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Influence of ovary and ecdysteroids on pheromone biosynthesis in *Drosophila melanogaster* (Diptera: Drosophilidae)

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Abstract. The influence of ovary and ecdysteroids on pheromone biosynthesis was investigated in *D. melanogaster*. Strains mutant for *ovo* produced enhanced amounts of cuticular hydrocarbons in both sexes. Female sex pheromone production was not affected by the mutation, except homozygous females for ovo^{D1rs1} which exhibited a higher proportion of the pheromone, 7,11 heptacosadiene.

The amount of hydrocarbons in wild-type (CS) flies was stable at 21 and 25°C but dramatically increased at 29°C whereas the cuticular hydrocarbon pattern was unchanged. In contrast, *ecdysone-less* 1^{ts} (*ecd-1*) flies showed an increase in overall hydrocarbon production at both permissive and restrictive temperatures. At restrictive temperature they showed also a decrease in pheromonal dienes production for females with an increase in monoenes and an inversion of the 7 tricosene-7 pentacosene ratio for males. These effects were partially reversible since a switch from 29 to 21°C restored an intermediary hydrocarbon pattern.

It is suggested that the regulation of female sex pheromone might be under the control of the *ecd-1* gene.

INTRODUCTION

The pheromone sexual dimorphism of *Drosophila melanogaster* builds up about one day after emergence. Very young flies of both sexes have the same hydrocarbon pattern characterized by very long hydrocarbons (29 to 37 carbons) present on the cuticular surface. These hydrocarbons disappear at sexual maturity and shorter compounds appear (23 to 29 carbons) (Antony & Jallon, 1981; Pechiné et al., 1988). At the same time a sexual dimorphism appears: 23 and 25 carbon compounds become predominant in males, 27 and 29 carbon compounds in females. Among cuticular compounds unsaturated hydrocarbons with at least one double bond ω 7 are predominant. ω 7 monoenes with 23 and 25 carbons represent 41% and 15% of all hydrocarbons, respectively in mature Canton S males (Antony & Jallon, 1982) and might have behavioral roles (Jallon, 1984; Scott & Jackson, 1988).

The established female pheromonal system of *Drosophila melanogaster* consists of long chains ω 7,11 dienes with 27 and 29 carbons (Antony et al., 1985). These hydrocarbons are to induce male precopulatory behavior together with other cuticular compounds, ω 7 monoenes with 27 ± 2 carbons (Antony et al., 1985). In mature Canton S females, 7,11 heptacosadiene is the major cuticular hydrocarbon (22%) and the most potent aphrodisiac (Antony & Jallon, 1982).

In the housefly the production of the female sex pheromone 9 tricosene starts at sexual maturation and requires a vitellogenic ovary (Dillwith et al., 1983). In ovariectomized flies

the pheromone biosynthesis does not take place but can be induced by reimplanting ovaries or by injection of 20-hydroxyecdysone (Adams et al., 1984).

In *Drosophila melanogaster*, preliminary experiments suggested the control of various endocrine factors on the female pheromone biosynthesis (Jallon et al., 1986). Cephalic neuroendocrine factors are necessary since females which were decapitated early after emergence produced reduced levels of pheromonal dienes. The effect depended on the age of decapitation, was maximal when decapitation occurred the first hour after emergence and null when it occurred at three days of age (Wicker & Jallon, 1994). This effect was clearly not linked to juvenile hormone which however controls the switch between young fly singular compounds and mature fly sex-specific compounds (Wicker & Jallon, 1994).

In this paper advantage was taken of mutants to study the influence of ecdysone and functional ovary on the female sex pheromone.

MATERIALS AND METHODS

Stocks and culture conditions

Drosophila melanogaster of the Canton S strain were used as a reference. Two alleles of ovo^{Dr}, provided by M. Mével-Ninio, were tested. They were maintained in a balanced state over *FM0:* ovo^{rM1} *(M1)* and *FM3*: ovo^{D1rs1} (ovo^{r}). Homozygous females for the amorphic, ovo^{r} allele show germ cell death during early embryogenesis; the phenotypes produced by the partial loss-of-function allele *M1* have defective oogenesis resulting in the laying of few eggs with gross abnormalities (Mohler, 1977; Oliver et al., 1987).

