desat1: A Swiss army knife for pheromonal communication and reproduction?

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A Swiss army knife for pheromonal communication and reproduction?

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Abbreviations: *PRR*, putative regulatory région; *RA, RC, RE, RB, RD*, five first alternative exons of *desat1; PPR(RB)*, *PRR* of *RB* transcript with *RB* alternative exon; *RD*$^{+}$, *PRR(RD)* + 662 base pairs; AL, antennal lobe; EB, ellipsoid body; FB, fat body; MB, mushroom body; SOG, sub-esophageal ganglion; PI, *pars intercerebralis*; Acc.gland, accessory gland; Malpighi, malpigian tubule; Rect.pap, rectal papilla; Ej.bulb, ejaculatory bulb; CH, cuticular hydrocarbon; C5-CH, cuticular hydrocarbon desaturated on C5; C7-CH, cuticular hydrocarbon desaturated on C7; 5-T, 5-tricosene; 7-T, 7-tricosene; 7,11-HD, 7,11-heptacosadiene; 5,9-HD, 5,9-heptacosadiene; 21C, CH with 21 carbons; *EP*, transposable element containing the upstream activating sequence; *RNAi*, interferential RNA; GRN, gustatory receptor neuron; ORN, olfactory receptor neuron; DA1, dorso-posterior glomerulus of the AL; Cs, canton-S strain

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The *desat1* gene possess an extraordinary—maybe unique—feature in the control of sensory communication systems: it codes for the two principal and complementary aspects—the emission and the reception—of Drosophila sex pheromones. These two complex aspects depend on separate genetic control indicating that *desat1* pleiotropically acts on pheromonal communication. This gene also control other characters either related to reproduction and to osmoregulation. Such a functionnal pleiotropy may be related to the molecular structure of *desat1* gene which combines a highly conserved coding region with fast evolving regulatory regions: it produces at least five transcripts all giving rise to the Δ9-desaturase enzyme.

Introduction

The *desat1* gene separately acts on the emission and on the reception of *Drosophila melanogaster* sex pheromones and also affects other characters related to reproduction and to osmoregulation.[1] We discuss the possibility that *desat1* functionnal pleiotropy is related to the molecular structure of this gene underlying its complex pattern of expression which may be currently evolving.

Structure of the desat1 Gene

The *desat1* gene has five regulatory regions with five first alternative exons (*RA, RC, RE, RB, RD*) which—when merged with the exons 2–5 (common to all transcripts and corresponding to the complete coding region; **Fig. 1**)—yield five transcripts (*RA-RD*; **Fig. 2A**). These five alternative transcripts all give rise to a Δ9-desaturase enzyme. This mode of expression differs from other courtship genes such as *fruitless* (*fru*) which produces sex-specific alternative transcripts coding for a variety of sex-specific product involved in multiple aspects of reproduction.[2] In contrast, *desat1* shows no sex-specific transcript or product, but only a slight quantitative sex-difference for the levels of some transcripts.[3] We speculate that the functionnal pleiotropy is linked (1) to each putative regulatory region (*PRR*) driving *desat1* expression in a variety of tissues involved in the various phenotypes and (2) to the first (non-translated) alternative exon of each transcript maybe providing a information on its stability, its efficiency to be translated, and its sub-cellular localization, even if this remains unknown.

The multiplicity of transcripts—separately regulated—and the diversity of tissue-specific phenotypes —all determined by the same product—may explain *desat1* functionnal pleitropy. To functionnally dissect *PRRs*, we used several tools: (1) alleles resulting of the un/precise excision of a *PGal4* element[3,4] (inserted in the *PRR(RB) = 1573-Gal4*; **Fig. 2A**), (2) *EP* deregulating elements inserted in the different *PRRs* and driven by *1573-Gal4*,[5] (3) a *desat1 RNAi* transgene driven by the *Gal4* transgenes made with each *PRR*.[1] All these approaches showed that the production and the perception of sex pheromones are controlled by separate *PRRs*. In particular, the misexpression of *desat1* in the oenocytes [driven by *PRR(RE)*] affected the production of pheromonal CHs whereas its misexpression in discrete regions of the

