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The Consequences of Regulation of desat1 Expression for Pheromone Emission and Detection in *Drosophila melanogaster*

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ABSTRACT

Sensory communication depends on the precise matching between the emission and the perception of sex- and species-specific signals; understanding both the coevolutionary process and the genes involved in both production and detection is a major challenge. *desat1* determines both aspects of communication—a mutation in *desat1* simultaneously alters both sex pheromone emission and perception in *Drosophila melanogaster* flies. We investigated whether the alteration of pheromonal perception is a consequence of the altered production of pheromones or if the two phenotypes are independently controlled by the same locus. Using several genetic tools, we were able to separately manipulate the two pheromonal phenotypes, implying that *desat1* is the sole gene responsible, exerting a pleiotropic effect on both transmission and detection. The levels of the five *desat1* trancripts, measured in the head and body of manipulated flies, were related to variation in pheromone production. This suggests that the pleiotropic action of *desat1* on pheromonal communication depends on the fine regulation of its transcriptional activity.

PHEROMONES are frequently used for sexual communication by vertebrate and invertebrates alike (Bradbury and Vehrencamp 1998; Wyatt 2003). In particular, pheromones play an important role in attraction and discrimination of sexual partners (Johansson and Jones 2007) and can be subjected to sexual selection (Darwin 1883). In moths, pheromones can affect premating behavior (Roelofs and Rooney 2003; Smadja and Butlin 2009). Pheromones have also been studied in several groups of Drosophila species (Toolson and Kupersimbron 1989; Tompkins *et al.* 1993; Higgie *et al.* 2000; Ferveur 2005), including *Drosophila melanogaster*, a model species used to dissect the molecular, genetic, physiological, neural, and evolutionary basis of pheromonal communication (Coyne *et al.* 1994; Jallon and Wicker-Thomas 2003; Lacaille *et al.* 2007; Koganezawa *et al.* 2010).

In moths, sex pheromone communication has evolved to make use of complex blends of relatively simple long-chain fatty acid precursors and species specificity is derived from the introduction of double bonds into exact locations along the hydrocarbon backbone of fatty acids (Rooney 2009). In *D. melanogaster*, the predominant hydrocarbons on the cuticle of mature flies radically differ between the sexes in both their structure and effect. Males produce cuticular hydrocarbons (CHs) with one double bond (monoenes), which stimulate conspecific females and inhibit conspecific males (Jallon 1984; Ferveur and Sureau 1996; Grillet *et al.* 2006; Lacaille *et al.* 2007). Females produce dienes (with two double bonds), which slightly stimulate conspecific males but strongly inhibit males of the sibling *D. simulans* species (Savarit *et al.* 1999). The biosynthesis of these sexually dimorphic CHs requires several desaturase enzymes that show sex- and species-specific expression (Legendre *et al.* 2008). We previously characterized the mutational effects of a specific *PGal4* transposable element inserted in the regulatory sequence of the *desat1* gene (1573-1). This mutation affected not only the production of monoenes and dienes in male and female flies (Marcillac *et al.* 2005a) but also the male discrimination of sex pheromones (Marcillac *et al.* 2005b). The precise excision of the transposon completely rescued the two phenotypes, demonstrating that *desat1* can simultaneously affect the emission and the reception of sex pheromones.

To explore the genetic relationship between these two pheromonal phenotypes, we used flies (i) homozygous for *desat1* excision alleles (Marcillac *et al.* 2005a), (ii) combining *PUAS* deregulation elements (Ep) (Rorth 1996) inserted in the *desat1* regulatory region, driven or not by a *PGal4 desat1* enhancer trap element. Two *PGal4 desat1* enhancer traps, containing either a complete or a deleted *desat1* coding region (Marcillac *et al.* 2005a), were used to induce Ep deregulation in these two *desat1* backgrounds. The effect of genetic deregulation was also measured on the relative levels of the five *desat1* transcripts.

Supporting information is available online at http://www.genetics.org/cgi/content/full/genetics.110.117226/DC1.

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MATERIALS AND METHODS

***D. melanogaster* stocks and crosses:** All *D. melanogaster* strains were raised on yeast/cornmeal/agar medium and kept at 24 ± 0.5° with 65 ± 5% humidity on a 12-hr light:12-hr dark cycle. Dijon2000 (DIJ) and Canton-S (Cs) are wild-type strains used as controls. Crosses were performed using standard techniques and genetic tools (Lindsley and Zimm 1992). We used 11 homozygous derivative excision lines of $desat1^{1573\text{-}1}$ (1573-1) (Marcillac *et al.* 2005b; Figure 1A). Among these excision alleles, 6 retained a substantial fragment (11–3.5 kb) of the original *PGal4* transposon. The fragment was long in the L_1–L_4 alleles (*A4*, *C1*, *F3*, and *H6*, respectively) and of medium size in M_1 and M_2 alleles (*L6* and *H3*). In these alleles, the *mini-white* sequence was partially or totally removed. In some alleles, the *pbluescript* sequence was also partly removed, whereas the *Gal4* sequence was not or only slightly affected. In two other alleles, S_1 and S_2, the transposon had a small size (*F7* and *F4*, respectively), since most of the transposon was removed at the exception of the "feet" (0.15 kb). The three other alleles, P_1–P_3, resulted from the precise excision of the transposon (*O5*, *A′ 2*, and *N2*, respectively).

We also used 10 desat1 Ep deregulation lines that contain a *PUAS* transgene inserted in the 5′ noncoding region of the *desat1* gene and whose expression can be deregulated under the conditional activation of Gal4 (Rorth 1996). These transgenes were named according to both the position and orientation of the *Ep* element in the *desat1* regulatory sequence. (The letter indicates the regulatory region of the corresponding transcript: capital letter = sens; lower case letter = antisens; Figure 1B). Most strains were purchased from Genexel with the exception of EpB_2 (EpGSV6) (Aigaki *et al.* 2003), which was kindly provided by T. Aigaki (University of Tokyo). The *Ep* transgenes were tested in four combinations. (1) The dominant effect of the *Ep* transgenes was measured in heterozygous flies combining one copy of each *Ep* transgene with the *desat1–P₃* precisely excised allele (P_3/Ep) (Marcillac *et al.* 2005b). (2 and 3) To potentially affect *desat1* transcriptional activity and deregulate its expression, each *Ep* transgene was driven either by the hypomorph *desat1 1573-1–Gal4* allele (1573-1>Ep) or with the null *desat1 1573-C′1–Gal4* allele (C′1>Ep) (Marcillac *et al.* 2005b). This allowed us to compare the effect of *desat1* deregulation in hetero- and hemizygous *desat1* genetic backgrounds, respectively. (4) We also measured the recessive effect of these *Ep* transgenes in homozygous flies (Ep/Ep) and their complementation in double heterozygote flies.

Behavior: All flies were isolated 0–4 hr after eclosion under CO_2 anesthesia. Tester male flies (*i.e.*, those whose sexual response to target flies was measured) were held individually in fresh glass food vials for 5 days before testing. Target flies were similarly treated but they were held in groups of five for the same period. All tests were performed in a room at 24 ± 0.5° with 65 ± 5% humidity. Tester males were individually aspirated (without anesthesia) under a watch glass used as a courtship observation chamber (1.6 cm^3). After 5 min, necessary for the tester male to habituate to the chamber, the two control target flies (a male and a female) were introduced and the observation period started.

To characterize male discrimination of sex pheromones, we measured the proportion of time spent by tester males in actively courting (wing vibration, licking, and attempting copulation; total = courtship index, CI) each target. For each male, we obtained two values corresponding to the CI directed to the male (CIm) and to the female target (CIf). Tests were carried out under a dim red light (25W with a Kodak Safe-Light Filter n°1) to remove all visual stimuli (Boll and Noll 2002) and with decapitated target flies to remove most acoustic and behavioral signals (Ferveur *et al.* 1995).

Besides the parametric CI parameters providing a global average measure of behavioral activity, we also designed, for each genotype, a new parameter to obtain a picture of the distribution of sexual discrimination in individual males on the basis of their respective CIf and CIm. We named this parameter "distribution of courtship index discrimination" [DCID = (CIf − CIm)/(CIf + CIm)]. Three classes of discrimination behavior were delimitated according to the dominant sexual orientation of each tester male: (1) heterosexual (DCID ≥ +0.35), or (2) homosexual (DCID ≤ −0.35), or (3) bisexual (−0.35 < DCDI < +0.35). Since low CI values could yield a biased ratio, we considered only individual males with a total CI (CIm + CIf) > 10%.

We measured the locomotor activity of single 5-day-old males. Each fly was introduced in a courtship chamber placed over a pattern delimitating 12 equal areas. After a habituation period of 5 min, we counted the number of lines (separating the areas) crossed during five periods of 10 sec and the total count number (during 50 sec) was used as the individual locomotor activity index (LAI) (Balakireva *et al.* 2000). This measurement was simultaneously and sequentially performed on four flies of different genotypes during a total period of 5 min. All tests lasted 5 min and took place 1–4 hr after lights on. For each strain, tests were performed over several days.

Cuticular hydrocarbons: Cuticular hydrocarbons (CHs) were extracted from 5-day-old intact individual flies by gas chromatography following a brief wash in hexane according to standard procedures (Ferveur 1991). Analyses were performed with a Varian CP3380 chromatograph, equipped with a Cp-sil 25-m capillary column with hydrogen as the carrier gas. All the *D. melanogaster* predominant CHs have already been identified and characterized (Antony and Jallon 1982; Pechine *et al.* 1985). Twenty-four CHs were systematically detected in female flies, and 14 in male flies, both with a chain length ranging from 23 to 29 carbons (Marcillac *et al.* 2005b). Each CH was characterized both by (i) its percentage relative to the sum of all CHs ($\sum$CHs) and (ii) to the area of an internal standard (hexacosane) used to calculate its absolute amount (in nanograms). For the sake of clarity, we show only four principal CH parameters: (1) the total amount of CHs (in ng; $\sum$CHs), the total percentage of (2) desaturated CHs ($\sum$% Desat), (3) linear saturated CHs ($\sum$% Lin), and (4) ramified CHs ($\sum$% Br). The percentages (parameters 2–4) were calculated relative to $\sum$CHs (Note that $\sum$% Desat + $\sum$% Lin + $\sum$% Br =100% and corresponds to $\sum$CHs).