The temperature-sensitive, recessive female sterile and lethal mutant, *ecd-1 st ca* was isolated by Garen et al. (1977). *ecd-1* flies were kept at 21°C. For the experiments, newly eclosed adults (0–2 hr post emergence) were sexed and maintained as separate sexes in rearing bottles at either 21°C (permissive temperature) or 29°C (restrictive temperature). A second *ecd-1* strain w^{118}, *ecd-1 st ca*, constructed from *ecd-1 st ca* by J. Deutsch, was also used to study an eventual role of the genetic background.

Rearing medium consisted of standard yeast-cornmeal-agar medium.

Analytical procedures

Hydrocarbons were extracted from groups of 5 flies immersed in hexane with an internal standard, as previously described (Antony et al., 1985; Wicker & Jallon, 1994). Samples were then evaporated and stored in a freezer until analysis.

A Girdel 300 gas chromatograph with a Flame-Ionization Detector was used for the analyses. The gas chromatograph was equipped with a BP1 capillary column (25 m, 0.22 mm i.d., SGE). The oven temperature was programmed from 185 to 320°C at 3°C/min.

Data analysis

In this study we only considered among cuticular hydrocarbons those which have pheromonal properties, that is monoenes for males and dienes and monoenes for females. Hydrocarbons were mainly characterized by their percentages relatively to the total sum of hydrocarbons (with 23 to 29 carbons). As great quantitative differences were also noted between hydrocarbons of mutant strains, the absolute amounts of total hydrocarbons were also measured in ng / fly.

All statistical analyses were done using Student's t test.

RESULTS

Hydrocarbons of the *ovo* strains

High amounts of total cuticular hydrocarbons were observed in both *ovo* alleles tested whichever the sex, although data for males are not shown (Table 1). For three day-old homozygous flies, the levels were higher in ovo^{r} than in M1. From 3 to 6 days, these amounts remained approximately constant for ovo^{r} and were multiplied by a factor three for *M1* flies, where large intergroup variations were observed.

The percentages of dienes were usually not very different in *CS* and *ovo* strains. However a marked increase in the content of 7,11 heptacosadiene occurred in ovo^r homozygous females between 3 and 6 days but not in heterozygotes. Low values for the ratio 7 heptacosene / 7,11 heptacosadiene were observed in all mutant females (Table 1).

TABLE 1. Influence of *ovo* mutation on mature female cuticular hydrocarbons. Amounts of total cuticular hydrocarbons (ng / fly) and relative percentages of 7,11 heptacosadiene, 7 heptacosene, 7,11 heptacosadiene + 7 heptacosene (27 C di + mono), 7,11 nonacosadiene and ratio of 7 heptacosene / 7,11 heptacosadiene (27 C mono / di) in females of wild-type (CS) or mutant for different alleles of *ovo*. Strains were reared at 25°C. Values ± SEM are means of 6 groups of 5 flies.

