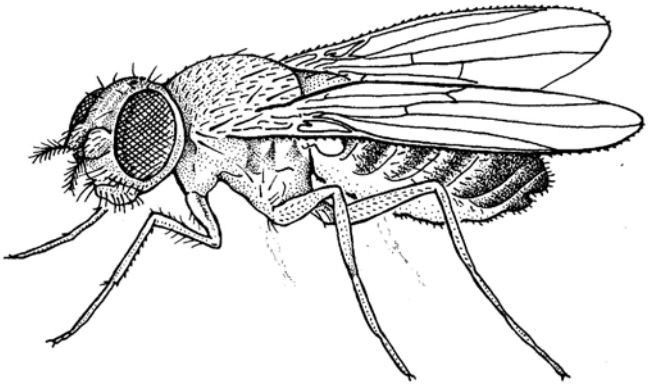
# Drosophila Information Service



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# Preface

Drosophila Information Service (known as "DIS" by those in the field) was first printed in March, 1934. Material contributed by *Drosophila* workers was arranged by C.B. Bridges and M. Demerec. As noted in its preface, which is reprinted in Dros. Inf. Serv. 75 (1994), Drosophila Information Service was undertaken because, "An appreciable share of credit for the fine accomplishments in *Drosophila* genetics is due to the broadmindedness of the original *Drosophila* workers who established the policy of a free exchange of material and information among all actively interested in *Drosophila* research. This policy has proved to be a great stimulus for the use of *Drosophila* material in genetic research and is directly responsible for many important contributions." Since that first issue, DIS has continued to promote open communication.

The production of Dros. Inf. Serv. volume 95 could not have been completed without the generous efforts of many people. Robbie Stinchcomb, Carol Baylor, and Clay Hallman maintained key records and helped distribute copies and respond to questions. Except for the special issues that contained now-dated stock lists and similar material, all current and past issues are now freely-accessible from our web site: www.ou.edu/journals/dis. Each year's issue is uploaded for free access at this site soon after its formal publication at the end of a calendar year.

We continue to encourage all researchers to consider submitting articles that use *Drosophila* for teaching as well as articles that report new techniques, research results, and interesting new mutations. In the interests of honoring the long-standing philosophy of open exchange of ideas, we sometimes accept articles that have unusual perspectives. We thank the many contributors from around the world who sent material for this issue, and we invite your submissions as well as any suggestions you have for maintaining this as a useful *Drosophila* research community resource.

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# Research Notes

## New record of *Drosophila nebulosa* Sturtevant, 1916 (Diptera, Drosophilidae) in western Argentina extends its southern distribution.

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*D. nebulosa* Sturtevant is a member of the *willistoni* species group of the subgenus *Sophophora*. It is rather common in tropical and partly subtropical America (Pavan, 1946), prefering open vegetal formations (Val *et al.*, 1981; Martins, 1987) and xeric environments (Da Cunha *et al.*, 1953), displacing *D. willistoni* as the dominant species at the dry savanna regions (Dobzhansy, 1962).

*D. nebulosa* was previously reported occurring from the southern United States (Texas and Florida; Patterson and Wagner, 1943) into the Caribbean, and throughout South America to the La Plata river area; as far south as Buenos Aires (34º 36' 14" S, 58º 22' 54" W) and Montevideo (34° 54' 21" S, 56° 11' 29" W) in the east (Da Cunha *et al.*, 1953; Valente *et al.*, 1996) and the Tucumán province (26° 49' 50" S, 65° 12' 13" W) in the west (Da Cunha *et al.*, 1953) (Figure 1). However, information regarding the actual geographic range and ecological conditions where this species occurs is scarce and frequently anecdotal.

In the present work we are reporting for the first time the presence of *D. nebulosa* in San Juan province enlarging in a south-western direction its Andean distributional range. Our sampling site, San Agustín del Valle Fértil (30° 38' 1" S, 67° 27' 59" W) is located at 250 km north-east of the capital city of the province, San Juan. This zone belongs to the Phytogeographic Province known as Monte (Cabrera, 1971) with a vegetation typical of a shrub steppe adapted to warm and arid weather, with periodic droughts of 6 to 9 months a year and mean annual precipitation of 200-250 mm (Mirré, 1976). The vegetation is of a xeric environment, with scarce arboreal vegetation, mainly represented by "algarrobos" (genus *Prosopis*), and cacti of *Echinopsis* (cardon) and *Opuntia* (prickly pear) genera.

