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**Dietary rescue of altered metabolism gene reveals  
unexpected *Drosophila* mating cues**

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## Abstract

To develop and reproduce, animals need long-chain Mono- and PolyUnsaturated Fatty Acids (MUFAs; PUFAs). Although some UFAs can be synthesized by the organism, others must be provided by the diet. The gene *desat1*, involved in *Drosophila melanogaster* UFA metabolism, is necessary for both larval development and for adult sex pheromone communication. We first characterized *desat1* expression in larval tissues. Then, we found that larvae in which *desat1* expression was knocked down throughout development died during the larval stages when raised on standard food. By contrast pure MUFAs or PUFAs—but not saturated FAs—added to the larval diet rescued animals to adulthood with the best effect being obtained with oleic acid (C18:1). Male and female mating behavior and fertility were affected very differently by preimaginal UFA-rich diet. Adult diet also strongly influenced several aspects of reproduction: flies raised on C18:1-rich diet showed increased mating performance compared to flies raised on standard adult diet. Therefore, both larval and adult *desat1* expression control sex-specific mating signals. A similar nutrigenetics approach may be useful, in other metabolic mutants, to uncover cryptic effects otherwise masked by severe developmental defects.

Key-words: lipid; fitness; hydrocarbon; cis-Vaccenyl acetate

Running title: Dietary rescue of metabolic mutation and reproduction

## INTRODUCTION

Lipids are widely used by plants and animals. In particular, fatty acids (FAs) are components of the cell membrane and are also involved in cellular signaling (1-3). In animals, FAs are necessary for many functions related to development and reproduction but the link between these two aspects is not clear (4-6). FAs can vary both in their carbon chain length and in their level of unsaturations (i.e., number of double bonds on the carbon chain). FAs can either have no double bond (called Saturated FAs; SFAs), or have one or more double-bond(s) (Mono- and PolyUnsaturated FAs, called MUFAs and PUFAs, respectively). If animals can only synthesize some UFAs, they are auxotroph for others, which they must therefore find in their diet. Desaturase enzymes often play a pivotal role in the FA metabolism of plants, vertebrates and invertebrates (7-9). Genetic alteration of FA-metabolism enzymes can induce severe human diseases (10-12), underscoring their importance.

The high genetic conservation between *Drosophila melanogaster* and vertebrates makes it a valuable experimental model organism to study metabolic functions, including lipid metabolism (7, 13, 14), however with some limitation (15). This invertebrate species, which is particularly suitable to study the relationship between nutrients and gene function (“nutrigenetics, nutrigenomics”), is also used as a translational model (16). A good picture of nutrigenetic conservation is provided by a peculiar dietary treatment (“Lorenzo oil”, a mixture of UFAs), which can partially cure adrenoleukodystrophy (ALD), a fatal X chromosome-linked brain disease resulting in the accumulation of very long chain FAs (VLCFAs) (17). Interestingly, a *Drosophila* type of “Lorenzo oil” made with UFAs can cure a ALD-related neuronal degenerative model in the fly (18).

In *D. melanogaster*, the  $\Delta^9$ -desaturase gene *desat1* is required both for development and reproduction: null *desat1* mutants die during larval life (19, 20) whereas hypomorphic *desat1* mutant flies show altered sex pheromone production and perception. The pleiotropic effect of the *desat1* gene is reflected by its varied adult expression in neural and non-neural tissues (21, 22).

We investigated the relationship between lipid-dependent metabolism and adult reproduction. We first characterized larval tissue-specific expression driven by each *desat1* regulatory region. Then, we used *desat1* full regulatory region (6908bp) to target a *desat1*-RNAi transgene. Knockdown of *desat1* throughout larval life was lethal to animals fed on standard food. Interestingly, however, we found that lethality could be rescued by supplementing the larval diet with fatty acids (FAs). We compared the ability of 6 FAs to rescue larvae to adulthood and their effect on mating and fertility performance. The effect of oleic acid added to the adult diet on mating behavior and on the production of sex pheromones was also measured.

## RESULTS

### 1) *desat1* tissue-specific expression during larval development

In early third instar larvae (L3), *desat1* expression was explored by targeting the fluorescent UAS-CD8-GFP reporter transgene with each of the five *desat1* putative regulatory region = PRR (RA; RC; RE; RB; RD; Table 1 & Fig. S1). We found that PRR(RA) targeted the gonads, CNS, and gut, PRR(RC) the fat body, and PRR(RE) the oenocytes; PRR(RB) targeted the Malpighian tubules, hindgut, and anterior fat body; PRR(RD) labeled the spiracular glands, the proventriculus, and a small subset of neurons in the CNS. The larval

expression driven by each PRR is somewhat consistent with the adult expression targeted by corresponding PRRs (21).

## 2) Developmental lethality and adult rescue with fatty acids

Similarly to what occurs in the null homozygous mutant alleles, *desat1-1573-C'1* (19), and 11A; (20), we found that the double transgenic genotype combining a copy of the full *desat1* regulatory region (6908bp) fused with Gal4 driving a copy of the UAS-*desat1*-IR transgene (+/6908-Gal4; UAS-*desat1*-RNAi/+; this genotype is hereafter designated as “IR”) died during second instar stage (L2) when raised on standard lab food (corn/yeast/agar). Therefore, this IR genotype produced no viable adult progeny (Fig. 1A-C). Probing early second instar larvae by qPCR revealed that the level of *desat1* RNA was drastically decreased in IR transgenics compared to both transgenic parental controls, but was still significantly higher than the levels of the null *desat1* 11A mutant allele (Table S1).