Desat1 transcripts: To find a relationship betwen the alteration of the two pheromonal phenotypes and the deregulation of *desat1*, we measured the relative amount of the five *desat1*transcripts (RA, RC, RE, RB, and RD) separately in heads and in headless bodies of 5-day-old males. The nine tested genotypes include four experimental genotypes (*C′1>1573-1*, *>Epb*, *>Epd*, and *>EpB_2*; which induced the strongest phenotypic effects; see results) and five control genotypes combining the precisely excised P_3 allele with the five alleles present in the experimental genotypes. Note that RE was never detected in the head. All measures were compared with those obtained with P_3 control males.

RNAs were extracted by the Trizol method (GIBCO BRL) and treated with RNase-free DNase to avoid contamination by genomic DNA. Total RNA (2 μg) was reverse transcribed with the iScript cDNA synthesis kit (Biorad). Quantative PCR reaction were performed with the IQ SYBR Green supermix (Biorad) in a thermal cycler (MyIQ, Biorad) according to the procedure recommended by the manufacturer. The qPCR reaction was done in a volume of 20 μl, by 40 cycles (95° for 30 sec, melting temperature (Tm) for 30 sec, and 72° for 30 sec), preceded by a 3-min denaturation step at 98°, and followed by a 1-min elongation step at 72°. TM of the

hybridization step depends on the primer pair used. Generally, we used a TM of 60° for RC (RC forward: GGACGTGTGCTTTCGCCACT; RC reverse: CTGC GATCAGTGAGTCTGAGAT), RE (RE forward: GATACAACA TCCTAAACAAATCGGG; RE reverse: CTGCGATCAGTGA GTCTGAGAT), RB (RB forward: TAATGGCCCCATCCTGGT; RB reverse CTGCGATCAGTGAGTCTGAGAT), RD (RD forward: CGAAACGGCTTGTTAATTTCTAGC; RD reverse: CTGCGAT CAGTGAGTCTGAGAT), and control actine 5C (Act60 forward: TAACAAATTCAAGGCGTGAAA; Act60 reverse: TTCAGTCG GTTTATTCCAGTCA), except for RA amplification where 62.5° was used (RA forward: GCCATCACTAAACCAGGA GAATA; RA reverse: CGGTGGTTTCCACATCGCACTCGAA) and control actine 5C (Act62.5 forward: CAGATCATGTTC GAGACCTTCAA; Act62.5 reverse: ATCTTCATCAGGTAGTC GGTCAA). Each reaction was performed in triplicate and the mean of the three independent biological replicates was calculated. All results were normalized to the Actine5C mRNA level.

Statistics: We compared CH levels between genotypes with a Kruskal–Wallis test. Within each strain, the difference between CIm and CIf was measured with a Student's *t*-test. The CI toward each target was tested between genotypes with a ANOVA completed by a multiple pairwise comparison using Bonferroni post hoc tests. DCIDs were compared between genotypes with a Khi2 homogeneity test. Only *P*-values <0.05 were considered to be statistically significant. Statistical analyses were performed using XLSTAT software. Significant differences in transcript level ratios between control and sample strain (body and head) were detected with the Relative Expression Software Tool (REST, REST-MCS beta software version 2 (Pfaffl 2001), where the iteration number was fixed at 2000. This test is based on the probability of an effect as large as that observed under the null hypothesis (no effect of the treatment), using a randomization test (pairwise fixed reallocation randomization test; Pfaffl *et al.* 2002).

RESULTS

Our experimental strategy principally consisted of measuring the production of CHs and male sexual behavior in two series of genetically manipulated flies. First, we used flies homozygous for 11 excision alleles resulting from the un/precise remobilization of the *PGal4* transposon (Figure 1A). Second, we measured the effect of 10 *PUAS* transgenes (*Ep*) inserted in the *desat1* regulatory region (Figure 1B) in various genetic combinations (see MATERIALS AND METHODS). Our goal consisted of determining whether and to what extent the two phenotypes can be separately affected.

Effect of *desat1* excision alleles on cuticular hydrocarbon production: For the sake of clarity, we show only the total absolute amount of CHs ($\sum$CHs; in ng) and the total relative levels of desaturated CHs ($\sum$% Desat), of linear CHs ($\sum$% Lin), and of methyl branched CHs ($\sum$% Br). The variation of individual CHs is not shown since it was similar to that of their principal group.

The production of male CHs was strongly affected in the three excision alleles containing the largest transposon fragment inserted (L_1–L_3; Figure 2A; see also supporting information, Table S1A). Their $\sum$% Desat was very reduced (7%), whereas their $\sum$% Lin was strongly increased (71%) compared to control males (62% and 18%, respectively). L_1 and L_2 males also showed strongly increased $\sum$CHs (3466 and 2716 ng, respectively). Overall, the CH levels of L_1–L_3 males were very similar to those of the original 1573-1 mutant (10% Desat, 69% Lin; $\sum$CHs = 3215 ng). Male CH levels were less affected in the five other imprecise excision alleles: their $\sum$% Desat was generally higher than $\sum$% Lin. P_1–P_3 males showed rescued CH profiles. Homozygous females of excision lines showed CH profiles very similar to respective excision males (Figure 2B; Table S1B). Note that S_1 and S_2 females showed a higher $\sum$% Lin than sibling males. Overall, these data reveal a relationship between the degree of alteration of male and female hydrocarbon profiles and the size of the transposon fragment inserted in the *desat1* regulatory region.

Effect of *desat1* excision alleles on male courtship and sexual discrimination: The second principal phenotype controlled by *desat1* is the male discrimination of sex pheromones. To determine this, we measured the amount of courtship, or courtship index (CI) that single males directed toward a pair of immobilized control target female and male flies presented simultaneously. For each genotype, we compared their mean CI to the target female (CIf) and to the target male (CIm). Besides CIs, which represent the mean (±SEM) intensity of each tester male genotype toward each sex target, we designed a new parameter showing the distribution of individual male ability to discriminate (DCID; see MATERIALS AND METHODS).

Control and homozygous P_1–P_3, L_2, L_3, M_2, and S_2 males showed a normally high discrimination: their CIf was significantly higher than the CIm ($P < 0.001$; Figure 2C). On the other hand, L_1, L_4, and M_1 males showed no discrimination. In comparison to the DCID of control males, fewer M_1 males showed heterosexual orientation, whereas M_2 males were more often bisexual and less often heterosexual (Figure 2D). Therefore, no relationship was found between the gravity of the discrimination phenotype and the size of the transposon fragment.

***desat1* deregulation and the production of male cuticular hydrocarbons:** We measured the potential dominant and recessive effect of each *Ep* transgene in Ep hetero- and homozygous flies, respectively. Some *Ep* transgenes were tested in double heterozygous flies to assess for complementation with regard to each phenotypes. To measure their effect on deregulation, each *Ep* transgene was also driven by either *1573-1* or *C′1* enhancer trap. Both drivers contain a similar *Gal4* element inserted at the same *desat1* genomic site (*e.g.*, able to reveal the activity of the same *desat1* regulatory sequences), but C′1 is a null allele resulting from the total excision of the Desat1 coding region. This allowed us to compare the effect of the deregulation in a hypomorphic (1573-1) and in a null (C′1) *desat1* context.

P_3/Ep males showed no or very slight CH difference with P_3 control males (Figure 3A; Table S1A). Their $\sum$%