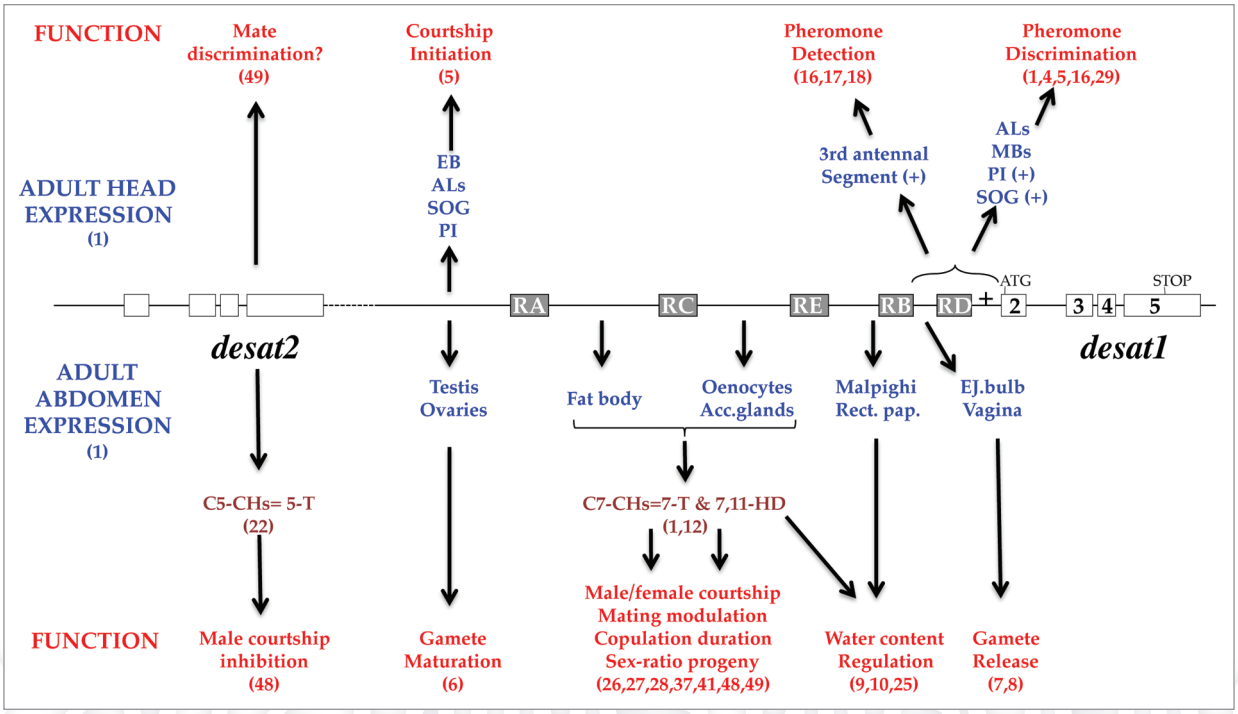

Figure 1. Molecular structure, tissue-specific expression and functions of desat1 gene. Shematic representation of the desat1 and desat2 loci. The *desat1* gene has five first alternative exons (*RA*, *RC*, *RE*, *RB*, *RD* shown as shaded box; not at scale) which all combine with the exons 2–5 corresponding to the coding regions (the codons for start and stop of translation are shown as ATG and STOP, respectively). Each of the five alternative exons (which will not be translated) has its own putative regulatory region (*PRR*; shown as the solid line upstream); the *RD*$^+$ includes the *PRR(RD)*, the *RD* exon, and a small 662 bp fragment (+) located downstream of the *RD* exon. The *desat2* gene, located 4 kb upstream (dotted line), has a similar coding region to *desat1*. Tissue-specific expression in adult flies driven by the different *PPRs* of *desat1* is shown (in blue) in head tissues (above gene structure; + indicates expression driven by RD$^+$ but not by RD; ALs, antennal lobes; EB, ellipsoid body; MBs, mushroom bodies; SOG, sub-esophageal ganglion; PI, *pars intercerebralis*), and in abdominal tissue (below gene structure; Acc.glands, accessory glands; Malpighi, malpigian tubules; Rect.pap, rectal papilla; Ej.bulb, ejaculatory bulb). Cuticular hydrocarbons desaturated on C5 or on C7 (C5-, C7-CHs) such as 5- and 7-tricosene (5-T, 7-T), and 7,11-heptacosadiene (7,11-HD) are shown in brown color. The function of *desat1* (and of *desat2*) in these different tissues is shown in red. Numbers between brackets refer to the reference list.

brain [including the antennal lobes = ALs; driven by *PRR*(*RD*$^+$)] affected the discrimination of sex pheromones.