### a) FA-preimaginal diet and adulthood rescue

In an attempt to rescue this lethal phenotype, which is likely caused by a metabolic defect blocking 2<sup>nd</sup> to 3<sup>rd</sup> instar molting (Fig. 1C, we fed IR larvae with standard lab food supplemented with six different long chain fatty acids (FAs; Fig. 1). These FAs, suspected to be some of the best candidates to rescue adulthood, have 16 or 18 carbon-chain and carry 0, 1, 2 or 3 unsaturated double-bonds (Fig.S2; (23)). The effect of each FA-rich diet—provided during the complete preimaginal development—was first measured with regard to their ability to rescue viability in IR pupae and adults (Figure 1B-D). Saturated FAs (SFAs; palmitic and stearic acids; C16:0 and C18:0) caused no developmental rescue of the IR genotype, whereas each parental transgene (namely +/6908-Gal4 or UAS-*desat1*-IR/+ alone) produced a substantial frequency of pupae and adults when raised on either SFAs (Table S2). Conversely,

when MUFAs (palmitoleic and oleic acids; C16:1 and C18:1, respectively), or PUFAs (linoleic and linolenic acids; C18:2, C18:3, respectively) were added in the larval food, they caused a high pupal and adult survival (87-94% and 77-88%, respectively) in IR individuals.

***b) Critical period of exposure to oleic acid***

We used C18:1—which induced a high adult rescue—to delimitate the period during which it is critical during larval development (Fig. 1E). Exposure to this fat diet either during the L1 (24-48 hours after egg laying = h AEL) or the L2 stage (48-72h AEL) had no effect. Feeding this diet during both L1 and L2 produced 59% pupae and 17% adults, but these adults were very small compared to wild-type flies (Fig. 1F). Pupal and adult survival rates increased when exposure to C18:1-rich diet was extended to 90h AEL. A full developmental rescue (with regular-size adults) was obtained when IR larvae were raised on fat diet until 96h AEL (corresponding to mid-L3 stage).

***c) Preimaginal UFA-rich diet and reproduction***

Preimaginal diet with different UFAs also differentially rescued several aspects of male and female reproduction (Figs. 2 and S3; Table S3). When paired with wild-type females, UFA-fed IR males showed a low copulation frequency (8-35%), whereas in the reciprocal cross, UFA-fed IR females showed a much higher performance (>50%), especially when fed on MUFAs (72-80%; Fig. 2A). However, these four UFA-rich diets induced differential effects on fertility with C18:1 resulting in the best performance in both female and male IR flies (Fig. 2B). Finally, the fecundity was always much higher in the crosses involving IR males than IR females (Fig. 2C). Overall, for a similar UFA-diet, IR males always yielded a higher number of adult progeny (per mating pair) than did IR females, and C18:1 allowed a high performance in both sexes (Table S3).

### 3) Effect of C18:1 in adult diet

Given the high rescue induced by C18:1 preimaginal feeding on adult reproductive characters, we further tested its effect when also provided in the adult diet. Mating performance and production of cuticular hydrocarbons—two characters related to those controlled by *desat1* (discrimination and production of cuticular pheromones; (19, 21, 24))—were compared in IR individuals raised on C18:1-rich food during either (i) preimaginal development only (IR<sup>-</sup>) or (ii) both preimaginal and adult stages (IR<sup>+</sup>).

#### a) Mating with wild-type females

We first tested mating pairs involving wild-type females (WT; Figs. 3 & S3B; Table S4; crosses #1-5). Within one hour, 75% WT pairs—preimaginally raised on standard food—mated to yield on average 80 viable adults (#1). WT pairs raised on C18:1-rich diet (preadult + adult) showed similar performances (n=136; 76% mating; 99 adults/mated female; (#1+C18:1, Fig. S3B; Table S4; #1\*). By contrast, IR males “adult-fed” on standard food (IR<sup>-</sup>) showed greatly decreased mating performance (35%; #2) but still showed a WT-like fertility. The intense courtship activity observed in most non-mating “WT females x IR<sup>-</sup> males” pairs suggests that their relatively low mating performance was caused by decreased female receptivity (25, 26). Since this effect may result from altered cuticular hydrocarbons (CHs) in IR<sup>-</sup> males ( (27); Table 2), we “perfumed” these males with WT male CHs. This significantly increased their mating performance and also the duration of their copulation (#3; Fig.3; Table S4). On the other hand, whereas IR<sup>+</sup> males (= adult-fed with C18:1; #4) showed a high mating performance (and a reduced fecundity), their “perfume” did not rescue the low mating performance of IR<sup>-</sup> males (#5).

### ***b) Mating with transgenic females***

Similarly treated males were paired with IR<sup>-</sup> and IR<sup>+</sup> females (Figs. 3 & S3 C,D). As observed for WT females, IR<sup>-</sup> females frequently mated with WT males (80%, #6), but not with IR<sup>-</sup> males (#7), whose performance was largely rescued with WT male “perfume” (#8). However, and differently from WT females, IR<sup>-</sup> females mated less frequently with IR<sup>+</sup> males (#9). This defect was rescued in females “perfumed” with WT females CHs (#10). However, similarly “perfumed” IR<sup>-</sup> females remained unreceptive to IR<sup>-</sup> males covered with IR<sup>+</sup> male “perfume” (#11). On the other hand, IR<sup>+</sup> females (#12-16) showed a similar mating pattern compared to WT females: they frequently mated with WT males (#12), and their low performance with IR<sup>-</sup> males (#13) was rescued with WT male CHs (#14). Although IR<sup>+</sup> males showed a high mating performance (#15), their “perfume” strongly decreased the mating performance of IR<sup>-</sup> males (#13 vs #16). Overall, IR females showed a very reduced fecundity (1-17 adult progeny/mated female), compared to WT females, independently of the male partner (Table S4).

## **4) Cuticular pheromones**

### ***a) Effect of genotype and perfume***

The comparison of cuticular hydrocarbons (CHs) in WT vs IR flies showed a large quantitative difference in their total amounts of saturated CHs ( $\sum$ Lin) and of unsaturated linear CHs (mono-unsaturated =  $\sum$ monoenes in both sexes; and di-unsaturated =  $\sum$ dienes in females; Table 2). Moreover, the inter-genotype variation for the overall amount of CHs ( $\sum$ CHs) was more important in males than in females. Overall, the CH profile of IR flies was very similar to that reported in other *desat1* altered genotypes (19, 21, 24). Note also that all WT and IR males produced low but substantial levels of dienes (which were previously thought to be exclusively produced by females).