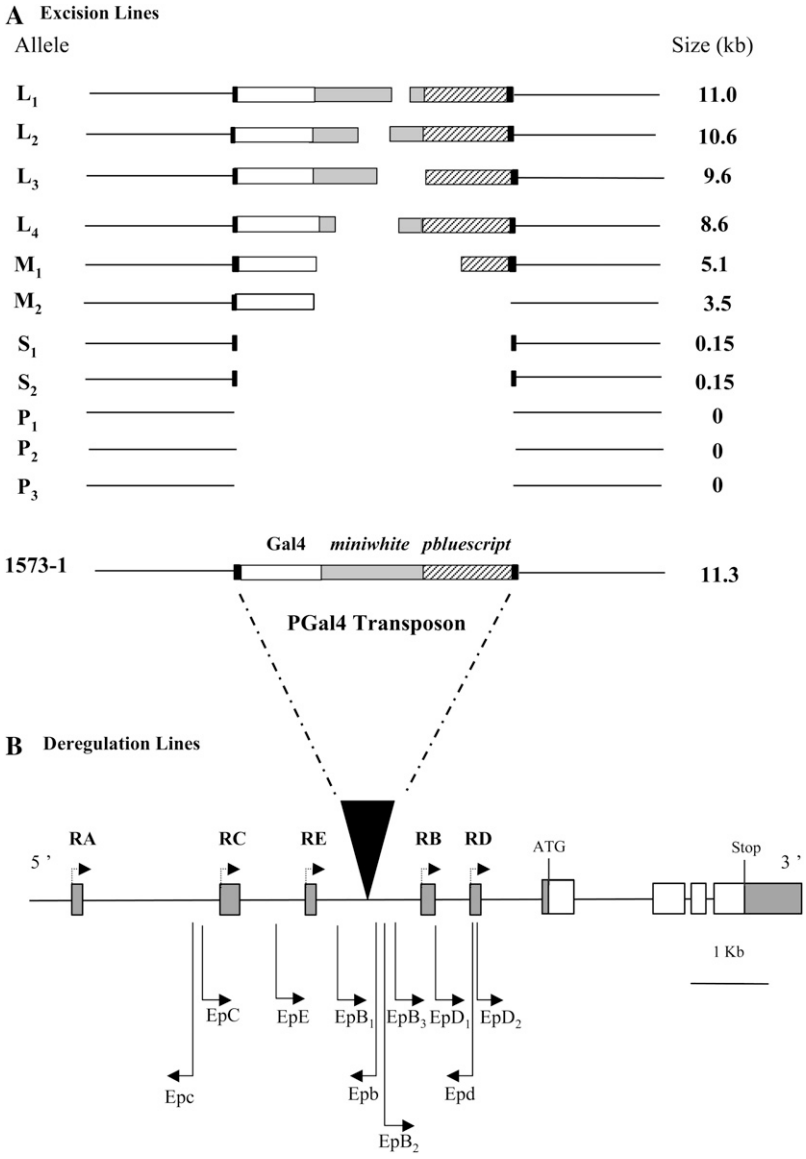

FIGURE 1.—Genetic tools used to dissect *desat1* functional pleiotropy. (A) Excision lines were derived from the remobilization of the *PGal4* transposon from the original 1573-1 allele. The transposon contains the *Gal4*, *miniwhite*, and *pbluescript* sequences (respectively shown as open, solid, and hatched horizontal bars). The two ends of the transposon are delimitated by two feet (shown as small dark bars). The remobilization (MARCILLAC *et al.* 2005a) induced either (i) precise excision of the transposon in the three P_1, P_2, and P_3 alleles or (ii) incomplete excision of the transposon in the eight alleles (L_1–S_2) shown from top to bottom, with a decreased size of the remaining transposon fragment (indicated in kb on the right). Excision alleles have been designated according to the approximate size of the remaining insertion: long (L_1–L_4), medium (M_1 and M_2), or short (S_1 and S_2). (B) Schematic representation of the *desat1* locus and Ep deregulation lines. The insertion position of the *PGal4* transposon (solid triangle) and of the *PUAS* (Ep transposons) is shown together with the Ep orientation relative to *desat1* transcription (arrow to the right, sense; arrow to the left, antisense). For Ep lines, the letter corresponds to the regulatory region of the exon in which the transgene is inserted, and its size to the orientation relative to the *desat1* gene transcription (uppercase, sense; lowercase, antisense). The index indicates that multiple Ep (only sense orientation) are inserted in the same regulatory region. The five solid boxes represent the five specific alternative 5′-UTR exons for each transcript (RA, RC, RE, RB, and RD), and the open boxes represent the translated *desat1* region. Note that the transposon and the *desat1* DNA are not shown at the same scale.

Desat (55–62%) and ∑% Lin (18–25%), respectively, corresponding to 700–900 ng (850 ng in P_3 males) and 200–350 ng (200 ng in P_3 males). Therefore, no *Ep* transgene had a dominant effect on male CH production.

The 1573-1>Ep males often showed a much higher ∑CHs than control 1573-1>P_3 males (Figure 3B). In particular, 1573-1>Epb and >Epd males produced the double ∑CHs (respectively, 3000 and 3200 ng) of control males (1500 ng). The two former males also strongly decreased their ∑% Desat/Lin ratio (12/65% and 10/74%, respectively) compared to control males (59/20%). Reciprocally, 1573-1>EpB_2 males showed a dramatically increased ∑% Desat/Lin ratio (80/3%). Concretely, 1573-1>Epd males produced 300 ng Desat CHs and 2300 ng Lin CHs against, respectively, 1400 and 60 ng in 1573-1>EpB_2 males (900 and 300 ng in controls). In summary, *1573-1* combined with *Epb* and *Epd* tended to decrease the ∑% Desat/Lin ratio whereas EpB_2 had a reciprocal effect on male CHs.

C′1>Epb and >Epd produced much higher ∑CHs (3400 and 3800 ng) than both control C′1> P_3 males (1700 ng) and C′1>1573-1 males (2600 ng). If the two former males also strongly decreased their ∑% Desat/Lin ratio (14/71% and 7/66%, respectively) compared to control males (57/24%) and to C′1>EpB_2 males (80/4%), they did not differ from C′1>1573-1 males (6/74%). Therefore, if *C′1* induced a very similar effect to *1573-1* with regard to respective *Ep* transgene, *C′1* tended to increase ∑CHs (+400–700 ng) compared to *1573-1* (when driving *Epc*, *EpE*, *Epb*, *Epd*, and EpD_2).

Homozygous Epb and Epd males showed strongly increased ∑CHs (2300 and 2600 ng) and decreased ∑% Desat/Lin ratio (21/59% and 20/63%, respectively) compared to control males (1400 ng; 62/18%, respectively). However, these variations were less important than in 1573-1 mutant males (3200 ng; 10/69%). Overall, the amplitude of the CH variation was always lower in homozygous Ep males than in 1573-1>Ep and

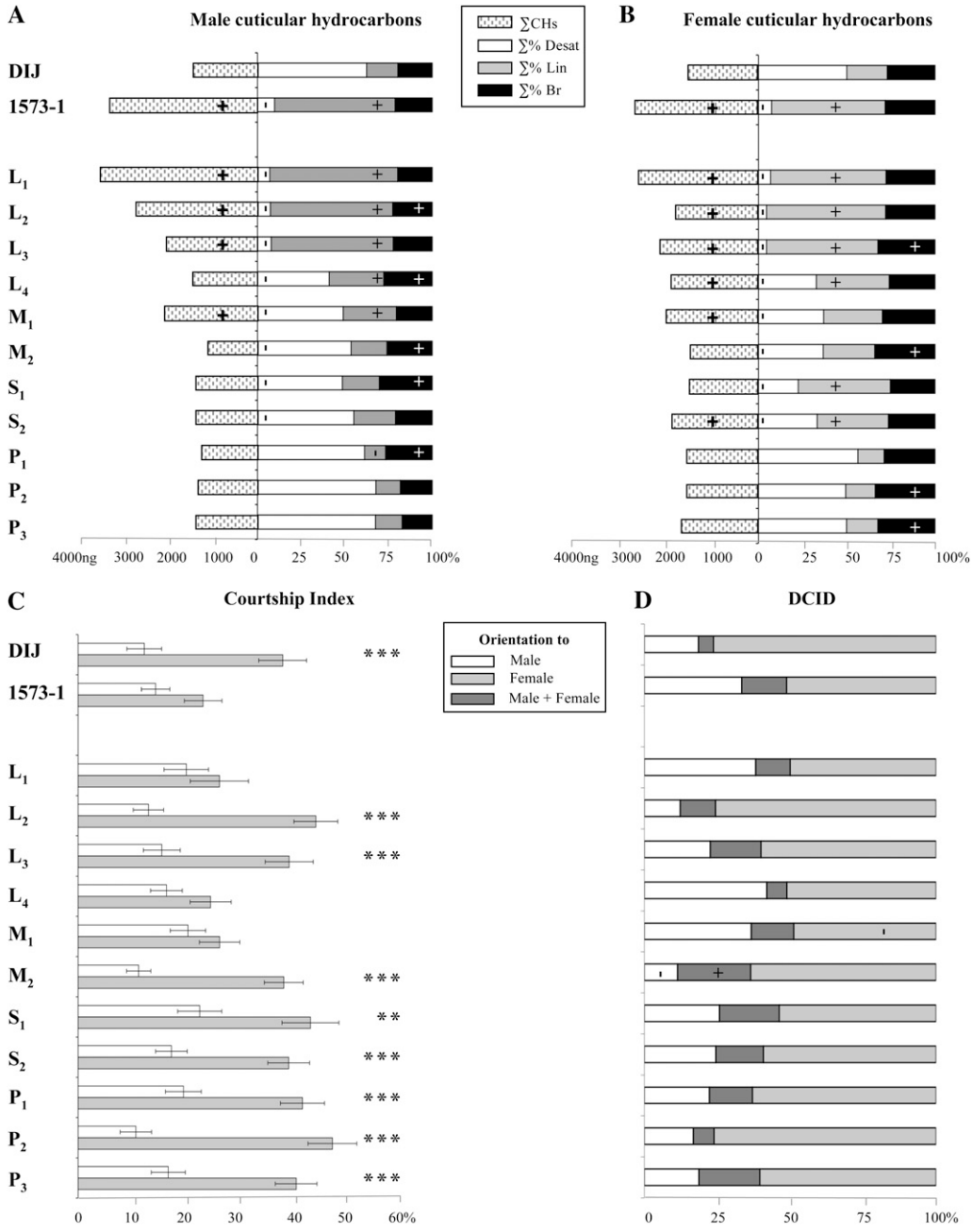

FIGURE 2.—Pheromonal phenotypes in flies homozygous for various excision alleles. The genotype of excision strains is indicated on the left (for the molecular structure of excision alleles, see Figure 1). Two reference strains were also used: wild-type Dijon strain (DIJ) and the original *desat1*$^{1573\text{-}1}$ mutant (1573-1). (A and B) represent the production of cuticular hydrocarbons in mature male and female flies, respectively. For each sex, dotted bars on the left indicate the mean total absolute amounts of hydrocarbons (∑CHs, in ng), and bars on the right represent the total percentage of desaturated hydrocarbons (∑% Desat, open bars), of saturated linear hydrocarbon (∑% Lin, shaded bars), and of ramified hydrocarbons (∑% Br, solid bars). Note that ∑% Desat + ∑% Lin + ∑% Br = 100% = ∑CHs. The symbol within each bar indicates a significant variation (+, increase; −, decrease; $P < 0.05$; Kruskal–Wallis tests) with the DIJ control strain. $N = 9$–18. (C) Mean (±SEM) male courtship index to control target male (open bars) and control target female (lightly shaded bars). Bars are paired since both decapitated male and female target flies were simultaneously presented to a single tester male, under red light, during a 5-min period. The statistical significance for male ability to discriminate sex targets within each genotype is shown above each pair of bars: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$. $N = 40$–50. (D) The distribution for courtship index discrimination (DCID) indicates predominant sexual orientation in individual males: homosexual (open bars); bisexual (darkly shaded bars); and heterosexual (lightly shaded bars). Only males with a total courtship index (CIf + CIm) ≥10 were kept. The symbols within bars indicate a significant variation (+, increase; −, decrease; $P < 0.05$; χ^2 test) of sexual orientation with the DIJ control strain. All flies were 5 days old. $N = 40$–50.