Adult Tissue-Specific Expression of desat1

To visualize *desat1* expression in the adult abdomen and head, we used a GFP reporter (thoracic expression was not explored; **Fig. 1**). In the abdomen, the different *PRRs* drove expression in a non-overlapping pattern of tissues either involved in reproductive functions or in osmoregulation. *PRR(RA)* drove expression in the testis and ovaries which are involved in the maturation of gametes,[6] and *PRR(RD)* in the ejaculatory bulb and in a vaginal structure. Both structures are presumably involved in the release of male and female gametes.[7,8] *PRR(RB)* drove expression in the malpighian tubules and in the rectal papilla both of which are involved in osmoregulation.[9,10] *PRR(RC)* and *PRR(RE)* were respectively expressed in the fat body (FB) and in the oenocytes, both of which are involved in the control of lipid metabolism[11] and in the maturation of cuticular hydrocarbons (CHs) by introducing a desaturation on carbon 7 (C7-CHs).[12-14]

In the head, *desat1* expression was found in the FB and in diverse neural regions involved in complex sex-specific behaviors (see below). *PRR(RD*$^+$*)* which affected pheromone discrimination—when driving the *desat1 RNAi*—was expressed in the *pars intercerebralis* (PI), in the ALs, the mushrooms bodies (MBs), and the sub-esophagial ganglia (SOG). ALs and MBs are necessary for chemosensory perception and learning (see below) whereas the SOG receives pheromone and food taste inputs from the proboscis and tarsa.[15,16] *PPR(RD*$^+$*)* expression in specific glomeruli of the ALs corresponds to the projection of third-antennal-segment olfactory-receptor neurons housed by large basiconic and trichoid sensilla—involved in pheromonal perception[17,18]— where *PPR(RD*$^+$*)* was also expressed. *PPR(RC)* labeled large epidermal cells which may control lipid metabolism in the third antennal segment. *PRR(RA)* drove expression in the PI, ALs and also in the ellipsoid body (EB; a region of the central complex), all involved in sex-specific behaviors, and in the SOG.

Multiple Functions of desat1

The high conservation of the *desat1* coding region among drosophilidae[19] may be explained by the crucial role that the Δ9-desaturase enzyme plays on lipid