The CH profiles of IR<sup>-</sup> flies “perfumed” with WT were somewhat intermediate between those of WT and IR<sup>-</sup> flies indicating that WT CHs—including 7-tricosene (7-T) and 7,11-heptacosadiene (7,11-HD), the major male and female CHs, respectively—were partly transferred. However, IR<sup>+</sup> “perfume” induced no CH difference between “perfumed” and “non-perfumed” IR<sup>-</sup> males. Note also that the amount of *cis*-Vaccenyl Acetate (cVA; (28, 29))—a non-CH male pheromone—was strongly decreased in all IR males irrespective of their adult diet status or perfume.

### ***b) Effect of C18:1-rich diet***

For each sex, intra-genotype comparisons revealed a differential effect of C18:1-rich food diet depending on the sex, genotype, and feeding period. In WT males, only adult C18:1-rich diet increased  $\Sigma$ CHs including 7-T (+46%) and 7- and 9-pentacosene (7-P, 9-P; Table S5A). The other CHs did not show such a diet-dependent variation. In contrast, in IR males, adult C18:1-rich diet tended to decrease  $\Sigma$ CHs and  $\Sigma$ Lin (-15%, -17%, respectively). Note also that cVA production strongly decreased with preimaginal C18:1-rich diet (-29 to -39%) in WT males. In females, adult C18:1-rich diet moderately increased  $\Sigma$ Lin for both genotypes, whereas desaturated CHs did so only for IR females (Tables 2 and S5B).

## **DISCUSSION**

FA-rich diet can rescue a genetic defect related to abnormal FA processing in *Drosophila*, and such diet has already been used to prevent neurodegeneration (in a VLCFA mutant; (18)) and to rescue adult viability (in a *Drosophila* Sterol Regulatory Element Binding Protein—dSREBP—mutant; (23)). Whereas these defects could be partially cured after preimaginal feeding with glyceryl trioleate oil (GTO) or with soy lipid extract (or C18:1), respectively, the

beneficial effect of the adult diet was only clear in the VLCFA mutant. Here, we describe the effects of both larval and adult FA-rich diet on the rescue of a variety of defects caused by the alteration of *desat1* function. As an alternative to the genetically-based conditional repression ( $\text{Gal80}^{\text{ts}}$ ), which allows gene expression to be modulated during a defined developmental period, our “nutri-transgenic” approach made use of different UFAs to induce a variable rescue for different phenotypes expressed by *desat1* knockdown transgenics. Together, our findings indicate that *desat1* expression is normally required in larval and adult tissues to permit normal development and sensory communication during courtship in both sex-partners.

#### ***Link between development and cuticular hydrocarbons***

The approach used in the present study allowed us to pinpoint several developmental and reproductive aspects related to *desat1* expression during larval and adult development. The embryonic and larval spatial expression of *desat1* prefigures its adult pattern of expression (21). In larvae, *desat1* is expressed in tissues related to metabolism (fat body, oenocytes, gut, Malpighian tubules), reproduction (genital discs) and behavior (nervous system). Absence or knockdown of function induced lethality in 2<sup>nd</sup> instar larvae supporting the idea that *desat1* is an essential gene (7). The rescue to adulthood by preimaginal UFA-rich diet suggests that lethality is caused by a metabolic deficit that prevents the synthesis, storage and/or use of the desaturated FAs necessary for larval development. However, providing a UFA-rich diet during preimaginal and adult stages did not rescue adult defective CH profile (Table 2). This indicates that FAs are required during both preimaginal and adult stages to allow both correct late preimaginal development and adult CHs biosynthesis. If the two phenotypes depend on different levels of FA precursors, it suggests that the amount of desaturated FA required for development is not sufficient for a normal production of adult desaturated CHs. If this were

true, it indicates that the production of desaturated CHs requires a larger amount of CH precursors than do saturated CHs. This may explain why most *desat1* mutations (decreasing the proportion of unsaturated CHs) dramatically increase the overall CH level ( $\Sigma$ CHs; (19, 24)). Alternatively, this dissociation could mean that the FA precursors necessary for development and for CHs are distributed in different tissues.

The dSREBP gene involved in FA transport showed a larval expression pattern which overlapped with that of *desat1* gene in several metabolic tissues (fat body, oenocytes, gut; (23)). Similarly to *desat1*, 2<sup>nd</sup> instar larval lethality induced by a dSREBP null mutation was rescued by feeding larvae with a C18:1-rich diet. This suggests that the two genes are co-expressed, or at least overlap, in some larval tissues involved in lipid processing. However, although development to adulthood was rescued by ectopic expression of dSREBP in the fat-body and gut, we do not yet know whether a similar tissue-specific expression of *desat1* is sufficient to rescue development and/or CHs.

### ***Preimaginal diet and adult reproductive ability***

Several aspects of reproduction (mating behavior, fertility and fecundity) were strongly decreased in *desat1* knockdown adults, and we found that UFAs added to the larval diet differently rescued these characters in males and females. Thus, preimaginal UFA-rich diet significantly rescued both female mating behavior and male fecundity, and induced differential effects on fertility. The four UFAs tested induced different effect on these characters, with C18:1 causing the greatest rescue for both sexes. This indicates that preimaginal FA-rich diet influences the development of larval tissues that will be involved—after complete metamorphosis—in female and male behavior and fertility. This is consistent with the fact that *desat1* larval expression can be detected in both neural and

metabolic/reproduction related tissues (Table 1; Fig. S1). Our preliminary dissection of male and female gonads did not reveal any obvious difference in WT vs. IR flies. We also found that adult—but not preimaginal—C18:1-rich diet decreased the fecundity of IR<sup>-</sup> males with WT females (compare #2 and 4).

### ***Effect of adult diet on mating signals***

The fact that C18:1-rich adult diet rescued mating behavior in both females and males (Figs. 2 & S3) indicates that *desat1* expression during adult development is normally required for the exchange of sensory signals between sexual partners. The behavioral effect of the adult diet is clearly apparent when comparing the performance of IR<sup>+</sup> pairs (#15) with IR<sup>-</sup> pairs (#7) or with reciprocal IR<sup>-</sup> / IR<sup>+</sup> pairs (#9, #13). The partial transfer of WT CHs (via WT “perfume”) allowed us to largely rescue the mating performance in both IR<sup>-</sup> males (#3,8,14) and females (#10). Taken together these results indicate that *desat1* is involved in the production of sensory cues used reciprocally during the courtship interplay preceding copulation (25, 30). Whereas IR<sup>+</sup> males showed a normal mating with WT and IR<sup>+</sup> females (#4,15), the rescue of the low mating observed between IR<sup>+</sup> males and IR<sup>-</sup> females (#9) was only induced by WT female “perfume” (#10). This indicates that female CHs changed the behavior of IR<sup>+</sup> males, which in turn performed a courtship behavior more potent to stimulate female sexual receptivity and copulation.

