Types of Nucleolar Chromatin Threads (NCT) in *Drosophila repleta* Wollaston

Two types of Nucleolar Chromatin Threads (NCT) were observed in *D. repleta* collected from Dwarahat in Kumaon region which are as follows:

**Type I (Figure 2A)**
Thread not visible, ramified and scattered throughout the nucleolus with granules concentrated towards the periphery of the nucleolus.

**Type II (Figure 2B)**
Thread very thin, ramified and scattered throughout the nucleolus with small light granules.


Figure 2. A-B, Types of Nucleolar Chromatin Thread (NCT) present in *Drosophila repleta*.

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**Genetic ablation of antennae or basiconic sensillia reduces *Drosophila melanogaster*’s ability to perceive odors from the fruit of *Morinda citrifolia***.

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**Abstract**

*Drosophila* use a variety of sensory organs to detect odorants in their environment. While recent work in *Drosophila melanogaster* has increased our knowledge of how these organs detect odorants, our understanding of how these organs function in an ecological context is limited. Here, I use several developmental mutations that ablate sensory organs in flies to see which are critical to *D. melanogaster*’s ability to perceive odorants from the fruit of *Morinda citrifolia*, which is the primary host of *D. sechellia*. I show that basiconic sensillia on antennae are particularly important to perceiving this fruit, although tarsal receptors may also play a role.

**Introduction**

Today we know that odor and taste perception in flies occurs via antennae, maxillary palps, proboscis, and tarsi (reviewed in Hallem, *et al.*, 2006). In the antennae, odorants pass through
cuticular pores in sensilla. These odorants are then bound by odorant binding proteins (Obps) and delivered to odorant receptors (Ors) on the surface of the insect odorant receptor neurons. Taste is perceived in an analogous manner, except that odorants are trafficked to gustatory receptors (Grs; Ebbs and Amrein, 2007). Odorant receptor neurons converge to spatially invariant antennal lobe glomeruli. From these glomeruli, neurons project into the mushroom body where higher order processing is believed to occur.

How sensing these odor cues results in ecologically important behaviors, however, is much less well understood. To better understand this problem, considerable work has focused on the host specialist, Drosophila sechellia and its generalist sister species, D. melanogaster. On its native islands, the Seychelles, D. sechellia almost exclusively uses the fruit of Morinda citrifolia (Morinda), a plant common around the Indian Ocean and Polynesia (Louis and David, 1986; Jones, 2005). D. sechellia has evolved strong preference for, and resistance to, the toxins in Morinda (Louis and David, 1986; R'Kha, et al., 1991; Jones, 1998, 2004, 2005). D. melanogaster, on the other hand, is a human commensal that originally arose in Africa (Lachaise and Silvain, 2004). Several compounds found in the Morinda fruit are toxic and noxious to D. melanogaster and other Drosophila. These include octanoic and hexanoic acid (Jones, 2005). D. melanogaster, as a result, typically avoids this plant. In contrast, D. sechellia responds positively to Morinda’s olfactory cues. When female D. sechellia detect Morinda, for instance, they increase egg production and ovipositioning (R'Kha, et al., 1991). Field experiments suggest that D. sechellia can detect Morinda at distances up to 50m (R'Kha, et al., 1991).

Several recent studies have investigated how D. sechellia perceives Morinda’s odor differently from D. melanogaster (Dekker, et al., 2006; Matsuo, et al., 2007; McBride, 2007). Dekker et al. (2006) recently suggested that D. sechellia differs from D. melanogaster in the numbers and types of sensilla and that this difference in the ability to perceive odors from Morinda leads to the behavioral differences between D. sechellia and D. melanogaster. Congruent with this observation, McBride (2007) has shown that several Ors and Grs appear to have become non-functional in D. sechellia. In contrast to the Dekker et al. and McBride result, Matsuo et al. (2007) have recently suggested that a change in Obp expression in the tarsi of D. sechellia is key to the behavioral difference between D. sechellia and other Drosophila.

Here I use a suite of mutations that affect sensory organs to determine which are critical for perceiving odors from the fruit of Morinda.