C′1>Ep deregulation males, with respect to *Ep* transgenes. *Epb* and *Epd* alleles did not complement each other since the general CH pattern (∑% Desat, ∑% Lin, and ∑CHs) of Epb/Epd males was similarly altered to that of either homozygous Epb or Epd males (Table S2A). EpE/EpB$_3$ males (combining two mild-effect transgenes) showed no marked difference with either parental male. Epb/EpB$_3$ and Epd/EpD$_2$ males (combining a mild- and a strong-effect transgene) showed hydrocarbon phenotypes intermediate between those of both parents. In summary, some *Ep* transgenes induced semidominant effect (on male hydrocarbon production) depending on their insertion position in the *desat1* regulatory region.

***desat1* deregulation and the production of female cuticular hydrocarbons:** P$_3$/Ep females showed no or slight CH difference with P$_3$ control females (Figure 4A; Table S1B). Their average ∑% Desat/∑% Lin ratio was

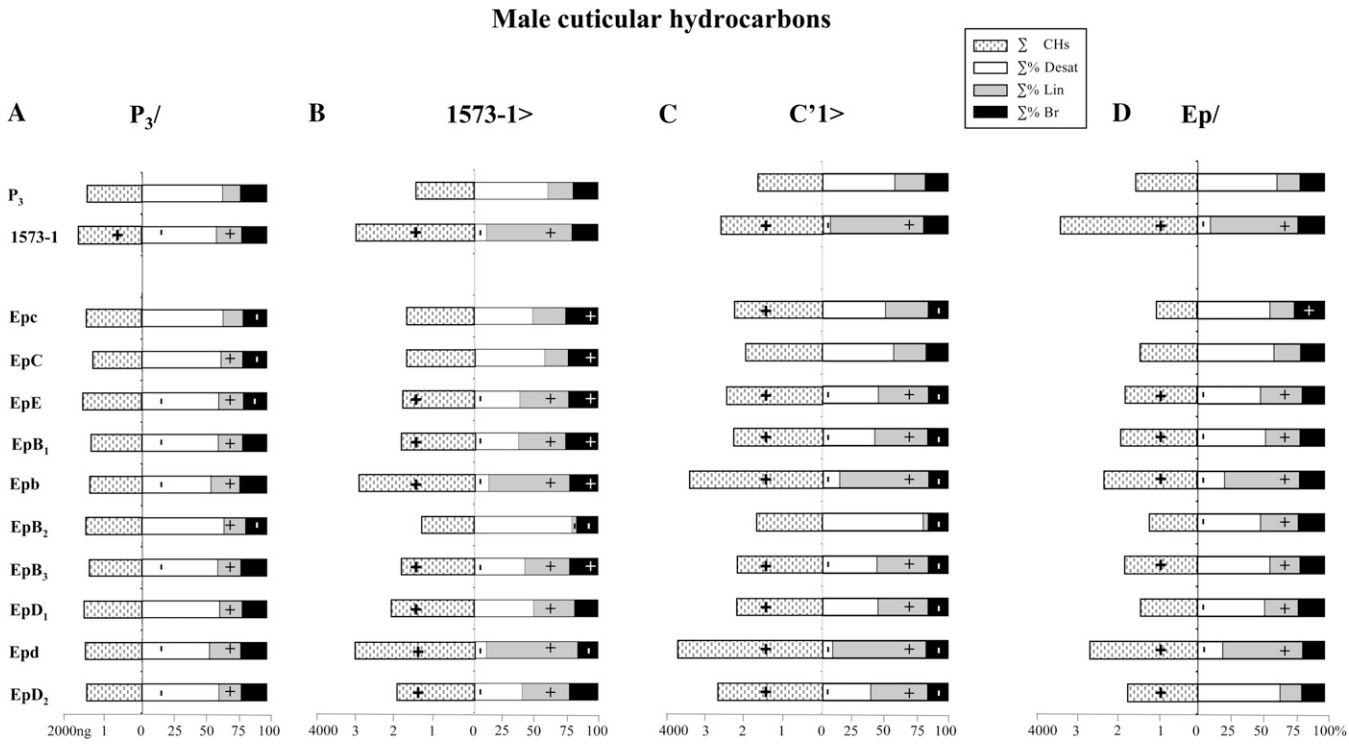

FIGURE 3.—Production of cuticular hydrocarbons in male flies of various strains combining *Ep* transgenes. The *Ep* transgenes used are indicated on the left (*Epc–EpD*$_2$) below two reference *desat1* alleles: the precisely excised *P*$_3$ allele and the original *1573-1* mutant. Reference and *Ep* alleles were tested in four combinations (shown on the top, from left to right): (A) P_3/ with *P*$_3$; (B) 1573-1> driven by *1573-1*; (C) C′1> driven by the null *C′1 desat1* allele; and (D) EP/homozygous. Dotted bars on the left indicate the total absolute amounts of hydrocarbons (∑CHs, in ng). Bars on the right represent the total percentage of desaturated hydrocarbons (∑% Desat, open bars), saturated linear hydrocarbon (∑% Lin, shaded bars), and ramified hydrocarbons (∑% Br, solid bars). The symbol within each bar indicates a significant variation (+, increase; −, decrease; $P < 0.05$; Kruskal–Wallis tests) with the respective P_3 control strain. For more information, see Figure 2 legend. $N = 5$–15.

51/19%. Therefore, no *Ep* transgene induced a dominant effect on female CHs.

1573-1>Epb and >Epd females strongly increased their ∑CHs (3200 ng) compared to control females (2000 ng; Figure 4B). Reciprocally, 1573-1>EpB_2 showed significantly reduced ∑CHs (1450 ng). In parallel to this variation, the ∑% Desat/Lin ratio was much lower in 1573-1>Epb and >Epd females (8/67% and 10/68%, respectively) than in control females (38/28%), whereas it strongly increased in 1573-1>EpB_2 (63/4%). Concretely, 1573-1>Epb females produced 250 ng Desat CHs and 2150 ng Lin CHs against 900 and 60 ng, respectively, in 1571-1>EpB_2 females (750 and 550 ng in control females). In summary, both *Epb* and *Epd* transgenes driven by *1573-1* tended to strongly decrease the ∑% Desat/Lin ratio, whereas *EpB*$_2$ had a reciprocal effect on female CHs.

C′1>Ep females also showed important variations (Figure 4C), which were, however, not always parallel to those detected in 1573-1>Ep females. If C′1>Epb females strongly increased their ∑CHs (3650 ng) compared to control females (2000 ng) this did not vary either in C′1>Epd females (1900 ng) or in C′1>EpB_2 (1670 ng). Nevertheless, C′1>Epb and >Epd decreased their ∑% Desat/Lin ratio (7/71%) similarly to C′1>1573-1 females (7/67%), compared to C′1>P_3 control females (40/28%), whereas C′1>EpB_2 females showed a strongly increased ratio (64/5%). Therefore, *C′1* induced a similar effect on female CHs as *1573-1*, except for Epd, which showed a less dramatic effect.

Homozygous Epb and Epd females produced much higher ∑CHs (2400 and 2250 ng, respectively), and much lower ∑% Desat/Lin ratio (7/67% and 7/71%, respectively) than control P_3 females (1450 ng; 50/23%, respectively; Figure 4D). The CH levels found in Epb and Epd females were close to those of 1573-1 homozygous females (2600 ng; 8/63%). Epb/Epd females and EpE/EpB_3 showed female hydrocarbon patterns resembling those of their respective parents (Table S2B). Epb/EpB_3 and Epd/EpD_2 females (combining a strong- and a mild-effect transgene) showed phenotypes intermediate between both parental females. Therefore, *Epb* and *Epd* transgenes showed a strong recessive effect and no complementation with regard to female CHs.

In conclusion, three *Ep* transgenes induced strong variation on the cuticular profiles of male and female flies. Both *Epb* and *Epd*—either homozygous, combined together, or with each *PGal4* driver—strongly decreased