The sensory effect of C18:1-rich adult diet remains largely unexplained. We found that adult FA-rich diet rescued a high mating performance in IR<sup>+</sup> males (except with “non-perfumed” IR<sup>-</sup> females; cross #9) and in IR<sup>+</sup> females. However, IR<sup>+</sup> male cues had no effect when transferred to IR<sup>-</sup> males (# 5 & 11). Furthermore, these cues strongly decreased the mating performance of IR<sup>-</sup> males paired with IR<sup>+</sup> females (#16). The low variation of chemical profile

between IR<sup>+</sup> and IR<sup>-</sup> males covered—and not—with IR<sup>+</sup> males “perfume” suggests that the performance of IR<sup>+</sup> males depends neither on CHs nor on cVA (Tables 2 & S4). Indeed, WT males preimaginally-raised on C18:1 produced less cVA but copulated slightly faster, at least during the first 30 min of the test, when compared to WT flies raised on regular food (#1+C18:1; Fig. S3B; (31)). We do not yet know the identity of the mating signal(s) involved here, but the strong negative effect observed in the cross #16 suggests that they are, at least partly, transmitted during the “perfuming” procedure. In all cases, our study reveals that the production and/or emission of these unknown cues depend on *desat1* expression during early adult life.

In summary, our study reveals that *desat1* is an essential gene for *Drosophila* development and is also involved in a variety of reproduction-related characters and determines several types of sensory mating cues that depend on its expression during both preimaginal and adult life.

## MATERIAL AND METHODS

### Strains

*Drosophila* strains were raised and tested at  $24 \pm 0.5^\circ\text{C}$  with a  $60 \pm 5\%$  humidity and a 12:12 L:D cycle. Stocks were maintained in 150 ml glass vials containing 50ml of our standard laboratory medium (agar 1.5%, yeast 10%, corn meal 9% and methyl para-hydroxybenzoate 0.4%). We used the Canton-S line (CS) as a control, the *UAS-desat1-RNAi* transgenic line to knock down *desat1* expression (#33338 ; VDRC, Austria), and the *UAS-CD8-GFP* transgenic line (*UAS-GFP*; stock #23300; Bloomington *Drosophila* Stock Center, Indiana University, USA) to visualize the expression driven by each *desat1* putative regulatory region

[*PRR(desat1)-Gal4*]. The construction of these GAL4 drivers, made with the fusion of the Gal4 sequence and each putative regulatory regions (PRR) of each of the 5 first alternatives *desat1* exons (*PRR(RA)* to *PRR(RD)*), is described elsewhere (21). We also used a Gal4 transgene containing the complete *desat1* sequence fused to GAL4 (*(6908-Gal4)*; *GreD-Fly Facility*, Clermont-Ferrand, France). Both the *UAS-desat1-RNAi* and the *6908-Gal4* transgenes were introduced in the genetic background of the  $w^{1118}$  strain deriving from the CS original strain. Our rationale was to knockdown *desat1* expression in tissues where *desat1* is normally expressed. If these transgenes also affect *desat1* in tissues where it is not expressed, this should have no functional consequence. The 1573-C'1 and 11A lines were generated by the imprecise excision of the *P* element, either from the original mutant *desat1*<sup>1573</sup> allele (1573-C'1 : (19)) or from the *desat1*<sup>EY07679</sup> allele (11A : (20)) using  $\Delta 2-3$  genomic source of transposase. These lines are missing the *desat1* gene coding region and are homozygous lethal. Each mutation was maintained with the balancer chromosome TM3, *Sb Kruppel-Gal4*; *UAS-GFP* (#5195 ; Bloomington); GFP fluorescence was used to discriminate between homozygous and heterozygous embryos.

For FA-diet rescue experiment, our initial assays involving both *desat1* null alleles showed highly variable rescue effects. This is why we used *desat1* RNAi knockdown (“IR”), which showed more stable effect with the different FAs tested.

## Diet

**Preparation of fatty acid-rich food.** To assess the diet-induced rescue of *desat1* transgenics, we used six pure fatty acids (FA) at concentrations which were previously tested with both larvae and adults (32). Stock FA solutions were prepared by diluting each FA in ethanol to produce a 3 M stock solution. These solutions were aliquoted and stored at -20°C. Each FA stock solution was added to heated standard media (60°C) to obtain a 5 mg / ml final

concentration (0.5%) and mixed using a metal stirrer. The media was then allowed to cool until solidified, stored at 4°C in a dark room to avoid oxidation, and used within one week. Palmitic acid (#P0500), palmitoleic acid (#P9417), stearic acid (#W303518), linoleic acid (#L1376) and linolenic acid (#L2376) were purchased from Sigma-Aldrich. Oleic acid (#06-4010) was purchased from Strem Chemicals Inc. (Bischeim, France).

**Determination of the critical period.** To synchronize larval age, gravid females were transferred every two hours onto fresh egg-laying medium, and larvae were regularly collected. First, or 2<sup>nd</sup> instar larva (depending on the test) progeny from the cross between *UAS-desat1-RNAi* females and *6908-Gal4* males were transferred in groups of 20 to tubes containing the FA-rich diet or to standard medium. After a variable period of time (depending on the test), larvae were washed twice in 0.1X PBS solution, then transferred to fresh standard medium. The number of 3<sup>rd</sup> instar larvae, pupae, and emerging adults were counted in each vial. Our data correspond to the mean of 6 replicates for each test.