Material and Methods

Stocks: The following D. melanogaster stocks were obtained from the Drosophila Stock Center in Bloomington, IN: OR-R, ant, ro, Dfd[3] red e/TM3, Sb[1], kni Dfd[9] e/TM3, Sb[1], th st Ki pb[4] pp/TM3, Sb[1], pb[27]/TM3] Sb[1], lz[3] and lz[77a7]. D. sechellia Line 1 ("Robertson" collected from Seychelles in 1981 by Tsacas and Bächli (1981)), D. sechellia Syn A (a wild-type non-isofemale line; courtesy of J. Coyne), and D. simulans sim6 (an isofemale line from Winters CA, courtesy D. Begun) were used for most comparisons. Except where noted, all stocks were reared on agar-yeast-cornmeal medium at room temperature.

Preference assay—oviposition: Following Jones (2004), oviposition-site preference was scored by presenting inseminated, ovipositing females with a choice of oviposition substrates, one tainted with octanoic acid and one untainted. Media was prepared using Drosophila Instant Medium (Carolina Biological Supply Co.). The toxic media was 0.07% octanoic acid by weight (Sigma Chemical Co.). This dose does not kill susceptible flies (Jones, 2001).

Each female was placed in a chamber with the two types of media. She was allowed to oviposit for two days, after which the number of eggs laid on each type of media was counted. The
female was then shifted to a fresh pair of tainted and untainted media. After 2 more days, her preference was scored again. All assays were conducted in a constant temperature room at 20°C with relative humidity between 50-70%.

Egg counts were converted to a preference index by the following formula:

\[
\text{Preference Index} = \frac{(N_{\text{Eggs on toxic}} - N_{\text{Eggs on control}})}{N_{\text{Both}}}
\]

Positive values indicate preference for tainted media, whereas negative values indicate avoidance of tainted media. Unless otherwise noted, data were pooled across both days.

High throughput assays of preference were performed in test chambers (2L) containing two standard fly bottles (Genesee Scientific, San Diego CA, USA); either one bottle of control media and one bottle of Morinda toxin media or two bottles of control media. Control media was 44 ml of water with 8.5 of Carolina 4-24 instant media (Carolina Biological Supply, NC, USA). Morinda toxin media was made by combining 44 ml of water with 8.5g of Carolina 4-24 instant media with 90 µl octanoic acid and 30 µl of hexanoic acid (Arcos Organics, NJ, USA). The combination was gently agitated to ensure even distribution of the hydrophobic octanoic and hexanoic acids. Morinda fruit has a 3:1 ratio of octanoic to hexanoic acid (Legal, et al., 1994). The concentration of Morinda toxins in the media was low relative to what is typically observed in nature, but not outside the normal range. This concentration was necessary to minimize mortality in D. simulans.

Roughly 90 one-day-old females were collected and allowed to mate ad libitum with males of their own species for three days. Females were then separated and allowed to recover for one day. They were then lightly sedated and placed in test chambers, which were then placed in an environmental chamber with constant humidity and temperature (65%; 25°C). They were allowed to roam the test chamber freely and to choose media to oviposit on.

Genetic ablation of sense organs: We used developmental mutants to ablate sense organs in order to determine which organs are important for oviposition-site preference. To establish the role of the proboscis, we used the proboscipedia (pb) mutation which transforms the proboscis into a (non-functional) leg. To produce these flies, we crossed th st Ki pb[4] pp/TM3, Sb to pb[27]/TM3, Sb. we collected and tested the resulting F1 pb[4]/pb[27] females. To test the effect of maxillary palp ablation, we crossed Dfd[3] red e/TM3, Sb to kni Dfd[9] e/TM3, Sb. A fraction of the F1 Dfd[3]/Dfd[9] females have greatly reduced maxillary palps. Finally, we tested the preference of D. melanogaster flies homozygous for a mutation that renders them antennaless (ant). This mutation normally causes the loss of one or both antennae; we used only those flies missing both antennae. ant has no known effect on tarsi.

As ant had a large effect in the oviposition assay, we wanted to see if ablating the antennae had the same effect in our choice-no choice assay. Unfortunately, the ant stock was lost and is no longer available from either the Bloomington or Kyoto Stock Centers. Instead, we used alleles of lozenge (lz[3] and lz[77a7]) that remove the antennal sensillia thought to be important to detecting fatty acids in Morinda.