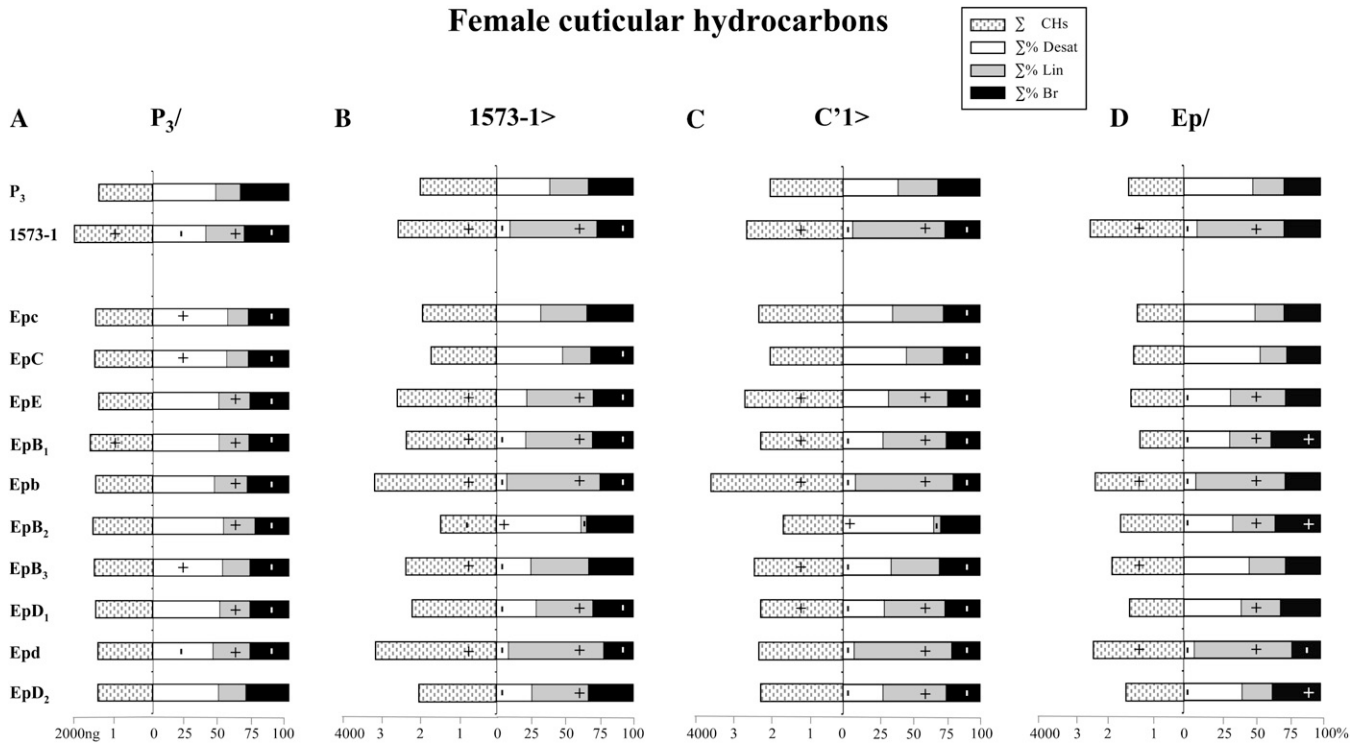

FIGURE 4.—Production of cuticular hydrocarbons in females flies of various strains combining *Ep* transgenes. The *Ep* transgenes (*Epc–EpD*$_2$) and the two reference *desat1* alleles used (*P*$_3$, *1573-1*) are indicated on the left. These alleles were tested in four combinations: (A) P$_3$/with *P*$_3$; (B) 1573-1> driven by *1573-1*; (C) C′1> driven by the null *C′1 desat1* allele; and (D) EP/homozygous. For more information, see legends for Figures 2 and 3. $N = 8$–15.

the ∑% Desat/Lin ratio and increased the ∑CHs. On the other hand, *EpB*$_2$ combined with either *PGal4* driver strongly increased the ∑% Desat/Lin ratio.

Desat1 deregulation and male discrimination behavior: P$_3$/Ep males showed a high discrimination ability similar to that of control P$_3$ males ($P < 0.01$–0.001; Figure 5A). The males with the lowest discrimination ability (P3/Epc, P3/EpC and P3/EpD$_1$) showed significantly decreased CIf ($t = 3.12$–3.54; $P = 0.0023$–0.0013). No CIm variation was detected between genotypes. Therefore, no *Ep* transgene induced a strong dominant effect with regard to male discrimination ability.

The 1573-1>Epb, >EpB$_2$, and >EpD$_2$ males did not discriminate the sex of their partners ($P > 0.05$; Figure 5A). The decreased CIf of 1573-1>EpB$_2$ males (28 *vs.* 50; $F_{10,468} = 2{,}177$; $P = 0.018$) could explain their strongly increased frequency of homosexual males (DCID) compared to control males (47 *vs.* 7%; $P < 0.01$; Figure 5B). Several other genotypes also showed an increased frequency of homosexual males. Therefore, *Ep* deregulation driven by *1573-1* could strongly affect male discrimination, especially with *EpB*$_2$ transgene.

C′1>Epb, >EpB$_2$, and >EpB$_3$ males showed no sexual discrimination and C′1>EpD$_1$ and >EpD$_2$ a slightly reduced discrimination ability ($P < 0.05$; Figure 5A). Their DCID revealed more frequent "homosexual" males. The frequency of "heterosexual" males only decreased in C′1>Epb. If *1573-1* and *C′1* drivers induced similar effects on male discrimination when combined with *Epb* and *EpB*$_2$, *C′1* induced a stronger effect with *EpB*$_3$ and a weaker effect with *EpD*$_2$.

Only homozygous EpB$_1$ males showed no discrimination ability similarly to homozygous 1573-1 mutant males (Figure 5A): Their CIm increased (23 and 27, respectively) compared to control males (10; $F_{11,541} = 2{,}575$; $P = 0.0034$). Strikingly, Epc males showed a high discrimination ability but dramatically reduced CIf and CIm. This low sexual activity was not related to a general behavioral defect since Epc males exhibited a locomotor activity index (LAI = 54), which was not significantly different from that of control P3 males (LAI = 60) and of 1573-1 mutant males (LAI = 48; $K_{2df} = 9.277$, $P = 0.01$). Moreover, if 55% of Epc males did not court during the observation period (5–11% in the other genotypes), the 45% courting males generally showed a delayed courtship latency (data not shown). Fewer "heterosexual" and more "bisexual" males were found in the EpD$_2$ genotype (Figure 5B). No complementation (no discrimination) was shown by Epd/EpD$_2$ males, which were less frequently "heterosexual" compared to Epd males (but not to EpD$_2$ males; Figure S1). On the other hand, EpE/EpB$_3$ and Epb/EpB$_3$ males showed no significant alteration of their discrimination phenotype. In summary, two *Ep* transgenes differently

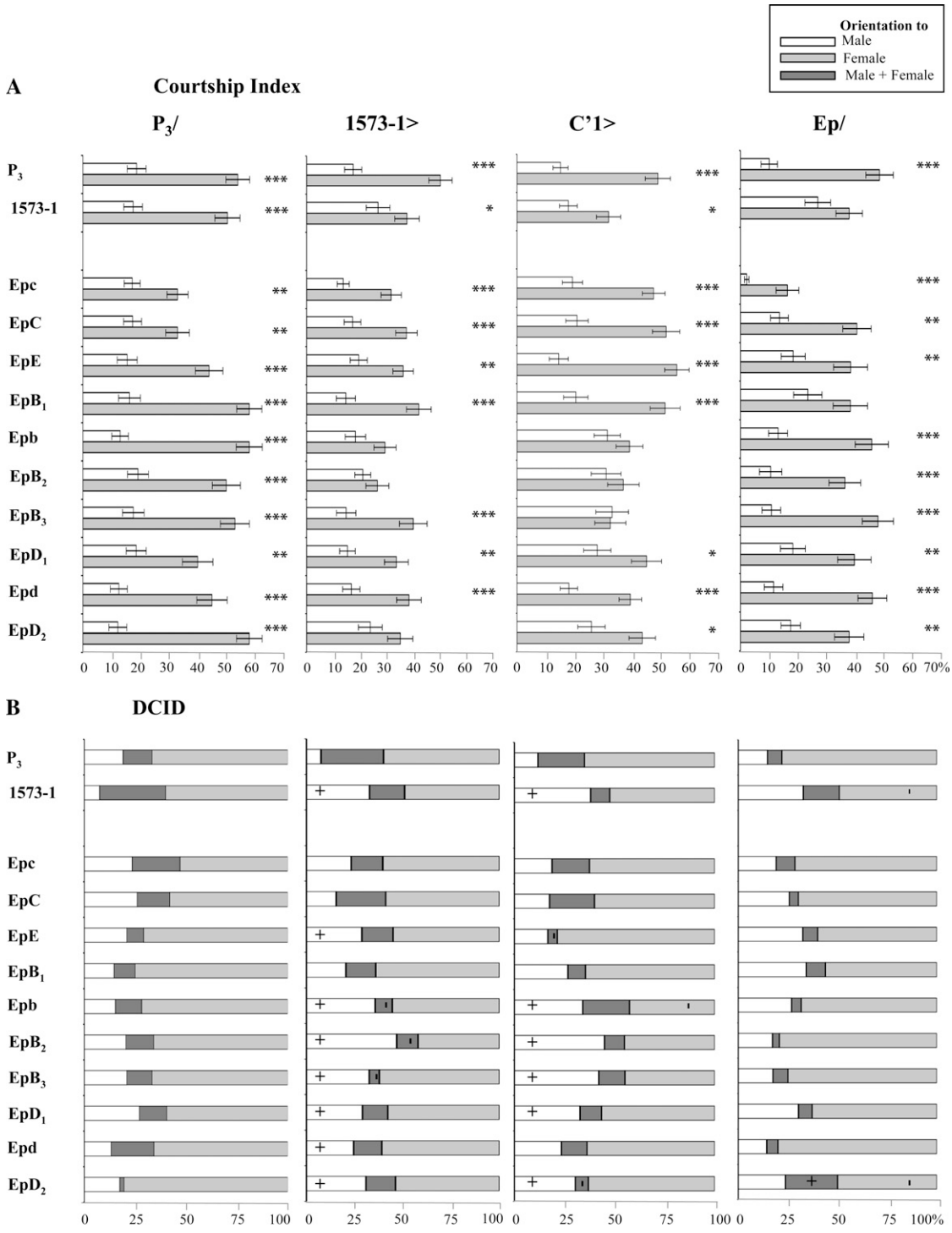

FIGURE 5.—Courtship behavior in males of various transgenic strains. The *Ep* transgenes (*Epc–EpD*$_2$) and the two reference *desat1* alleles (*P*$_3$ and *1573-1*) used are indicated on the left. Reference and *Ep* alleles were tested in four combinations (shown on the top) with *P*$_3$ (P$_3$/), with *1573-1* (1573-1>), with the null *C′1 desat1* allele (C′1>), and homozygous (Ep/). (A) Mean (±SEM) male courtship index to control target male (open bars) and female (lightly shaded bars). A pair of decapitated control target flies (a male and a female) was simultaneously presented to a single tester male, under red light, during a 5-min period. The male ability to discriminate sex targets within each strain is shown above each pair of bars: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$. (B) The distribution for courtship index discrimination (DCID) is a newly designed parameter representing the distribution of predominant sexual orientation in individual males: homosexual (open bars); bisexual (darkly shaded bars); and heterosexual (lightly shaded bars). Only males with a total courtship index ≥10 were kept. The symbol within each bar/sexual orientation indicates a significant variation (+, increase; −, decrease; $P < 0.05$; $\chi^{2)}$ with each respective P$_3$ control strain. For more information, see legends for Figures 2 and 3. $N = 38$–59.