We used a mix of commercial yeast and mashed banana as bait. The baits were located in plastic buckets in shadowed areas beneath trees and bushes in rocky sloped terrain and near a creek bank. Flies were collected by net sweeping around the buckets. Alongside *D. nebulosa*, we captured other drosophilids such as the cactophilic *D. buzzatii* and *D. koepferae*, the widespread *D. simulans*, and also other dipterans such as Neriid flies.

*D. nebulosa* was identified following Markow and O'Grady (2006). Briefly, along with its sibling *D. fumipennis* Duda, *D. nebulosa* presents a conspicuous trait: it is the only species of the *willistoni* group that presents wings with infuscations that give them the typical dark coloration (Figure 2). However, *D. nebulosa* presents a broader carina and differs from *D. fumipennis* in the relative length of the cephalic setae (Markow and O'Grady, 2006).

The isofemale lines founded from the collected females inseminated in the wild are kept in the stocks of the Laboratory of Evolution at the Buenos Aires University in a rearing medium based on cornmeal and commercial yeast.

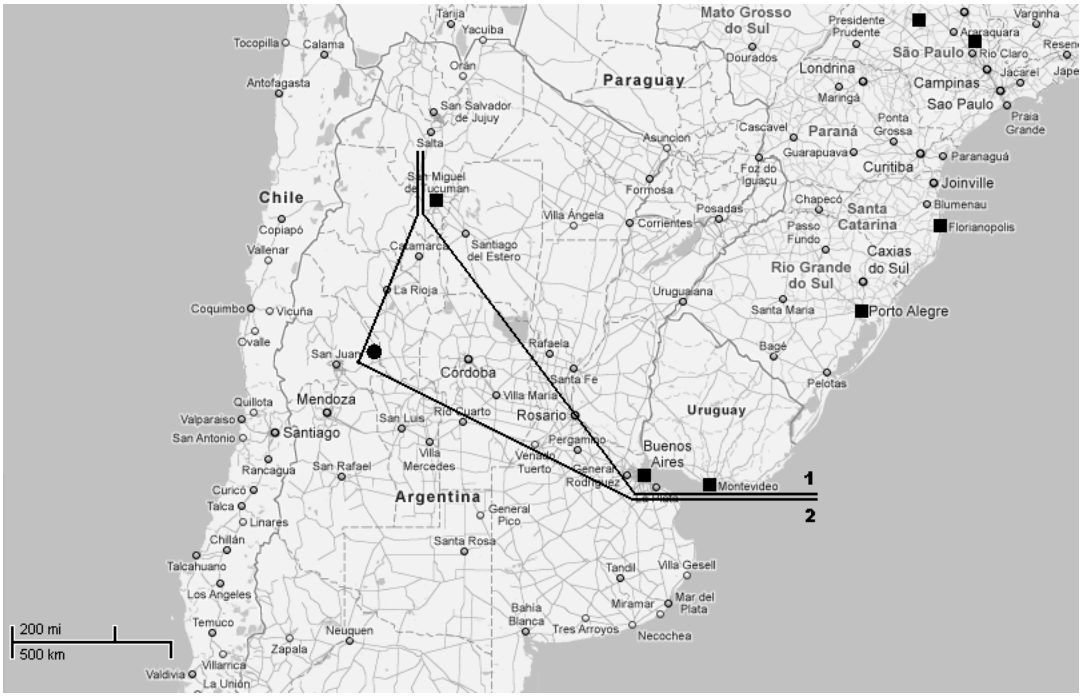


Figure 1. Geographic range of *Drosophila nebulosa* in southern South America. Squares represent localities reported elsewhere (see references). The circle points the location of this new record. Previous (1) and actualized (2) putative southern distributional limits are also shown.