## Reproduction

**Preparation of flies.** Wild-type and transgenic flies were sexed within 1±1 hour of emergence under light CO<sub>2</sub> anesthesia and kept in groups of 10 same-sex flies in vials with fresh, plain or FA-rich, food. For “perfuming” experiments, cuticular compounds were passively transferred between donor and receiver flies kept in a restricted space. For this male and female flies used as donors were kept in vials in groups of 50, until 4-6 days old. For the perfuming between WT and IR flies, we used donors from the *w<sup>1118</sup>* line (CS with white eyes) to allow separation from transgenic receivers (*w<sup>+</sup>*, with red eyes). For the experiment involving the passive transfer from IR<sup>+</sup> donors to IR<sup>-</sup> receivers, one wing of the donors was clipped to allow separation under binocular microscope. To transfer cuticular compounds, 8-10 receiver flies

were introduced with the 50 donors the day before the test and kept overnight. A few hours before the test donors and receivers to be tested were separated on the basis of either eye color or wing shape (see above). All experiments were performed at  $24 \pm 0.5^\circ\text{C}$  and  $60 \pm 5\%$  humidity. Tests were completed over several days and took place 1-4h after lights on.

***Mating behavior, fertility and fecundity.*** One male was aspirated (without anesthesia) under a watch glass used as an observation chamber ( $1.6 \text{ cm}^3$ ). After 10 minutes, a virgin female was introduced. Each test was performed for 60 minutes under white light and the overall frequency of copulation was measured for each treatment. We calculated the cumulative proportion of flies mating during this observation period. We also noted the duration of copulation (time between copulation onset and separation). Females from the mating pairs were then kept isolated (the male was discarded) and the presence (fertility) and number (fecundity) of adult progeny noted. The sex ratio of the progeny was also determined.

### **Histology**

Larvae (2<sup>nd</sup> and 3<sup>rd</sup> instar) were dissected in PBS and each dissected tissue was placed between a coverslip and a glass microscope slide in Vectashield (Vector). The developmental stage of larvae was determined based on the shape of their mouth hooks. Histological observations were carried out with a Leica TCS SP2 AOBS confocal microscope (a Arg laser used to excite the Alexa Fluor 488 was coupled with a He/Ne laser used to excite Alexa Fluor 594) and the images were analyzed using the Leica Confocal Software on the Dimacell platform (Université de Bourgogne).

### **Pheromones**

5-day-old flies were frozen for 5 min at  $-20^{\circ}\text{C}$  and individually extracted for 5 min at room temperature using  $30\mu\text{l}$  of a mixture of hexane and methylene chloride (50/50; vol/vol), in order to extract a high variety of compounds. The solution also contained  $3,33\text{ ng}/\mu\text{l}$  of C26 (*n*-hexacosane) and  $3,33\text{ ng}/\mu\text{l}$  of C30 (*n*-triacontane) as internal standards. Cuticular hydrocarbons and *cis*-Vaccenyl acetate were quantified by gas chromatography using a Varian CP3380 gas chromatograph fitted with a flame ionization detector, a CP Sil 5CB column (25 m x 0,25 mm internal diameter;  $0,1\ \mu\text{m}$  film thickness; Agilent), and a split–splitless injector (60 ml/min split-flow; valve opening 30 sec after injection) with helium as carrier gas (50 cm/sec at  $120^{\circ}\text{C}$ ). The temperature program began at  $120^{\circ}\text{C}$ , ramping at  $10^{\circ}\text{C}/\text{min}$  to  $140^{\circ}\text{C}$ , then ramping at  $2^{\circ}\text{C}/\text{min}$  to  $290^{\circ}\text{C}$ , and holding for 10 min. Individual CH profiles were determined by integration of 46 peak areas in males and females, representing all the peaks that could consistently be identified in all individuals. The chemical identity of the peaks was checked using gas chromatography – mass spectrometry equipped with a CP Sil 5CB column, and yielded data that were consistent with those obtained in our previous study (29). The amount (ng/insect) of each component was calculated on the basis of the readings obtained from the internal standards. Twenty flies were tested per condition.

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## LEGENDS

**Table 1. Summary of *desat1* tissue-specific expression in 3<sup>rd</sup> instar larvae.**

Tissue-specific expression was performed in genotypes combining each *desat1*-*PRR*-Gal4 transgene (*PRR(RA)*, *PRR(RC)*, *PRR(RE)*, *PRR(RB)*, *PRR(RD)*) with *UAS-CD8-GFP* (see Fig S1). For each *PRR*, the tissue expression shown corresponds to the compilation of several transgenic lines bearing different insertions of the same transgene, which were all compared to the expression of the *UAS-CD8-GFP* transgene by itself (no *PRR* activation). The relative intensity of GFP expression detected in each tissue is indicated as follows: ++ = strong expression; + = weak expression; - = no expression. \* = expression in the fat body in the anterior part of the body. N = 6–12 for each genotype.

**Table 2. Production of cuticular hydrocarbons and *cis*-Vaccenyl acetate in wild-type and transgenic flies.**

(A) Male flies; (B) Female flies. The genotype (WT, IR), larval and adult diet (+ = C18:1), and the type of perfume (WT, IR<sup>+</sup>) are shown on the four top rows (for details, please refer to Fig. 4). The mean absolute amount (in ng) for the major products is shown for each condition:  $\Sigma$ CHs is the total amount of cuticular hydrocarbons;  $\Sigma$ Lin is the total amount of linear (saturated) CHs;  $\Sigma$ Monoenes and  $\Sigma$ Dienes are the total amounts of mono- and di-unsaturated CHs, respectively;  $\Sigma$ Branched represent the total amount of methyl-branched (ramified) CHs. In males, the amount of 7-tricosene (7-T) and of cVA (a non-CH compound) is also shown. In females, the amount of 7,11-heptacosadiene (7,11-HD) is shown. (Please see Table S3 for detailed results). For each group of compound, significant differences (using Kruskal-Wallis test with  $p < 10^{-3}$ ) are indicated by different letters. N = 20.

