Dietary rescue of altered metabolism gene reveals unexpected Drosophila mating cues

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Abstract To develop and reproduce, animals need long-chain MUFAs and PUFAs. Although some unsaturated FAs (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 UFAs-rich diet. Adult diet also strongly influenced several aspects of reproduction: flies raised on a C18:1-rich diet showed increased mating performance compared with 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.—Bousquet, F., I. Chauvel, J. Flaven-Pouchon, J-P. Farine, and J-F. Ferveur. Dietary rescue of altered metabolism gene reveals unexpected Drosophila mating cues. J. Lipid Res. 2016. 57: 443–450.

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Lipids are widely used by plants and animals. In particular, 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 bonds [called saturated FAs (SFAs), or have one or more double-bond(s) (MUFAs and PUFAs, respectively). If animals can only synthesize some unsaturated FAs (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, 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 an adrenoleukodystrophy-related neuronal degenerative model in the fly (18).

In D. melanogaster, the Δ9-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

Abbreviations: AEL, after egg laying; C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; CH, cuticular hydrocarbon; CS, Canton-S; cVA, cis-vaccenyl acetate; dSREBP, Drosophila sterol regulatory element-binding protein; IR, desat1 RNAi knockdown; L1, L2, L3, first, second, and third larval instars; PRR, putative regulatory region; SFA, saturated FA; UFA, unsaturated FA; VLCFA, very long chain FA; 7,11-HD, 7,11-heptacosadiene; 7-T, 7-tricosene.

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of the desat1 gene is reflected by its varied adult expression in neural and nonneural 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 (6,908 bp) 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 FAs. We compared the ability of six FAs to rescue larvae to adulthood and their effect on mating and fertility performance. The effect of oleic acid (C18:1) added to the adult diet on mating behavior and on the production of sex pheromones was also measured.

MATERIAL AND METHODS

Strains

Drosophila strains were raised and tested at 24 ± 0.5°C with a 60 ± 5% humidity and a 12:12 light:dark cycle. Stocks were maintained in 150 ml glass vials containing 50 ml of our standard laboratory medium (agar 1.5%, yeast 10%, cornmeal 9%, and methyl para-hydroxybenzoate 0.4%). We used the Canton-S (CS) line as a control, the UAS-desat1-RNAi transgenic line to knock down desat1 expression (#33338; VDRC, Austria), and the UAS-CDS8-GFP transgenic line (UAS-GFP; stock #22300; Bloomington Drosophila Stock Center, Indiana University, Bloomington, IN) to visualize the expression driven by each desat1 putative regulatory region (PRR) [PRR(desat1)-Gal4].

The construction of these GAL4 drivers, made with the fusion of the Gal4 sequence and each PRR of five of each first alternative desat1 exon [PRR(RA) to PRR(RD)] is described elsewhere (21). We also used a Gal4 transgene containing the complete desat1 sequence fused to GALA [(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 w1118 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 1575-C1 and 11A lines were generated by the precise excision of the P element, each from the original mutant desat1575 allele [1575-C1 (19)] or from the desat111A allele [11A (20)] using A2-3 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 (#5199; Bloomington); GFP fluorescence was used to discriminate between homozygous and heterozygous embryos.

For the 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 a more stable effect with the different FAs tested.

Diet

Preparation of FA-rich food. To assess the diet-induced rescue of desat1 transgensics, we used six pure FAs at concentrations which were previously tested with both larvae and adults (23). 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 medium (60°C) to obtain a 5 mg/ml final concentration (0.5%) and mixed using a metal stirrer. The medium was then allowed to cool until solidified, stored at 4°C in a dark room to avoid oxidation, and used within 1 week. Palmitic acid (C16:0) (#P0500), palmitoleic acid (C16:1) (#P9417), stearic acid (C18:0) (#W30318), linoleic acid (C18:2) (#L1376), and linolenic acid (C18:3) (#L2376) were purchased from Sigma-Aldrich. C18:1 (#06-4010) was purchased from Strem Chemicals Inc. (Bisheim, France).

Determination of the critical period. To synchronize larval age, gravid females were transferred every 2 h onto fresh egg-laying medium, and larvae were regularly collected. First or second 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 0.1× PBS solution, then transferred to fresh standard medium. The number of third instar larvae, pupae, and emerging adults was counted in each vial. Our data correspond to the mean of six replicates for each test.

Reproduction

Preparation of flies. WT and transgenic flies were sexed within 1 ± 1 h of emergence under light CO2 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 w1118 line (CS with white eyes) to allow separation from transgenic receivers (w with red eyes). For the experiment involving the passive transfer from IR donors to IR receivers, one wing of the donors was clipped to allow separation under binocular microscope. To transfer cuticular compounds, eight to ten 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 ± 0.5°C and 60 ± 5% humidity. Tests were completed over several days and took place 1–4 h 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 cm3). After 10 min, a virgin female was introduced. Each test was performed for 60 min 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 males were discarded) and the presence (fertility) and number (fecundity) of adult progeny noted. The sex ratio of the progeny was also determined.

