso-thorax. *Antp* is characterised by the presence of two promotors, one (P1) distal to the other (P2) (Laughon *et al*., 1986). The dominant gain-of-function allele $Antp^{73b}$ results from the misregulation of the second promoter, P2, and is characterised by a normal *Antp* expression in the thorax and an ectopic accumulation of *Antp* transcripts in the head, where they are normally not found (Frischer *et al*., 1986). Flies mutant for this allele did not show any significant

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modification of their hydrocarbon pattern. The hypomorphic *Antp*^{17} and amorphic *Antp*^{D42} alleles affect the ability of the P1 promotor to initiate transcription, resulting in more or less decrease in Antp product (Laughon *et al*., 1986). The defects associated with these alleles are a transformation of the anterior part of the dorsal mesothorax into prothorax in adults. Both alleles had no clear effect on male hydrocarbons. In females, the amorphic allele induced marked effects: diene increases and monoene decreases of similar amplitudes (40%); similar effects were also obtained for the hypomorphic allele, but of lower amplitude. They might be due to the presence of a regulatory factor, either originating from the mesothorax and acting negatively or originating from the prothorax and acting positively on the diene formation.

(iii) *Influence of BX-C on pheromone biosynthesis*

In this study, we analysed two sets of progeny for each allele, differing in the origins of their sex chromosomes and of their maternal cytoplasm. For two crosses, more pronounced effects were observed when the maternal strain was wild type. Scott and Richmond (1988) also observed a maternal effect on the production of male hydrocarbons. In our case, the maternal effect of these mutant alleles would lead to a mortality of embryos, especially at the first stages, when the low production of wild-type BX-C RNAs cannot be compensated by the maternal ones. This would result in a selection of less-strong phenotypes.

Female unsaturated compounds were markedly affected by the BX-C locus. *DfP9* flies, which have only one copy of *Ubx* and *abd-A*, had less dienes than *DpP5* flies, which have three complete copies of BX-C (*Ubx*, *abd-A* and *Abd-B*). In *DfP9* flies, abdominal segments two to eight are more or less transformed into copies of the first (Karch *et al*., 1985). These data support an important role for the abdomen in diene synthesis, as suggested by mosaic studies (Jallon & Hotta, 1979; Coyne & Oyama, 1995; Ferveur *et al*., 1997).

BX-C is organised into three domains, *Ubx*, *abd-A* and *Abd-B*, which are responsible for the assignment of thoracic and abdominal segments (Lindsley & Zimm, 1992; Fig. 1). *Ubx* is required to specify thoracic segments 2 and 3 (Lewis *et al*., 1980). It acts by repressing *Antp* expression in these segments (Carrol *et al*., 1986). *Ubx*^{1} and *Ubx*$^{bx\text{-}34e}$ are amorphic and hypomorphic alleles, respectively (Bender *et al*., 1983; Peifer & Bender, 1988). *Ubx*^{1} females had markedly decreased amounts of dienes, with no change of the monoene levels. *Ubx*$^{bx\text{-}34e}$ *Abd-B*^{Mc} also resulted in a decrease in diene level, which was paralleled with a monoene increase; *Abd-B*^{Mc} contains a partial duplication of the *Abd-B* domain (*Dp(3; 3)Mc*) and leads to defects in the head and thorax (Hopmann *et al*., 1995). The cuticular phenotypes of these two mutants show the importance of the thorax in the control of hydrocarbon production.

Two *abd-A* mutations were examined, which show more or less defects in abdominal segments one to seven (Busturia *et al*., 1989); both were associated with *Ubx* or *Abd-B* alleles. The *DfP10* heterozygous flies are mutant for both *Ubx* and *abd-A* but show a characteristic *Ubx* phenotype (Lewis *et al*., 1980). They have reduced abd-A product, especially in the *cis*-regulatory region infra-abdominal 2 (iab2), which is required for the identity of the second abdominal segment (Karch *et al*., 1990). The second allele *abd-A*^{C1} has a large deletion with breakpoints in introns of *Ubx* and *abd*-A, resulting in a hybrid *abd-A–Ubx* gene that produces low amounts of both gene products (Macias *et al*., 1990). It is associated, in the variant strain studied, with the recessive mutation *Abd-B*^{M1}, which affects abdominal segments five to eight (Sanchez-Herrero & Crosby, 1988). The double mutant *abd-A*$^{-C1}$ *Abd-B*^{M1}, in which *Ubx*, *abd-A* and *Abd-B* are affected, shows a transformation of all the abdominal segments in homozygous embryos, but the resulting heterozygous flies are viable and fertile, suggesting that the abdominal mutant phenotype is not strong. In our study, the hydrocarbon profiles of both types of mutants were not significantly modified either, with only a moderate increase in monoenes. This result suggests that the first abdominal segments have only a moderate influence on hydrocarbon production.

The *Abd-B* domain is required for the proper identity of the fifth to eighth abdominal segments (corresponding to parasegments 10–14). The morphological difference between these four segments is due to the differential expression of *Abd-B*, which is achieved by the action of the regulatory regions iab-5, iab-6, iab-7 and iab-8 (Lindsley & Zimm, 1992). The mutation *Mcp1* has a deletion of a segment from the region between the iab-4 and iab-5 *cis*-regulatory domains (Karch *et al*., 1994), resulting in the transformation of abdominal segment four to segment five (Karch *et al*., 1994). The deletions occurring in *iab6* and *iab7* have also been mapped (Karch *et al*., 1985). Although the *iab7* mutation led to no significant differences in diene biosynthesis, the diene defects occurring in *iab6* and *Mcp1* females were larger in both crosses (−60% on average) and were more pronounced than those observed in other BX-C mutants. In both cases, they paralleled large increases in monoene levels (+47% monoenes on average). These results emphasize the importance of Abd-B products to control the production of female dienes.

In the female housefly *Musca domestica*, the study of hydrocarbon production by isolated thoracic and abdominal segments has shown that the abdomen was

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the site of hydrocarbon synthesis and that the major production was associated with segments 2–5 (Dillwith *et al*., 1981). Our study supports the functional specificity of the abdominal central segments (around A4 and A5) to produce female dienes in *D. melanogaster*.

(iii) *Implication of the sex-determination genes in ANT-C and BX-C phenotypes*

The present study did not show any significant effect of ANT-C and BX-C genes on the production of male hydrocarbons even though many genetic variations in the system affected the female hydrocarbons, mainly the diene:monoene ratio. It is suggested that they mainly affect the conversion of monoenic fatty acids into dienic fatty acids before their elongation and decarboxylation into dienes.

However, the control of this cuticular hydrocarbon production by genes of the sex determination program has been well documented (Jallon *et al*., 1986; Tompkins & McRobert, 1989; Tompkins & McRobert, 1995; Ferveur *et al*., 1997). Jallon *et al*. (1988) were the first to provide evidence for the role of *doublesex* (*dsx*) on that production. The more thorough studies of Waterbury *et al*. (1999) have demonstrated that *dsx* induced the downstream target for the *Sxl* → *tra* → splicing cascade. They have shown that *dsx* female form Dsx^F was a dominant regulator of diene production.

An interesting question for future studies is how the homeotic and sex-determination sets of genes interact in the control of sex-specific hydrocarbon production. Another clear sex-dimorphic phenotype, abdominal pigmentation, is regulated by the same two genetical pathways and involves an integrator gene, *bric-a-brac* (Kopp *et al*., 2000). The possible involvement of genes with a similar integrator function to control the production of cuticular hydrocarbons has to be searched for.

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