### Figure 1. Preimaginal diet and developmental rescue.

(A) All individuals tested here express the UAS-*desat1*-RNAi transgene under the control of the complete *desat1* regulatory region fused with Gal4 (*6908-Gal4*) driver (named “IR”). (B) When IR animals were fed during all preimaginal development with standard food either alone or mixed with palmitic or stearic acids (C16:0; C18:0; two saturated fatty acids), they produced no pupae or adults. (C) Development of a wild-type (left) and transgenic larva (right), raised on standard food and observed at 48, 90 and 112 hours after egg laying (AEL; 48AEL larvae corresponds to L2). (D) When IR animals were raised on standard food supplemented with a pure UFA (palmitoleic, oleic, linoleic, or linolenic acid: C16:1, C18:1, C18:2, C18:3, respectively), a relatively high frequency of pupae and adults survived. (E) C18:1 was given during a limited period of larval development (in alternation with standard food) and the probability of pupal and adult rescue was noted. (F) When C18:1-rich food was supplied until 72 to 90 hours AEL, resulting adults had a smaller size (\*) than wild-type flies (left).

### Figure 2. Preimaginal UFA-rich diet and reproduction.

Three reproduction-related characters were examined in either IR<sup>-</sup> females paired with wild-type males (WT; top histograms) or in IR<sup>-</sup> males paired with WT females (bottom histograms). IR<sup>-</sup> flies were fed during the complete preimaginal period on different UFA-rich diet. Each pattern indicates the UFA added in the preimaginal diet (indicated in the box; see also Fig. 1D). (A) The frequency of cumulative copulation was measured during 60 min. Significant differences between diets and sexes, tested by a homogeneity test, are indicated by different letters ( $K\chi^2_{(7df)}=141.6$ ;  $p<10^{-4}$ ). (B) Fertility was measured as the capacity to

produce (at least one) adult progeny and was only determined in the females mating in #A. Statistics were performed as in #A ( $\text{Khi}^2_{(7\text{df})}=62.4$ ;  $p<10^{-4}$ ). (C) Fecundity is the number of adult progeny produced by each fertile female (see #B). We found very significant effects between the sexes using a Kruskal-Wallis test ( $\text{KW}_{(7\text{df})} = 95.42$ ;  $p<10^{-4}$ ). Bars indicate the mean ( $\pm\text{sem}$ ). N = 34-137.

### Figure 3. Effect of adult C18:1-rich diet on mating behavior and fecundity

**(Top)** Histograms representing the mating frequency of pairs of male and female during 60 minutes. Both males (3 top rows) and females (3 bottom rows) were subjected to varied manipulations. Their genotype was either wild-type (WT) or transgenic (IR; see Figs 1 and 2; IR flies were always fed during preimaginal development with C18:1). “C18:1 food” indicates that young emerging adult males and females were fed (+) or not (-) on C18:1-rich food until the test. “Perfume” means that flies were kept, during the night preceding the test, together with same-sex WT flies (WT) or with IR<sup>+</sup> males (raised during adult life on C18:1-rich food). A cross # is provided for each “male x female” combination to ease the reading.

**(Bottom)** Histograms representing the mean adult progeny ( $\pm\text{sem}$ ) produced by mated females from the pairs described above, calculated using the “fertility x fecundity” product (For other parameters, see Table S4). For each series of graphs, different letters indicate statistical difference. We used a homogeneity test to compare mating success:  $\text{Khi}^2_{(15\text{df})}=222.01$ ;  $p<10^{-4}$  ; for fecundity, we used a Kruskal-Wallis test:  $\text{KW}_{(15\text{df})}=271.9$ ;  $p<10^{-4}$ .

Putative Regulatory Region	PRR(RA)	PRR(RC)	PRR(RE)	PRR(RB)	PRR(RD)
<b>Brain hemispheres</b>	++	-	-	-	-
<b>Ventral ganglion</b>	+	-	-	-	+
<b>Peripheral nervous system</b>	-	+	-	-	-
<b>Oenocytes</b>	-	-	++	-	-
<b>Fat body</b>	-	++	-	+ *	-
<b>Posterior spiracle glands</b>	-	-	-	-	++
<b>Proventriculus</b>	-	+	-	-	+
<b>Midgut</b>	+	+	-	-	-
<b>Hindgut</b>	-	-	-	++	-
<b>Malpighian tubules</b>	-	-	-	++	-
<b>Ovaries/Testis</b>	+	-	-	-	-

Table 1: GFP expression driven by each putative regulatory region (PRR) of *desat1* gene.

GENOTYPE	WT	WT	WT	IR	IR	IR	IR
LARVAL DIET	-	+	+	+	+	+	+
ADULT DIET	-	-	+	-	+	-	-
PERFUME	-	-	-	-	-	WT	IR <sup>+</sup>
$\Sigma$ CHs	2329 <sup>a</sup>	2312 <sup>a</sup>	3009 <sup>b</sup>	3986 <sup>c</sup>	3375 <sup>b</sup>	3223 <sup>b</sup>	3421 <sup>bc</sup>
$\Sigma$ Lin	226 <sup>a</sup>	260 <sup>a</sup>	320 <sup>b</sup>	2894 <sup>c</sup>	2390 <sup>cd</sup>	2001 <sup>c</sup>	2489 <sup>de</sup>
$\Sigma$ Monoenes	1141 <sup>d</sup>	1274 <sup>d</sup>	1833 <sup>e</sup>	462 <sup>b</sup>	398 <sup>a</sup>	619 <sup>c</sup>	389 <sup>a</sup>
$\Sigma$ Dienes	24 <sup>b</sup>	24 <sup>b</sup>	42 <sup>c</sup>	38 <sup>c</sup>	24 <sup>b</sup>	17 <sup>a</sup>	17 <sup>a</sup>
$\Sigma$ Branched	178 <sup>a</sup>	192 <sup>a</sup>	272 <sup>b</sup>	347 <sup>c</sup>	329 <sup>c</sup>	334 <sup>c</sup>	337 <sup>c</sup>
7T	769 <sup>d</sup>	870 <sup>e</sup>	1267 <sup>f</sup>	23 <sup>b</sup>	14 <sup>a</sup>	241 <sup>c</sup>	12 <sup>a</sup>
cVa	577 <sup>d</sup>	410 <sup>c</sup>	350 <sup>c</sup>	65 <sup>ab</sup>	60 <sup>ab</sup>	65 <sup>b</sup>	48 <sup>a</sup>

Table 2A- Production of major cuticular hydrocarbons and pheromones in wild-type and transgenic male flies