		ng / fly	Percentages				
Genotype	Age (days)	23 C – 29 C hydrocarbons	27 C 7,11-diene	27 C 7-monoene	29 C 7,11-diene	27 C di + mono	27 C mono / di
CS	3	1,646 ± 137	21.4 ± 0.6	9.6 ± 0.0	17.1 ± 0.6	31.0 ± 0.6	0.45
	6	1,485 ± 87	24.7 ± 1.1	11.1 ± 0.4	14.1 ± 0.8	35.8 ± 0.6	0.45
ovo^r / ovo^r	3	4,487 ± 217	22.0 ± 1.8	3.4 ± 0.7	17.8 ± 3.1	25.4 ± 1.1	0.15
	6	5,218 ± 938	31.4 ± 1.1	1.6 ± 0.3	16.0 ± 1.8	32.8 ± 1.0	0.05
$ovo^r / FM3$	3	4,320 ± 167	19.1 ± 0.6	4.3 ± 0.4	20.7 ± 2.4	23.2 ± 0.4	0.23
	6	3,217 ± 678	23.1 ± 2.2	4.8 ± 1.8	16.2 ± 3.2	28.0 ± 1.2	0.21
M1 / M1	3	2,370 ± 140	23.0 ± 1.0	2.7 ± 1.6	18.1 ± 2.9	25.7 ± 1.3	0.12
	6	7,557 ± 3,799	25.6 ± 0.5	1.6 ± 0.8	13.4 ± 1.8	27.3 ± 1.1	0.06
M1 / FM0	3	2,345 ± 360	25.0 ± 0.8	1.7 ± 0.7	14.1 ± 2.4	26.8 ± 1.3	0.07
	6	11,679 ± 4708	21.3 ± 3.3	0.8 ± 0.6	6.0 ± 1.5	22.2 ± 3.1	0.04

Influence of temperature on the amount of cuticular hydrocarbons

In the wild-type CS strain the amount of hydrocarbons remained constant between 3 and 6 days at 21°C. Meanwhile at 29°C there was a 2-fold and 3-fold increase in males and females, respectively (Table 2). Thus the temperature itself can modulate the quantity of hydrocarbons in wild animals. However the cuticular pattern does not seem affected (data not shown).

TABLE 2. Amounts of C23–C29 hydrocarbons in wild-type (CS) and mutant (*ecd-1*) flies reared from emergence at 21 or 29°C. Values ± SEM are means of 6 groups of 5 flies.

Strain	Rearing temperature	Age (days)	Hydrocarbons (ng / male)	Hydrocarbons (ng / female)
ecd-1	21°C	3	1,923 ± 278	1,835 ± 157
		6	10,295 ± 511	16,466 ± 6,407
	29°C	3	4,740 ± 628	3,721 ± 598
		6	3,427 ± 592	4,645 ± 755
CS	21°C	3	1,718 ± 138	1,646 ± 137
		6	2,232 ± 270	1,718 ± 119
	29°C	3	3,855 ± 139	4,734 ± 765
		6	4,211 ± 251	6,089 ± 321

In three day-old *ecd-1* flies reared at 21°C, quantities of cuticular compounds were not significantly different from those of CS flies (Table 2). But, unlike CS flies, there was at least a 5 fold increase in the total quantities of hydrocarbons at 6 days, whichever the sex.

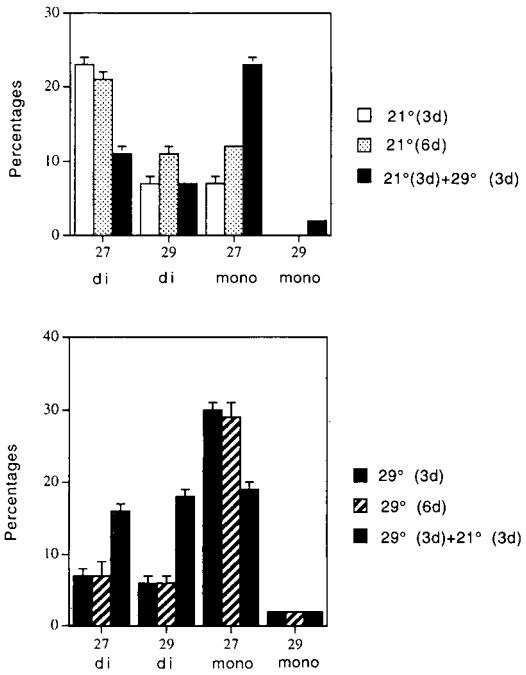

Fig. 1. Effects of the *ecd-1* mutation on female cuticular unsaturated hydrocarbons: 7,11 heptacosadiene (27 di), 7,11 nonacosadiene (29 di), 7 heptacosene (27 mono) and 7 nonacosene (29 mono) in *ecd-1* females maintained at different temperatures from emergence. Columns show the mean value of 4 measurements of 5 females + SEM.