Histology

Larvae (second and third 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 AOPS confocal microscope (an 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).
**RESULTS**

**The 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 PRRs (RA, RC, RE, RB, and RD; Table 1, supplementary Fig. 1). 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).

**Developmental lethality and adult rescue with FAs**

Similarly to what occurs in the null homozygous mutant alleles, desat1-1573-C1’ (19) and 11A (20), we found that the double transgenic genotype combining a copy of the full desat1 regulatory region (6,908 bp) 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 quantitative PCR revealed that the level of desat1 RNA was drastically decreased in IR transgenics compared with both transgenic parental controls, but was still significantly higher than the levels of the null desat1 11A mutant allele (supplementary Table 1).

**FA-preimaginal diet and adulthood rescue.** In an attempt to rescue this lethal phenotype, which is likely caused by a metabolic defect blocking second to third instar molting (Fig. 1C, we fed IR larvae with standard lab food supplemented with six different long chain FAs (Fig. 1). These FAs, suspected to be some of the best candidates to rescue adulthood, have a 16 or 18 carbon chain and carry 0, 1, 2, or 3 unsaturated double-bonds (supplementary Fig. 2). The effect of each FA-rich diet (provided during the complete preimaginal development) was first measured with regard to its ability to rescue viability in IR pupae and adults (Fig. 1B–D). SFAs (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 SFA (supplementary Table 2). Conversely, when MUFA’s (C16:1 and C18:1), or PUFA’s (C18:2 and C18:3) were added in the larval food, they caused high pupal and adult survival (87–94% and 77–88%, respectively) in IR individuals.

**Critical period of exposure to C18:1.** We used C18:1, which induced a high adult rescue, to delimitate the period during which it is critical during larval development (Fig. 1E).

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**Table 1. Summary of desat1 tissue-specific expression in third instar larvae**

| Tissue                        | PRR(RA) | PRR(RC) | PRR(RE) | PRR(RB) | PRR(RD) |
|-------------------------------|---------|---------|---------|---------|---------|
| Brain hemispheres             | ++      | -       | -       | -       | -       |
| Ventral ganglion              | +       | -       | -       | -       | -       |
| Peripheral nervous system     | -       | +       | -       | -       | -       |
| Oenocytes                     | -       | -       | ++      | -       | -       |
| Fat body                      | -       | -       | -       | +       | -       |
| Posterior spiracle glands     | -       | -       | -       | -       | ++      |
| Proventriculus                | -       | -       | -       | -       | +       |
| Midgut                        | +       | +       | -       | -       | -       |
| Hindgut                       | -       | -       | -       | ++      | -       |
| Malpighian tubules            | -       | -       | -       | ++      | -       |
| Ovaries/testis                | +       | -       | -       | -       | -       |

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 supplementary Fig 1). 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 with 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.
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. Matting performance and production of CHs, two characters related to those controlled by desat1 ([discrimination and production of cuticular pheromones (19, 21, 26)], were compared in IR individuals raised on C18:1-rich food during either:

1) Preimaginal development only (IR/H11002); or
2) Both preimaginal and adult stages (IR + ).

Matting with WT females. We first tested mating pairs involving WT females (Fig. 3, supplementary Fig. 3B, supplementary Table 4; crosses #1–5). Within 1 h, 75% of WT pairs, preimaginally raised on standard food, mated to yield, on average, 80 viable adults (cross #1). WT pairs raised on a C18:1-rich diet (preadult + adult) showed similar performances [n = 136; 76% mating; 99 adults per mated female (cross #1 + C18:1, supplementary Fig. 3B; supplementary Table 4, cross #1*)]. By contrast, IR males “adult-fed” on standard food (IR/H11002) showed greatly decreased mating performance (35%; cross #2) but still showed a WT-like fertility. The intense courtship activity observed in most nonmating “WT females × IR males” pairs suggests that their relatively low mating performance was caused by decreased female receptivity (27, 28). Because this effect may result from altered CHs in IR males (29 [Table 2]), we perfumed these males with WT male CHs. This significantly increased their mating performance and

Exposure to this fat diet either during the L1 (24–48 h after egg laying (AEL)) or the L2 stage (48–72 h 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 with WT flies (Fig. 1F). Pupal and adult survival rates increased when exposure to a C18:1-rich diet was extended to 90 h AEL. A full developmental rescue (with regular-size adults) was obtained when IR larvae were raised on a fat diet until 96 h AEL (corresponding to mid-L3 stage).