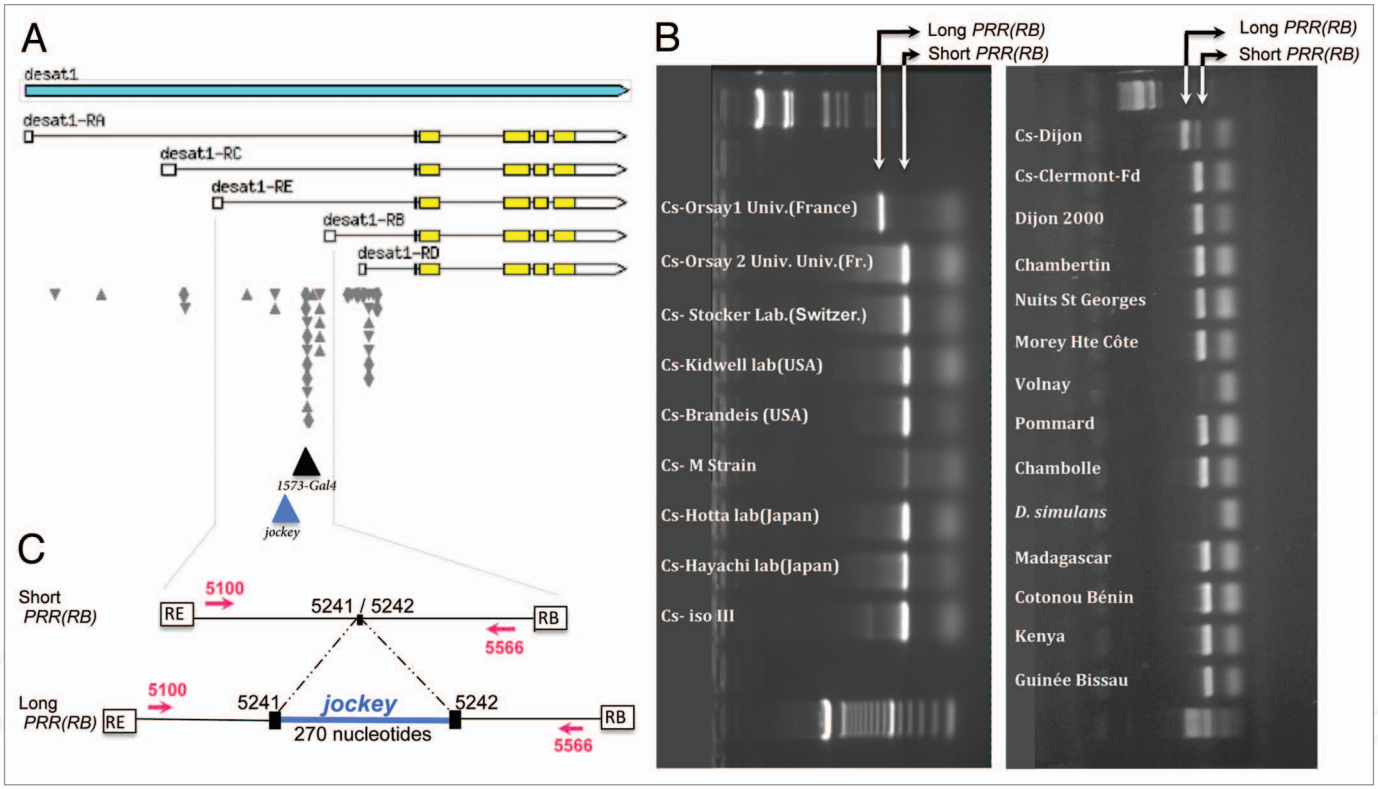

Figure 2. Transcripts, inserted elements and intraspecific variability of desat1. (A) The representation of *desat1* locus (from FlyBase) shows the five transcripts and various inserted elements (shown as shaded arrowheads). The insertion site of the *1573-Gal4* and the fragment of the transposable *jockey* element identified are highlighted (in black and blue, respectively). (B) The genomic DNA of each line was extracted from a pool of 50 *D. melanogaster* (or *D. simulans*) flies (25 males and 25 females). PCR performed with 5,100 and 5,566 primers (C) revealed, after separation by agarose gel electrophoresis, the presence of a short *PRR(RB)* and/or a long *PRR(RB)* (bottom corresponds to the 100 bp DNA Ladder). Lines shown on the left part are all Canton-S lines (Cs) from differents labs or universities (from top to bottom): Orsay University (lines 1 and 2; France), Reini Stocker lab (Switzerland), Margaret G. Kidwell lab (USA); Brandeis University (USA), M-strain (Bloomington), Hotta and Hayachi labs (Japan), Cs-III iso (Bloomington); On the right part (from top to bottom): Cs-Dijon (initiated with Cs-Orsay 1 line), Cs-Clermont-Ferrand, Dijon 2000 (France), six strains sampled in Burgundy vineyards in 2002 (Chambertin, Nuits St-Georges, Morey Hautes-Côtes, Volnay, Pommard and Chambolle; France), *Drosophila simulans* flies (Seychelles line), and four lines sampled in Africa in the 1980s (Madagascar, Benin, Kenya and Guinea-Bissau). Short and long *PRRs(RB)* were found in flies of both sexes in the Cs Dijon line. The short *PRR(RB)* transcript was faintly detected in Cs-M strain and in Volnay flies but not in *D. simulans* flies probably due to four mismatches with the 5,100 primer in the *D. simulans* sequence. (C) Detail of the Putative Regulatory Region *RB* [*PRR(RB)*] located between the first alternative *RE* and *RB* exons. Primers used to amplify this region of genomic DNA (shown in red) allowed its cloning and sequencing (5,100: 5'-ACA TTC CGA ATT TTG AAT ATA GCA G-3'; 5,566: 5'-ACA GTG AGA GAG GGA GAG AGA AAA AGC-3'). The 270 nucleotides fragment of the transposable element *jockey* (shown in blue) was found in both Cs-Orsay1 and Cs-Dijon lines.