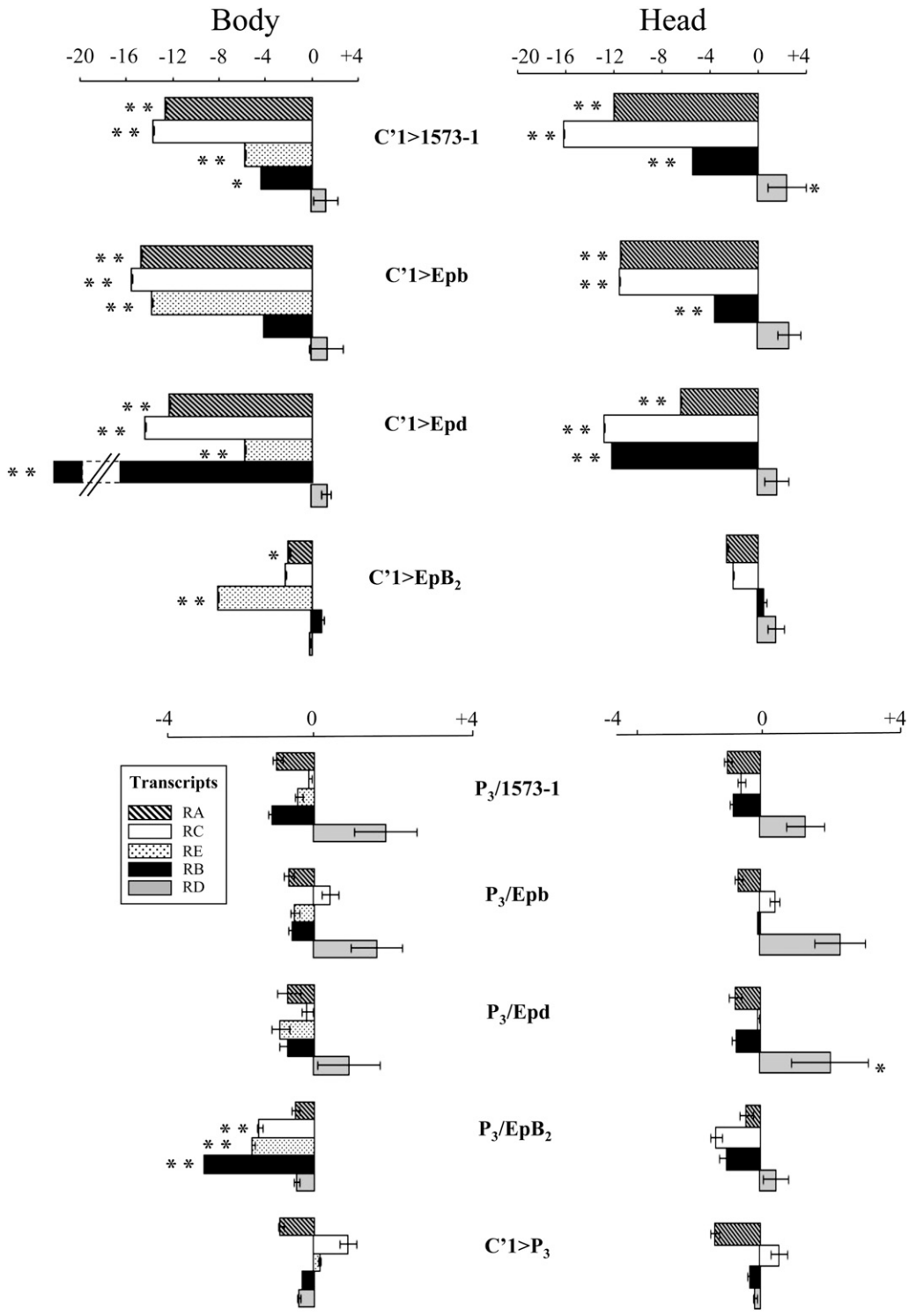

FIGURE 6.——Relative levels of *desat1* transcripts in various transgenic males. Transcripts were quantified with q-PCR separately in the body (left) and in the head (right) of nine transgenic males. The top four experimental genotypes combine the *C′1* allele (C′1>) with either *1573-1*, *Epb*, *Epd*, or EpB_2. The five bottom males represent control genotypes combining the *P3* rescued allele with the five transgenes present in the four experimental genotypes. Bars represent the mean (±SEM) for the relative levels of the five *desat1* transcripts: RA (hatched), RC (open), RE (dotted; not detected in the head), RB (solid), and RD (shaded). Negative and positive values, respectively, indicate significantly decreased or increased levels compared to P_3 control genotype (**$P < 0.01$; *$P < 0.05$). RB was not detected in the body of C′1>Epd males. Note that the Log2 scales differ between experimental and control genotypes. For each biological extraction, q-PCR was replicated three times. $N = 3$ for each tissue and genotype.

affected male sexual behavior: *Epc* altered general sexual activity, whereas EpB_1 altered male discrimination. Moreover *Epd* and EpD_2 transgenes did not complement each other with regard to male discrimination.

Desat1 deregulation and the transcript levels: Our data reveal a very mild transcriptional variation in control genotypes but a strong variation in experimental genotypes (Figure 6). In experimental males, RA and RC strongly decreased in the head and body of C′1>1573-1, >Epb, and >Epd males. RB also decreased in the head of these males ($P < 0.01$) and in the body of C′1>1573-1 and >Epd males ($P < 0.05$ and 0.01, respectively). RE level also strongly decreased in the body of the four experimental genotypes ($P < 0.01$) but was never detected in the head of either experimental or reference *P3* genotypes. Therefore, *desat1* deregulation seems to strongly affect most transcripts in the head and body of males combining *C′1* with *Epb*, *Epd*, or *1573-1*

TABLE 1

Synthesis of the principal results obtained in this study

A. Excision lines		1573-1	L_1	L_2	L_3	L_4	M_1	M_2	S_1	S_2	P_1	P_2	P_3
CHs	Σ% Desat	−	−	−	−	−	−	−	−	−			
	ΣCHs	+	+	+	+	(+)	+			(+)			
Discrimination		−−−	−−−			−−−	−−−		−				

B. Ep lines			1573-1	Epc	EpC_1	EpE	EpB_1	Epb	EpB_2	EpB_3	EpD_1	Epd	EpD_2
CHs	Σ% Desat	1573-1>	−			(−)*	−	−	(+)	−	(−)	−	−
		C′1>	−			(−)*	−	−	(+)	−	(−)	−	−
		Homozygous	−			−	−	−	−		(−)*	−	(−)
	ΣCHs	1573-1>	+			+	+	+	(−)	+	(+)*	+	(+)*
		C′1>	+	(+)*		+	+	+		+	+	(+)*	(+)*
		Homozygous	+			(+)*	(+)*	+		+		+	(+)*
Discrimination		1573-1>	−−			−		−−−	−−−		−		−−−
		C′1>	−−					−−−	−−−	−−−	−−		−−
		Homozygous	−−−		−	−	−−−				−		−

(A) Excision alleles and (B) *Ep* transgenes (driven by 1573-1 = 1573-1>; driven by 1573-C′1 = C′1>; homozygous). For the production of cuticular hydrocarbons (CHs), we only show the total CHs (∑CHs), and the total relative amounts of desaturated CHs (∑% Desat). The "+" and "−" signs indicate an increase and a decrease of the CH level. These variations were parallel in both sexes except when indicated as: ( ) = only in female; ( )* = only in male. For male discrimination, the number of minus (−) signs indicate the degree of alteration for the ability to discriminate: "−" = slight reduction; "−−" = important reduction; "−−−" = no discrimination. For both phenotypes, the absence of sign indicates no significant variation relatively to control genotypes.

transgenes, whereas this effect is less important (and restricted to the body) in C′1>EpB_2 males. Control genotypes only showed lower and sporadic decrease of transcript level. In summary, the quantitative variation of some transcripts could be related to the alteration of CH production but not with that of male discrimination.

DISCUSSION

Desat1 induces pleiotropic effects on *D. melanogaster* pheromonal communication: After showing that a single mutation in the *desat1* gene affected both the production of cuticular pheromones and the male discrimination of these pheromones (Marcillac *et al.* 2005a,b), the present study reveals that these two phenotypes are independently regulated by *desat1* (Table 1). First, the simultaneous rescue of both "production" and "discrimination" phenotypes in P_1–P_3 alleles confirms that the *PGal4* transposon inserted in *desat1* induced both defects. Second, several genetic manipulations allowed us to separately rescue each phenotype (Table 1; see below). The absence of a complete positive relationship between the two phenotypes indicates that they are independently controlled by *desat1*.

Qualitative and quantitative effects on the production of cuticular hydrocarbons: The comparison of male and female CH profiles induced by *desat1* excision alleles revealed a positive relationship between the gravity of this phenotype and the size of the transposon fragment inserted in *desat1* regulatory region: Alleles with the larger fragment showed a very low Desat/Lin ratio whereas shorter-fragment alleles showed a Desat/Lin ratio ≥1. Such a similar "molecular–phenotypic" relationship has been described with a series of *Voila-prospero* alleles (resulting from the remobilization of a *PGal4* transposon) (Grosjean 2002; Grosjean *et al.* 2003).

Three *Ep* transgenes strongly affected pheromone production: *Epb* and *Epd* (i) combined with each *PGal4* driver or (ii) homozygous or (iii) combined together showed strongly decreased Desat/Lin ratio. The EpB_2 transgene driven by either *PGal4* highly increased the Desat/Lin ratio. Since flies with strongly decreased Desat/Lin ratio often showed highly increased ∑CHs, this suggests that Desat CH biosynthesis requires more fatty acid precursors than Lin CH. This agrees with the finding that *desat1* mutation also affects the total levels and fatty acid composition of several phospholipid species in Drosophila (Kohler *et al.* 2009). The few exceptions noted in the "low ratio-high ∑CHs" rule in our study (Table 1; L_4 males and C′1>Epd females) suggest that *desat1* separately controlled quantitative and qualitative aspects of pheromonal production. Moreover, some sex-specific effects were noted (Table 1). They could be related to the influence of sex determination genes such as *doublesex*, which target *desat* genes and other genes involved in sex-specific characters (Waterbury *et al.* 1999; Shirangi *et al.* 2009).