Preimaginal UFA-rich diet and reproduction. Preimaginal diet with different UFAs also differentially rescued several aspects of male and female reproduction (Fig. 2, supplementary Fig. 3, supplementary Table 3). When paired with WT 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 rather 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 (supplementary Table 3).
Cuticular pheromones

The comparison of CHs in WT versus 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; 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, 26). 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/H11002 flies perfumed with WT were somewhat intermediate between those of WT and IR males, 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 + perfume induced no CH difference between perfumed and non-perfumed IR/H11002 males. Note also that the amount of cVA (30, 31), a non-CH male pheromone, was strongly decreased in all IR males irrespective of their adult diet status or perfume.

**Mating with transgenic females.** Similarly treated males were paired with IR - and IR + females (Fig. 3; supplementary Fig. 3C, D). As observed for WT females, IR - females frequently mated with WT males (80%, cross #6), but not with IR - males (cross #7), whose performance was largely rescued with WT male perfume (cross #8). However, and differently from WT males, IR - females mated less frequently with IR - males (cross #9). This defect was rescued in females perfumed with WT female CHs (cross #10). However, similarly perfumed IR - females remained unresponsive to IR - males covered with IR + male perfume (cross #11). On the other hand, IR + females (crosses #12–16) showed a similar mating pattern compared with WT females: they frequently mated with WT males (cross #12), and their low performance with IR - males (cross #13) was rescued with WT male CHs (cross #14). Although IR - males showed a high mating performance (cross #15), their perfume strongly decreased the mating performance of IR + males (cross #13 vs. cross #16). Overall, IR females showed a much reduced fecundity (1–17 adult progeny per mated female), compared with WT females, independently of the male partner (supplementary Table 4).

**CUTICULAR PHEROMONES**

**Effect of genotype and perfume.** The comparison of CHs in WT versus 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; 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, 26). 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 + flies perfumed with WT were somewhat intermediate between those of WT and IR - 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 + perfume induced no CH difference between perfumed and non-perfumed IR - males. Note also that the amount of cVA (30, 31), a non-CH male pheromone, was strongly decreased in all IR males irrespective of their adult diet status or perfume.

**Effect of a C18:1-rich diet.** For each sex, intra-genotype comparisons revealed a differential effect of a C18:1-rich food diet depending on the sex, genotype, and feeding period. In WT males, only adult C18:1-rich diet increased $\sum$CHs, including 7-T (+46%) and 7- and 9-pentacosene (supplementary Table 5A). The other CHs did not show such a diet-dependent variation. In contrast, in IR males,
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 CHs

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 knock-down of function induced lethality in second 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

**DISCUSSION**

A FA-rich diet can rescue a genetic defect related to abnormal FA processing in *Drosophila*, and such a 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 (25)]. Whereas these defects could be partially cured after preimaginal feeding with glyceryl trioleate oil 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 (Gal80 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.
that FAs are required during both preimaginal and adult stages to allow both correct late preimaginal development and adult CH 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, 26).

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 the desat1 gene in several metabolic tissues [fat body, oenocytes, gut (25)]. Similarly to desat1, second 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 rescued these characters differently 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 effects 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; supplementary Fig. 1). Our preliminary dissection of male and female gonads did not reveal any obvious difference in WT versus IR flies. We also found that adult, but not preimaginal, C18:1-rich diet decreased the fecundity of IR- males with WT females (compare cross #2 and cross #4).

Effect of adult diet on mating signals

The fact that a C18:1-rich adult diet rescued mating behavior in both females and males (Fig. 2; supplementary Fig. 3) 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+ pairs (cross #15) with IR- pairs (cross #7) or with reciprocal IR/IR+ pairs (cross #9 and cross #13). The partial transfer of WT CHs (via WT perfume) allowed us to largely rescue the mating performance in both IR- males (crosses #3, #8, and #14) and females (cross #10). Taken together, these results indicate that desat1 is involved in the production of sensory
cues reciprocally used during the courtship interplay preceding copulation (27, 31). Whereas IR' males showed a normal mating with WT and IR' females (crosses #4 and #15), the rescue of the low mating observed between IR' males and IR' females (cross #9) was only induced by WT female perfume (cross #10). This indicates that female CHs changed the behavior of IR' 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' males (except with non-perfumed IR' females; cross #9) and in IR' females. However, IR' male cues had no effect when transferred to IR' males (crosses #5 and #11). Furthermore, these cues strongly decreased the mating performance of IR' males paired with IR' females (cross #16). The low variation of chemical profile between IR' and IR' males, covered and not covered with IR' males' perfume, suggests that the performance of IR' males depends neither on CHs nor on cVA (Table 2; supplementary Table 4). Indeed, WT males preimaginally raised on C18:1 produced less cVA, but coped on the perfuming procedure. In all cases, our study reveals that CHs and long-chain FA are transmitted during the reproduction in a fruit-feeding butterfly: the role of fruit decay and biochemical pathogenesis of fatty aldehyde dehydrogenase deficiency. Mol. Genet. Metab. 90: 1–9.

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