At 29°C there was no difference in the total amounts of hydrocarbons for mutant and wild-type males at 3 and 6 days of age but a small increase in wild-type females between 3 and 6 days – not observed in mutant females.

Hydrocarbon levels in the *ecd-1* strain

ecd-1 females reared at 21°C exhibited percentages of monoenes and dienes similar to those of wild-type females (Fig. 1). When reared at 21°C and shifted at emergence to 29°C an important decrease in 7,11 heptacosadiene was observed (–70%), paralleled with an increase in 7 heptacosene (3 fold). Other experiments performed with the *w118*; *ecd-1 st ca* strain led to the same results, suggesting that the observed effect is due to the mutation itself (data not shown). When the temperature shift from 21 to 29°C was performed at 3 days of age, it resulted 3 days later in intermediate levels between those of control females reared either at 21°C or at 29°C from emergence (Fig. 1). These double changes were reversible and intermediate levels of both 7,11 heptacosadiene and 7 heptacosene were obtained when flies reared for 3 days at 29°C were shifted to 21°C and analysed for cuticular hydrocarbons 3 days later. The total amount of unsaturated compounds with 27 carbons remained approximately constant (35%) but the ratio 7,11 heptacosadiene / 7 heptacosene changed much.

DISCUSSION

In *Drosophila melanogaster* as in *Musca domestica*, the female specific sex pheromone is not present on the cuticle at emergence and builds up during sexual maturation. The data reported here confirm preliminary results (Jallon et al., 1982) and show a clear effect of the temperature sensitive mutation *ecd-1* on the biosynthesis of the female pheromone.

A shift of temperature from 21 to 29°C led to lower proportions of female pheromonal dienes. This phenomenon was reversible since a second shift to permissive temperature restored (although not completely) a more normal female cuticular pattern. This effect seems to be due to the *ecd-1* mutation because two different strains mutated in the *ecd-1*

gene but with different genetic backgrounds exhibited similar cuticular changes with temperature. As the ecdysone biosynthesis in this mutant has been demonstrated to be normal at 21°C and inhibited at 29°C (Garen et al., 1977), the observed modification of cuticular unsaturated hydrocarbons at 29°C in females may be due to a reduction of ecdysone level. The *ecd-1* mutation has pleiotropic effects, resulting in defective vitellogenesis and ovarian dysfunctions at restrictive temperature (Audit-Lamour & Busson, 1981; Pétavy, 1991). These ovarian abnormalities might not be responsible for the switch of hydrocarbon pattern because *ovo*^{r} strains displayed wild-type female hydrocarbon patterns.

The absence of functional ovaries in *ovo* mutants did not result in a deficiency in pheromones. In some cases there was even an enhanced production of 7,11 heptacosadiene together with an enhanced production of most hydrocarbons. However the levels in 7 heptacosene are slightly lower in *ovo*, compared to wild-type females and the sum of 7 heptacosene and 7,11 heptacosadiene remains lower too.

ovo mutations were assessed to lead to defects only in females, causing female germ cell death (*ovo*^{r}) or defective oogenesis (*M1*) with the production of ovarian tumors and it has been suggested that female germ cells have been partially transformed to male germ cells (Oliver et al., 1990). Moreover *ovo* is transcribed in both males and females (Mével-Ninio et al., 1991).The results obtained in this study suggest that *ovo* could alter in some way the hydrocarbon production.

A last point to be discussed is the fact that a shift of temperature from 21°C to 29°C in *ecd-1* flies induced a marked decrease in 7,11 heptacosadiene, paralleled with a large increase in 7 heptacosene, while the sum of their percentages remained quasi constant. The same correlated modifications have been observed as result of early decapitation (Wicker & Jallon, 1994). Jallon (1984) has proposed that monoene and 7,11 diene biosynthetic pathways might share several enzymatic steps including one desaturation-present in both sexes- but that a female specific second desaturation might be responsible for the formation of 7,11 dienes. We suggest that this one step might be regulated by ecdysone and a cephalic factor.

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