Moreover, *Epb* and *Epd*—but not EpB_2—transgenes show an antisense orientation (relative to the direction of *desat1* transcription), and their deregulation affected some transcript(s) differently. If RE decreased in the

body of all experimental genotypes, RA and RC levels strongly decreased in the head and body (as well as RB in the head) of C′1>Epb, >Epd, but not of C′1>EpB_2. Interestingly, C′1>1573-1 and homozygous 1573-1 males, both genotypes with a strongly decreased Desat/Lin ratio, also showed a drastic general reduction of RA, RC, and RE (Marcillac *et al.* 2005a). Therefore, if the alteration of the pheromonal production is linked to the differential variation of RC, RA, and/or RE in the fly body, RE decrease could induce a general alteration of the CH profile, whereas RC and/or RA decrease could induce the decreased Desat/Lin ratio. Our current data suggest that RC is preferentially expressed in the fat body, while RA is likely expressed in the central nervous system (F. Bousquet and J.-F. Ferveur, unpublished data). Given that (i) the fat body contains the fatty acid precursors necessary for hydrocarbon biosynthesis (Savarit and Ferveur 2002; Zinke *et al.* 2002; Vihervaara and Puig 2008) and (ii) the negative relationship between the RC level and the $\sum$CHs variation in C′1>Epb and >Epd—but not in C′1>EpB_2—flies, the RC transcript may act to repress the overproduction of CHs. On the other hand, a brain hormonal factor (Wicker and Jallon 1995) linked with the strong decrease of RA in the head of C′1>1573-1, >Epb, and >Epd—but not in C′1>EpB_2—flies could change the Desat/Lin ratio. Note that RC, RE, and RB also decreased in the body of P_3/EpB_2 control males, which, however, showed no significant CH variation. This can be explained by the fact that (i) RA did not vary in the head of these control males and/or (ii) their RC decrease was much weaker than in C′1>Ep males.

Effects on male discrimination of sex pheromones and courtship: No clear relationship was found between the discrimination phenotype and the variation of (i) the transposon fragment size or (ii) the transcripts level. Males with severely affected discrimination often showed decreased CIf (≤40) and increased CIm (≥25). They may have lost the ability to perceive the pheromones produced by male and/or female flies (Table 1). Similar cases of reduced sex pheromones discrimination occurred after genetic misexpression in Drosophila chemosensory peripheral nervous system (PNS) (Svetec and Ferveur 2005; Xu *et al.* 2005; Park *et al.* 2006; Koganezawa *et al.* 2010) or central nervous system (CNS) (Ferveur *et al.* 1995). Given that *desat1* is expressed in the chemosensory PNS (sensilla on the proboscis, tarsae, antennae) necessary for pheromonal perception (Marcillac *et al.* 2005a), the fact that we did not detect any relationship between the variation of transcripts and of male discrimination could be explained if two or more transcripts are simultaneously expressed at a very low level in a small number of neural cells. Alternatively, the RNA variation potentially occurring in these cells may have been diluted in the larger global amount of tissue used for q-PCR analysis.

Surprisingly, Epc males showed high sexual discrimination but very low general sexual activity, likely resulting from their delayed courtship initiation (Figure 5A). This indicates that they still perceive sex pheromones but not other non-sex-specific pheromones, which normally elicit male courtship initiation (Savarit *et al.* 1999). Further experiments are needed to verify this hypothesis.

Evolution of desat1 regulation: Our data clearly show that distinct *desat1* regulatory regions and/or transcripts separately control the two major aspects—emission and perception—of pheromonal communication in *D. melanogaster*. Many Drosophila genes affecting behavior have pleiotropic effects. For example, *prospero* affects several aspects of the nervous system development from early embryogenesis to adult life as well as larval and adult behaviors (Knoblich *et al.* 1995; Grosjean *et al.* 2001; Behan *et al.* 2005; Guenin *et al.* 2007). The evolution of *cis*-regulatory elements of two pigmentation genes (*yellow* and *Bric-à-brac*) was shown to change not only the pigmentation pattern of the fly but also sexually dimorphic traits including courtship behavior (Prud'homme *et al.* 2006; Williams *et al.* 2008).

Several genetic mechanisms are associated with the evolution of chemical communication and courtship behavior in Drosophila such as (i) recruitment of new chimeric genes combining genetic sequences with unrelated functions (Dai *et al.* 2008) or (ii) coordinated transcription of functionally related genes organized in a operon-like manner (Ben-Shahar *et al.* 2007). A third evolutionary manner would consist of increasing the complexity of regulatory sequences controlling the expression of a unique product, as in *desat1*. This was the first gene described to control several aspects of sensory communication (Marcillac *et al.* 2005b). The rarity of this mechanism may be explained by the dominant theory that proposed that genes coding for the emission and for the reception of sensory signals should be different (Boake 1991; Andersson and Simmons 2006). Two more loci affecting several aspects of sensory communication have recently been described: The *color interfere* locus changes visual recognition between sex partners in the teleost fish Medaka (Fukamachi *et al.* 2009), and a single QTL linkage group influences acoustic communication between two Hawaiian cricket species (Shaw and Lesnick 2009).

The sequence of *desat1* is highly conserved in insects (Knipple *et al.* 2002) and desaturase genes may have played a key role in the formation of new species by creating new pheromonal molecules after the introduction of double bonds in new positions (Roelofs *et al.* 2002; Rooney 2009). In Drosophila, some very fast evolving *desat* genes (*desat2* and *desatF*) originating from the ancestral *desat1* gene may have been crucial in creating pheromonal diversity (Fang *et al.* 2009; Shirangi *et al.* 2009). If the primary function of desaturated cuticular hydrocarbons resides in the protection against

water loss (Gibbs 2002), their pheromonal role was acquired more recently. Since the *D. melanogaster* subgroup is thought to have evolved 3–4 millions years ago (Lachaise *et al.* 1988), the evolution of *desat1* regulatory elements involved in pheromonal perception may have occurred during that period.

In conclusion, our genetic dissection of *desat1* reveals that both complex pheromonal phenotypes (production and perception) depend upon separate genetic controls. Given that some traits of the two phenotypes (the Desat/Lin ratio and $\sum$CHs for the hydrocarbon production; discrimination and initiation for male behavior) can also be genetically dissociated, this suggests that they depend on the precise control of one or several *desat1* transcripts in specific tissues. Our next goal will consist of analyzing at a fine-grain resolution the expression of *desat1* transcript in these tissues.

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The Consequences of Regulation of desat1 Expression for Pheromone Emission and Detection in *Drosophila melanogaster*

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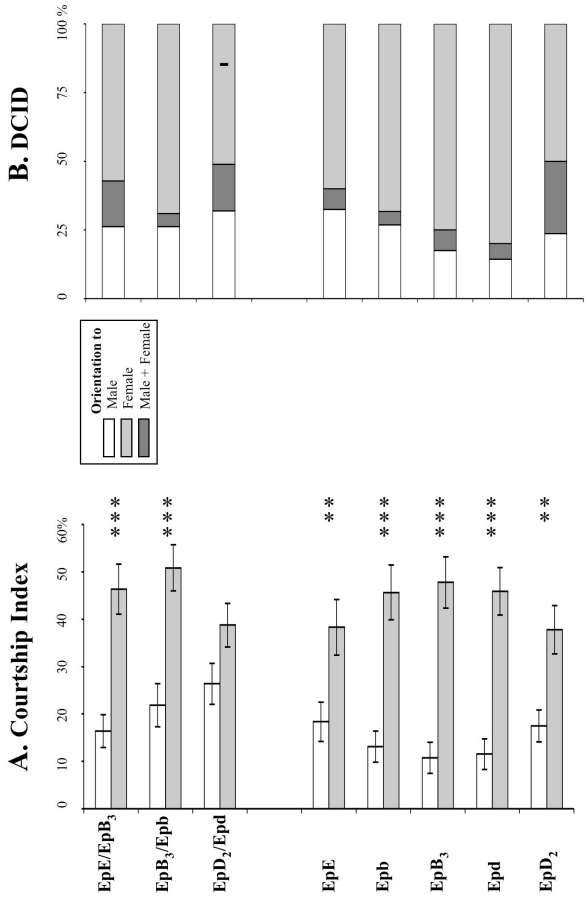

FIGURE S1.—Complementation effect of two different *Ep* transgenes on the male discrimination of sex targets. (A) Histograms show the mean (±sem) courtship index to control target male (empty bars) and female (light shaded bars). The statistic significance for male ability to discriminate sex targets, within each genotype, is shown above each pair of bars : *** : $p<0.001$; **: $p<0.01$; *: $p<0.05$. (B) Histograms represent the distribution for courtship index discrimination (DCID). This indicates the predominant sexual orientation in individual males: homosexual (empty bars) ; bisexual (dark shaded bars) ; heterosexual (light shaded bars). Only males with a total courtship index ≥ 10 were kept. Only Epd/ EpD_2 males showed a significant decrease ($p < 0.05$; Chi2) for the number of males with heterosexual orientation compared to Epd homozygous males. For other informations, see the legend of Figures 2 and 3. N = 42-47. The data shown for homozygous males correspond to those shown on Figure 5.