metabolism in these species. In *D. melanogaster*, the Δ9-desaturase is necessary for larval development: the complete elimination of Desat1 prevents the second-to-third instar larval molt.[3,20] This suggests that some of the desaturated lipids processed by the Δ9-desaturase are required for larval survival. The Δ9-desaturase is also involved in the biosynthetic pathway leading to the production of C7-CHs.[21,22] These substances cover not only the adult cuticle but also the embryonic chorion similarly to other diptera.[23] Long-chain hydrocarbons may increase embryonic and adult protection against xenobiotics[24] and may also help to keep the internal water balance constant during development.[25]

CHs produced by adult *D. melanogaster* flies include the male predominant pheromone-7-tricosene (7-T) and the female-specific pheromone-7,11-heptacosadiene (7,11-HD), both of which play multiple roles in courtship and mating behaviors. 7-T inhibits the courtship of other males and enhances female receptivity to mating: this substance may be perceived both by taste and olfactory organs.[26,27] Female CHs modulate reproductive functions before, during and after mating: higher amounts of 7,11-HD increase both the mating frequency and duration of copulation, and affects the sex ratio of the progeny.[28]

The 7-T/7,11-HD difference is used by male flies for sex-discrimination: when these pheromones—and visual/acoustic cues—are absent, males cannot discriminate.[4] Male discrimination of pheromonal CHs depends on both the peripheral detection and central integration of pheromonal inputs. At the periphery, gustatory receptor neurons (GRNs; housed in tarsal and proboscis sensilla) and olfactory receptor neurons (ORNs; mainly carried by the third antennal segment) are involved. The physiological activity of proboscis GRNs varied with the sex and genotype of the

CHs used for stimulation. Moreover, their activity increased in mutant *desat1* males (1,573; which cannot discriminate wild-type sex pheromones) compared with wild-type neurons.[16] Olfactory cells of the third-antennal-segment—possibly expressing *desat1*—are also involved in the perception of pheromonal CHs.[16]

The *desat1* gene is also expressed in several brain centers (ALs, MBs, PI, EB, SOG) involved in chemosensory-driven behaviors related to reproduction. In the ALs, *desat1* was expressed in several glomeruli including DA1 which process pheromonal inputs.[29] MBs are necessary for the memory associating chemical signals,[30] such as short-term courtship conditioning which involves 9-pentacosene.[31,32] Both EB and PI are implicated in the coordination of adult locomotion and also change sex-specific behaviors. The alteration of a serotonine receptor in the EB affected courtship and mating behaviors,[33] whereas the manipulation of PI affected sex-specific aspects of locomotor activity.[34] The PI is connected, via neurosecretory cells, with the *corpus allatum/cardiacum* complex, whose alteration in females affected behavior[35] (response to insemination; oviposition) and cuticular pheromones.[36]

The alteration of the transcription driven by a *EP* element inserted antisens in the *PRR(RC)* (theoretically affecting the *RA* transcript) induced a long delay in the onset (latency) of male courtship without affecting sex pheromone discrimination.[5] This defect maybe caused by the altered olfactory perception of non-sex specific pheromones potentially volatile (*ur*-pheromones).[37,38] Another *EP* element inserted antisens in the *PRR(RD)*, strongly decreasing the levels of *RA*, *RC* and *RB* transcripts in the head, did not induce a similar behavioral defect.[5] We believe that *PRR(RA)* and *PRR(RD)* are differently expressed in the PI since they differently affected male CHs, when combined with the *desat1 RNAi* transgene.[1]