GENOTYPE	WT	WT	WT	IR	IR	IR
LARVAL DIET	-	+	+	+	+	+
ADULT DIET	-	-	+	-	+	-
PERFUME	-	-	-	-	-	WT
$\Sigma$ CHs	2161 <sup>b</sup>	2341 <sup>bc</sup>	2512 <sup>cd</sup>	2335 <sup>c</sup>	2612 <sup>d</sup>	1874 <sup>a</sup>
$\Sigma$ Lin	260 <sup>a</sup>	281 <sup>a</sup>	373 <sup>b</sup>	1503 <sup>d</sup>	1688 <sup>e</sup>	1146 <sup>c</sup>
$\Sigma$ Monoenes	612 <sup>d</sup>	712 <sup>e</sup>	710 <sup>e</sup>	393 <sup>b</sup>	449 <sup>c</sup>	284 <sup>a</sup>
$\Sigma$ Dienes	1005 <sup>d</sup>	1026 <sup>d</sup>	1033 <sup>d</sup>	51 <sup>a</sup>	73 <sup>b</sup>	158 <sup>c</sup>
$\Sigma$ Branched	274 <sup>a</sup>	316 <sup>b</sup>	388 <sup>c</sup>	377 <sup>c</sup>	387 <sup>c</sup>	279 <sup>ab</sup>
7,11HD	604 <sup>c</sup>	608 <sup>c</sup>	589 <sup>c</sup>	15 <sup>a</sup>	20 <sup>a</sup>	95 <sup>b</sup>

Table 2B- Production of major cuticular hydrocarbons in wild-type and transgenic female flies

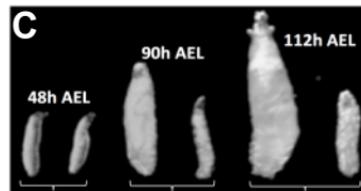
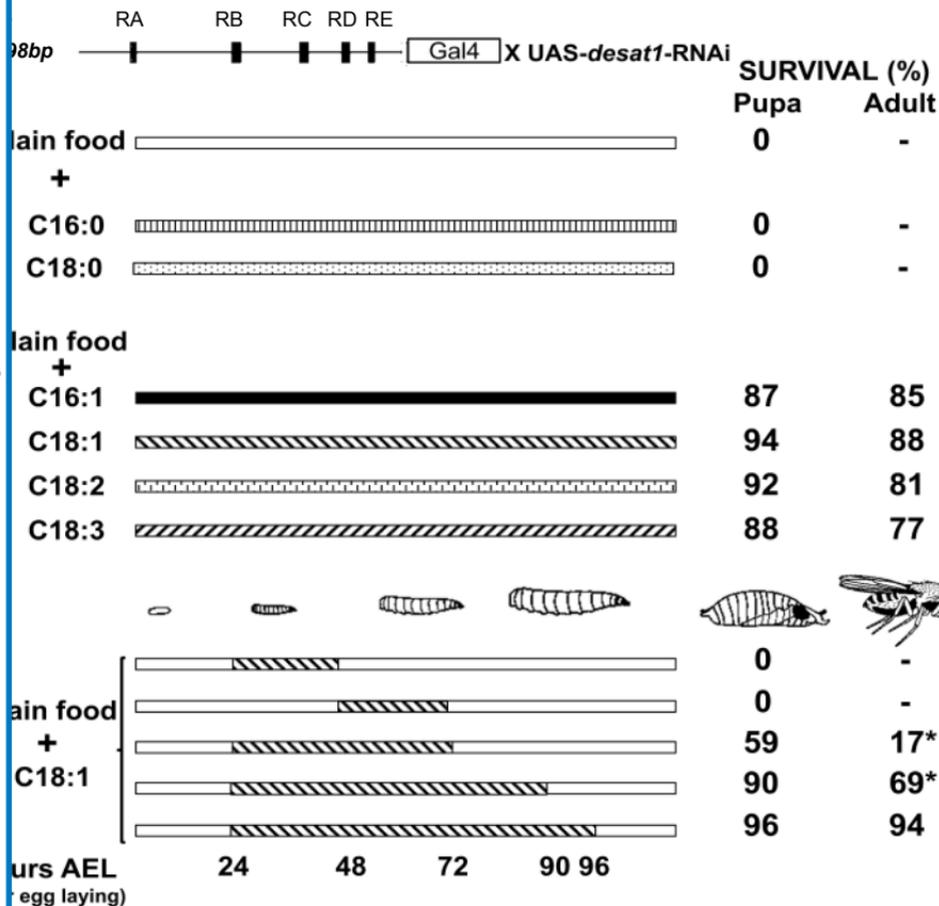