TABLE S1

Production of cuticular hydrocarbons in (A) male and (B) female mature flies of the experimental genotypes shown on Figures 2-4

A. MALE

Excision Lines		L_1	L_2	L_3	L_4	M_1	M_2	S_1	S_2	P_1	P_2	P_3
	∑ CHs	3466±137	2717±57	2003±108	1280 ±82	1580 ±112	1146 ±111	1467 ±67	1374 ±53	1200 ±92	1344 ±95	1372 ±91
	∑ Desat	240±26	193±16	147±15	535 ±47	759 ±49	617 ±47	724 ±65	756 ±33	736 ±61	913 ±69	926 ±64
	∑ Lin	2537±110	1900±10	1394±117	394 ±25	508 ±89	199 ±20	301 ±30	330 ±21	142 ±10	184 ±10	208 ±15
	∑ Br	689±32	623±49	463±47	351 ±20	311 ±13	330 ±46	442 ±15	228 ±11	321 ±26	247 ±17	238 ±17
1573-1>		**Epc**	**EpC**	**EpE**	**EpB_1**	**Epb**	**EpB_2**	**EpB_3**	**EpD_1**	**Epd**	**EpD_2**	
	∑ CHs	1769±34	1771±72	1866±53	1993±122	3018±90	1730±160	1950±90	2245±81	3157±165	2012±64	
	∑ Desat	831±22	1005±44	681±13	722±102	350±28	1388±142	793±36	1076±80	301±14	777±64	
	∑ Lin	469±18	334±19	733±34	744±49	1964±56	56±4	702±39	738±21	2325±134	764±18	
	∑ Br	469±26	432±30	452±18	527±31	704±37	286±22	455±23	431±19	531±43	471±34	
C'1>		**Epc**	**EpC**	**EpE**	**EpB_1**	**Epb**	**EpB_2**	**EpB_3**	**EpD_1**	**Epd**	**EpD_2**	
	∑ CHs	2302 ±89	2007 ±45	2504 ±72	2294 ±88	3417 ±176	1666 ±96	2284 ±45	2237 ±38	3792 ±395	2726 ±166	
	∑ Desat	1139 ±52	1106 ±35	1087 ±31	931 ±52	464 ±15	1335 ±82	970 ±60	969 ±39	269 ±20	1009 ±102	
	∑ Lin	773 ±32	501 ±23	975 ±38	944 ±50	2411 ±135	63 ±3	914 ±85	870±21	2486 ±306	1180 ±78	
	∑ Br	390±27	400±13	442±16	419±25	542±57	268 ±23	400±36	398±19	1037±331	537±49	
Ep/		**Epc**	**EpC**	**EpE**	**EpB_1**	**Epb**	**EpB_2**	**EpB_3**	**EpD_1**	**Epd**	**EpD_2**	
	∑ CHs	986±75	1374±69	1775±90	1840±131	2260±175	1114±65	1719±62	1333±48	2563±216	1685±65	
	∑ Desat	562±52	827±66	873±56	993±93	442±46	556±42	982±50	701±35	472±65	1092±52	
	∑ Lin	185±14	287±28	583±55	488±26	1343±223	328±20	398±12	350±12	1592±117	283±12	
	∑ Br	237±23	260±22	319±21	358±25	444±68	230±12	339±20	282±17	443±52	310±19	

B. FEMALE

Excision Lines		L_1	L_2	L_3	L_4	M_1	M_2	S_1	S_2	P_1	P_2	P_3
	∑ CHs	2557 ±118	2086 ±190	1804 ±251	1873 ±83	1969 ±104	1509 ±309	1561 ±92	1284 ±73	1572 ±54	1560 ±77	1618 ±50
	∑ Desat	171 ±11	96 ±10	82 ±14	623 ±52	731 ±45	652 ±210	359 ±61	624 ±37	884 ±39	759 ±36	809 ±33
	∑ Lin	1648 ±91	1404 ±189	1138 ±163	767 ±49	650 ±35	367 ±63	801 ±71	766 ±64	233 ±11	257 ±15	288 ±23
	∑ Br	688 ±21	587 ±51	584 ±75	483 ±17	587 ±17	491 ±100	400 ±38	494 ±17	455 ±45	527 ±33	527 ±14
1573-1>		**Epc**	**EpC**	**EpE**	**EpB_1**	**Epb**	**EpB_2**	**EpB_3**	**EpD_1**	**Epd**	**EpD_2**	
	∑ CHs	1937 ±45	1667 ±44	2552 ±19	2361 ±29	3201 ±70	1448 ±55	2381 ±43	2144 ±77	3171 ±52	2004 ±32	
	∑ Desat	643 ±23	778 ±21	588 ±2	523 ±9	248 ±13	915 ±37	614 ±13	641 ±16	302 ±13	527 ±12	
	∑ Lin	640 ±34	332 ±10	1214 ±18	1133 ±33	2154 ±77	59 ±4	991 ±32	867 ±61	2163 ±53	816 ±36	
	∑ Br	654 ±31	557 ±23	750 ±17	705 ±8	799 ±24	474 ±26	776 ±17	636 ±33	706 ±15	661 ±22	
C'1>		**Epc**	**EpC**	**EpE**	**EpB_1**	**Epb**	**EpB_2**	**EpB_3**	**EpD_1**	**Epd**	**EpD_2**	
	∑ CHs	2254 ±69	2011 ±52	2694 ±145	2280 ±88	3662 ±41	1672 ±60	2461 ±69	2339 ±51	1913 ±156	2243 ±37	
	∑ Desat	824 ±25	952 ±38	992 ±238	633 ±20	249 ±17	1070 ±38	718 ±28	685 ±22	114 ±13	659 ±26	
	∑ Lin	819 ±31	506 ±18	1084 ±119	1069 ±54	2633 ±29	91 ±4	1070 ±74	1041 ±32	1351 ±106	1014 ±32	
	∑ Br	611±28	553 ±17	618 ±26	578 ±23	780 ±14	511 ±27	673 ±30	613 ±38	448 ±83	570 ±19	
Ep/		**Epc**	**EpC**	**EpE**	**EpB_1**	**Epb**	**EpB_2**	**EpB_3**	**EpD_1**	**Epd**	**EpD_2**	
	∑ CHs	1205 ±73	1348 ±34	1393 ±65	1181 ±80	2391 ±312	1685 ±55	1947 ±118	1398 ±79	2249 ±294	1644 ±15	
	∑ Desat	636 ±46	753 ±29	478 ±31	408 ±49	187 ±14	606 ±30	934 ±69	585 ±43	196 ±30	676 ±86	
	∑ Lin	243 ±16	259 ±11	553 ±26	357 ±30	1637 ±239	519 ±22	506 ±24	397 ±18	1747 ±223	332 ±24	
	∑ Br	326 ±25	336 ±22	362 ±26	416 ±33	568 ±68	560 ±21	507 ±77	415 ±47	508 ±53	636 ±88	

All data represent the mean ± sem (in ng) for the total absolute amounts of hydrocarbons (∑CHs), and the total amounts of desaturated hydrocarbons (∑Desat), of saturated linear hydrocarbon (∑Lin), and of ramified hydrocarbons (∑Br). (From top to bottom) Excision lines correspond to flies homozygous for various *desat1* excision alleles (indicated above: L_1 to P_3); *1573-1>*, *C'1>*, *Ep/* correspond respectively to each *Ep* transgene (indicated above: *Epc* to *EpD_2*) either driven by *1573-1* or *C'1* allele or homozygous. N = 5-18.

TABLE S2

Complementation effects of two different *Ep* transgenes on the production of cuticular hydrocarbons.

A. Male cuticular hydrocarbon

	∑CHs		∑% Desat		∑%Lin		∑% Br	
EpE/EpB$_3$	*EpE*	*EpB$_3$*	*EpE*	*EpB$_3$*	*EpE*	*EpB$_3$*	*EpE*	*EpB$_3$*
	N.S.	**N.S.**	+	**N.S.**	-	**N.S.**	**N.S.**	**N.S.**
Epb/EpB$_3$	*Epb*	*EpB$_3$*	*Epb*	*EpB$_3$*	*Epb*	*EpB$_3$*	*Epb*	*EpB$_3$*
	-	-	+	-	-	+	**N.S.**	**N.S.**
Epb/Epd	*Epb*	*Epd*	*Epb*	*Epd*	*Epb*	*Epd*	*Epb*	*Epd*
	N.S.	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**	-	**N.S.**
Epd/EpD$_2$	*Epd*	*EpD$_2$*	*Epd*	*EpD$_2$*	*Epd*	*EpD$_2$*	*Epd*	*EpD$_2$*
	-	**N.S.**	+	-	-	+	+	+

B. Female cuticular hydrocarbon

	∑CHs		∑% Desat		∑%Lin		∑% Br	
EpE/EpB$_3$	*EpE*	*EpB$_3$*	*EpE*	*EpB$_3$*	*EpE*	*EpB$_3$*	*EpE*	*EpB$_3$*
	+	**N.S.**	+	-	**N.S.**	+	+	**N.S.**
Epb/EpB$_3$	*Epb*	*EpB$_3$*	*Epb*	*EpB$_3$*	*Epb*	*EpB$_3$*	*Epb*	*EpB$_3$*
	-	**N.S.**	+	-	-	+	+	+
Epb/Epd	*Epb*	*Epd*	*Epb*	*Epd*	*Epb*	*Epd*	*Epb*	*Epd*
	N.S.	**N.S.**	+	+	**N.S.**	-	**N.S.**	**N.S.**
Epd/EpD$_2$	*Epd*	*EpD$_2$*	*Epd*	*EpD$_2$*	*Epd*	*EpD$_2$*	*Epd*	*EpD$_2$*
	N.S.	**N.S.**	+	-	-	+	+	-

These effects were measured in 5-day-old males (A) and females (B). The comparison was carried out with both homozygous same-sex parent on the four following hydrocarbon parameters: the total absolute amounts of hydrocarbons (∑CHs, in ng), the total percentages of desaturated hydrocarbons (∑%Desat), of saturated linear hydrocarbon (∑%Lin), and of ramified hydrocarbons (∑%Br). For each combined genotype, the sign indicates a significant variation (+ = increase ; - = decrease ; N.S. not significant ; $p < 0.05$; Kruskal-Wallis tests) with either parent. N = 8-15.