Evolution of desat Genes and Pheromonal Communication

Many Diptera species including Drosophilidae use CHs for pheromonal communication.[39,40] In the *D. melanogaster* subgroup, mature adult flies of all species produce CHs with carbon chain length generally ranging from 21–29°C (rarely up to 33°C) with no, one or two double bonds on C5, C7, C9 and/or C11.[41-43] In particular, the two sibling and cosmopolitan species *D. melanogaster* and *D. simulans* which likely diverged in Central Africa 3–4 million years ago[44] differ for their production and their perception of sex pheromones: only *D. melanogaster* females produce 7,11-HD while *D. melanogaster* males and *D. simulans* flies predominantly produce 7-T. Moreover, *D. melanogaster* males are inhibited by 7-T and stimulated by 7,11HD whereas *D. simulans* males reciprocally respond to these pheromones.[37]

This suggests that the *desat* genes underlying pheromonal communication system have rapidely evolved.[45,46] This concerns the *desat1* and *desat2* genes which are located in tandem (4 kb apart) in the *D. melanogaster* genome (**Fig. 1**). Most *D. melanogaster* cosmopolitan strains (outside of sub-Saharan Africa and the Carribbean) carry a non-functionnal copy of *desat2* (caused by a partial deletion of the *PRR*) [47] and have lost the ability to produce C5-CHs (instead of C7-CHs for *desat1*). Flies of ancestral sub-Saharan Africa strains (recently introduced in the Carribbean), have functionnal *desat1* and *desat2* genes: females predominantly produce 5,9-heptacosadiene (5,9-HD) whereas males predominantly produce C7-CHs.[48] However, in Zimbabwean strains, both sexes produce substantial amounts of C5-CHs (5,9-HD in females; 5-tricosene = 5-T in males).[49] Zimbabwean females show a strong sexual isolation against cosmopolitan males which is partly due to the male CH profile.

Our unpublished data also indicates a fast intra-strain evolution of *desat1* in *D. melanogaster* lines. We compared the sequence of this gene in 21 wild-type strains collected worldwide including ten Canton-S (Cs) lines from different laboratories (**Fig. 2B**). The Cs strain, initially caught in the USA in 1935, was distributed worldwide in research centers. The fact that these Cs lines have been kept separated from each other, sometimes for decades, may have favored a lab-specific evolution due to bottleneck and genetic drift effects. If lab-acclimation during 50 generations did not reduce the genetic variability, nor affected the CH production or courtship and mating behaviors of wild-type freshly caught strains,[50] a longer «isolation» in specific lab condition may differently affect genes involved in sexual communication. In particular, we found a divergence for the length of the *PRR(RB) desat1* sequence (**Fig. 2B**). The DNA corresponding to the length increase corresponds to the insertion of a fragment of a transposable *jockey* element[51] (**Fig. 2C**). The two closely related lines Cs-Orsay1 and Cs-Dijon (Orsay1 was brought to Dijon in 1999) produced flies with a long *PPR(RB)*. If all Cs-Orsay1 flies are homozygous for the long *PPR(RB)*, most Cs-Dijon (80%) are heterozygous (short/long) and about 20% are homozygous (long/long or short/short). The *jockey* fragment was likely inserted during the last decades in the Cs-Orsay1 line and the heterogeneity of the Cs-Dijon population may result from a contamination with other *D. melanogaster* Cs lines, or alternatively of the excision of the *jockey* fragment. Its site of insertion corresponds to a «hot spot», where—or nearby—many transposable elements are inserted (**Fig. 2A**; FlyBase; http://flybase.org/cgi-bin/gbrowse/dmel/). The comparison of the CH profile between long and short *PPR(RB)* flies reveals a slight difference—to be confirmed—for the level of 5-T and 7,11-TD which are two quantitatively minor volatile pheromones. Therefore, the rapid molecular change of *PRR(RB)* may illustrate the recent and rapid acquisition of novel features of the *desat1* gene.

Conclusion

The ubiquitous presence of desaturases in living organisms supports the vital role of these enzymes in development and survival. The Δ9-desaturase coded by the *desat1* gene is also crucial for many aspects of pheromonal communication and reproduction. A better understanding of the relationship between the molecular structure of *desat1* (and eventually *desat2*), their tissu-specific expression and the variety of characters they control in more or less phylogenetically related animals (insects, diptera, drosophilidae, species of the *D. melanogaster* subgroup, *D. melanogaster*,

laboratory lines) should shed light on the some recent evolutionary processes which have shaped their system of pheromonal communication.

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