Figure 1

WT FEMALE  
x  
IR FEMALE  
x  
WT MALE  
x  
IR MALE

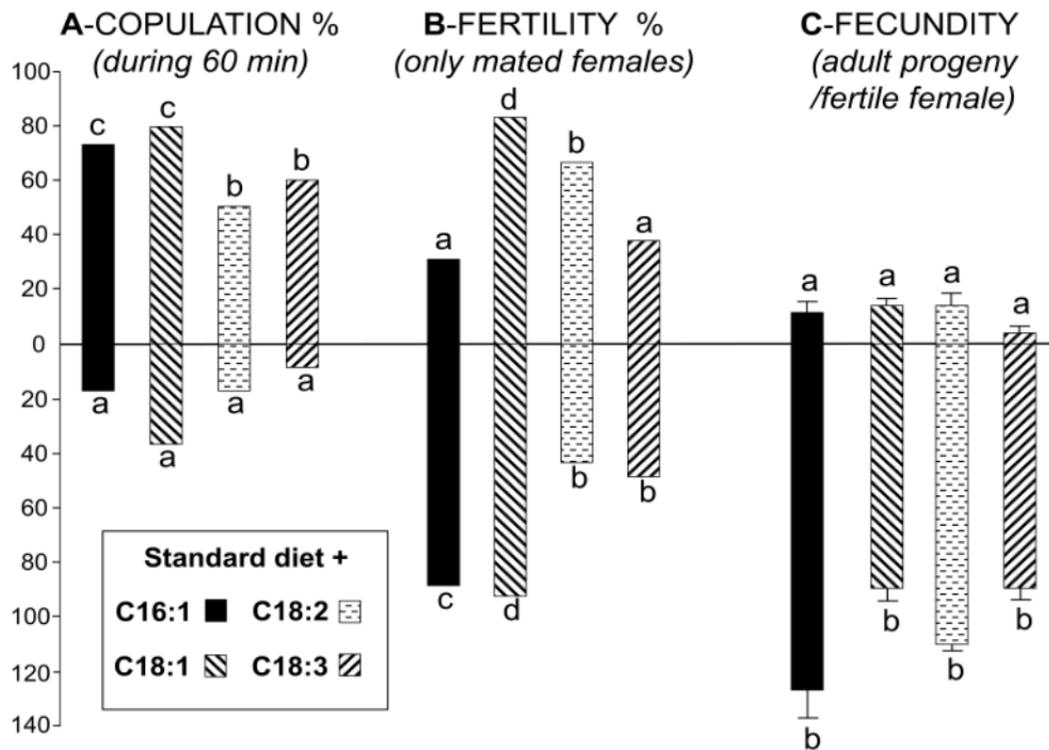
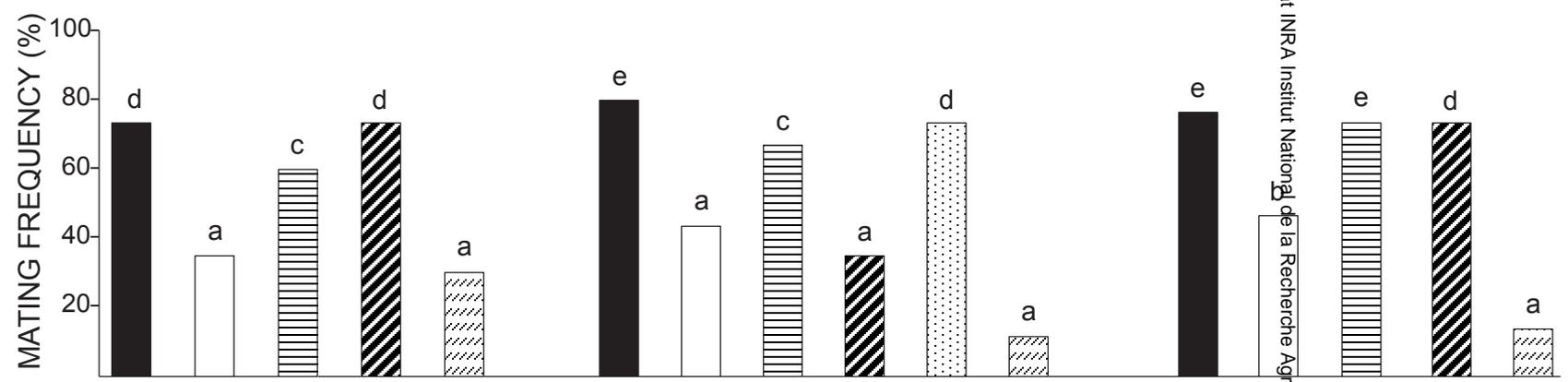


Figure 2



<b>Genotype:</b>	WT	IR	IR	IR	IR	WT	IR	IR	IR	IR	IR	WT	IR	IR	IR	
<b>C18:1 food:</b>	-	-	-	+	-	-	-	-	+	+	-	-	-	-	+	
<b>Perfume:</b>	-	-	WT	-	IR <sup>+</sup>	-	-	WT	-	-	IR <sup>+</sup>	-	WT	-	IR <sup>+</sup>	
<i>(Cross #)</i>	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
<b>Genotype:</b>	WT	WT	WT	WT	WT	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	
<b>C18:1 food:</b>	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	
<b>Perfume:</b>	-	-	-	-	-	-	-	-	-	WT	WT	-	-	-	-	

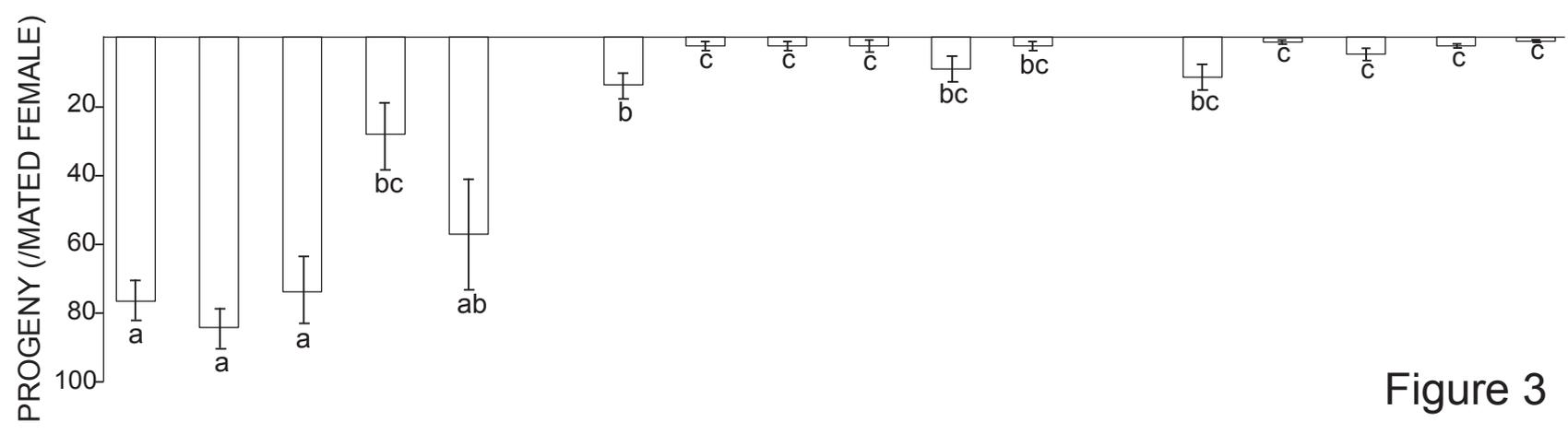


Figure 3