Results

Ablation of antenna removes preference: The electrophysiological data of Dekker et al. (2006) suggests that olfactory receptors on the antennae are important to a fly’s perception of Morinda fruit. In contrast, the genetic data of Matsuo et al. (2007) suggest that tarsal taste receptors are playing an important role in oviposition-site preference. To clarify this issue, we used D. melanogaster mutants lacking proboscis, maxillary palps or antennae to see if sense organs on the
head are important for preference behavior. If any of these organs are important, flies lacking these structures should be indifferent to the presence versus absence of the toxin (Preference Index ≈ 0). Only antennaeless (ant/ant) flies are indifferent (Figure 1; one sample sign tests: ant versus no preference: \( P = 0.4049 \); Dfd versus no preference: \( P < 0.0001 \); pb versus no preference: \( P = 0.0005 \)). This result suggests that the antennae play a role in the detection of Morinda.

![Figure 1.](image)

**Figure 1.** Comparison of oviposition-site preference across species and among sense-organ ablation lines. Oviposition-site preference was scored by presenting inseminated, ovipositing females with a choice of oviposition substrates, one tainted with octanoic acid and one untainted (see Methods). *D. sechellia* shows strong preference for tainted media (A). This behavior is recessive to the avoidance of *D. simulans* and *D. melanogaster*. Only ablation of the antennae causes a significant shift in fly behavior (B).

We confirmed the effect of antenna ablation using our choice-no choice assay. Unfortunately, the ant stock was lost and is no longer available. Instead, we used alleles of lozenge (lz[3] and lz[77a7]) to remove some or all of the basiconic sensillia—which are thought to be important to detecting the aromatics in Morinda (Dekker et al., 2006)—from the antennae. lz[3] antennae lack all basiconic sensillia and show a slight preference for Morinda media (61% of flies on Morinda media; \( N = 67 \)). lz[3] also have tarsal claw defects so it is difficult to wholly exclude an effect of taste receptors on the tarsi. lz[77a7] has severely reduced basiconic sensillia and no tarsal defect. These mutants have a tendency to avoid Morinda media more than the lz[3] flies (\( \chi^2 = 38.0; P < 0.0001 \)), but much less than wild-type (38% of lz[77a7] flies on Morinda media; \( N = 169; \chi^2 = 384.4; P < 0.0001 \)). At this point, it is unclear whether the difference between the two lz alleles results from the presence/absence of the tarsal claw defect, the residual basiconic sensillia in lz[77a7] flies, or genetic background effects. It is clear, however, that antennae are important to a fly’s ability to detect and respond to odorant from Morinda.
Discussion

My data show that antennae are important to *D. melanogaster*’s ability to detect and respond to odorants from Morinda fruit. In particular, the removal of the basiconic sensillia clearly reduces avoidance of this fruit, genetically confirming the electrophysiological data of Dekker et al. (2006). My data also suggest that tarsal taste receptors (*sensu* Matsuo et al., 2007) may influence behavior, although not as much as antennae. Interestingly, ablation of sensory organs in *D. melanogaster* is not sufficient to engender *D. sechellia*-like behavior (Figure 1). This result suggests that the host specific behavior is not simply a loss of the ability of *D. sechellia* to perceive its host.

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**Chromosomal aberrations in *Drosophila ananassae*.**

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*Drosophila ananassae* exhibits a high degree of chromosomal polymorphism. It harbors a large number of inversions in its natural populations (Singh, 1998). Out of these reported from various parts of the world, most have restricted distribution while the three cosmopolitan inversions namely, Alpha (AL) in 2L, Delta (DE) in 3L, and Eta (ET) in 3R show worldwide distribution (Singh, 1998). Population genetics of chromosomal polymorphism in Indian natural populations of *D. ananassae* has been extensively studied (for references see the review by Singh, 1998). The results have clearly shown that there is geographic differentiation of inversion polymorphism. Present communication gives the details of chromosomal aberrations detected from natural populations and laboratory stocks of *D. ananassae*. We have tried to include all detected chromosomal aberrations so far in *D. ananassae* in natural populations and laboratory populations to give the holistic picture of chromosomal variability as well as unusual mutational property.

The details of pericentric inversions and translocations detected in *D. ananassae* are given in Tables 1 and 2, respectively. The numbers of pericentric inversions and translocations are twenty one and forty eight, respectively. The occurrence of pericentric inversions (heterozygotes for pericentric inversions produce unbalanced gametes, their appearance, therefore, is opposed by natural selection) and translocations, which are rare in other species of *Drosophila*, reflect unusual mutational properties of *D. ananassae*.

The paracentric inversions are depicted in Figures 1-6 via line diagram. We have followed the improved edition of polytene chromosome reference photomap of *D. ananassae* (Moriwaki and