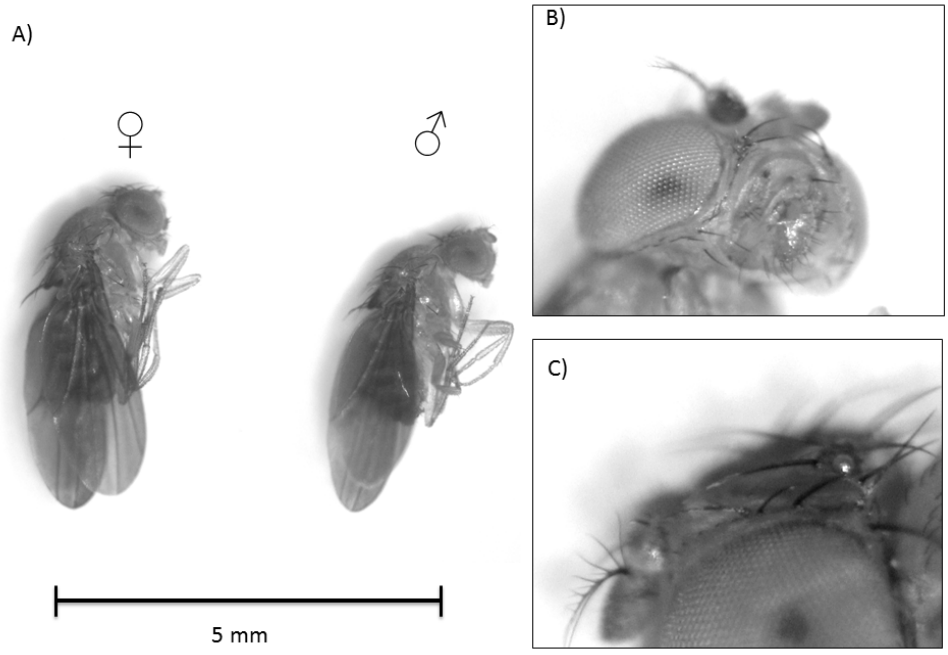


Figure 2. Lateral view of collected specimens of *D. nebulosa* (A) with a more detailed latero-frontal view of the head, showing the distinctive relative lengths of the oral (B) and orbital setae (C) (for further detail, see Markow and O'Grady, 2006).

This finding confirms previous observations that *D. nebulosa* is at its best in the savanna environments where dry seasons alternate with rainy ones (Dobzhansky, 1950), and that it occurs in dry habitats where no sibling species is found (Ayala *et al*., 1974). The finding here reported extends the putative region of ocurrence for this species in about 230,000 square kilometers and into the semi-arid regions of the Andean mountain range.

Finally, we would like to encourage further attempts to summarize and compile the distributional data of other Neotropical species of *Drosophila*, a task dearly needed for the better assessment of the biodiversity and too infrequently performed.

Acknowledgments: To M. Polihronakis who independently confirmed the species identification. This study was supported by CONICET, ANPCyT and Universidad of Buenos Aires. JP is recipient of a postgraduate scholarship of CONICET. IMS is a member of Carrera del Investigador Científico (CONICET).

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**Behavioral characterization of P-element insertion lines of *Drosophila melanogaster*.**

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## Abstract

As continuation of our previously published results (Ahsan *et al.*, 2008) we further characterized the *Drosophila* P-element insertion lines behaviorally in our standardized adult and larval olfactory behavioral paradigms for their olfactory response phenotype. We tested these lines with three chemicals - Ethyl acetate, Isoamyl acetate, and 1-Hexanol at concentrations varying from $10^{-1}$ to $10^{-9}$. We also compared the conditioned and unconditioned olfactory responses. We found insertion lines with varying degree of olfactory responses to the tested chemicals.

## Introduction

The fruit fly, *Drosophila melanogaster*, can smell and discriminate a wide variety of odors with remarkable sensitivity and specificity. Its olfactory system is somewhat similar to vertebrates, but indeed a lot simpler structurally, and may help unravel fundamental principles of chemosensation and contribute to understanding of the more complex process of olfaction in higher organisms. Because of its anatomical simplicity, the availability of genetic information, the well-established physiological and behavioral analysis techniques, and its ability to learn in simple olfactory-based associative learning paradigms, *Drosophila* has become an ideal model organism for studying olfaction (de Bruyne *et al.*, 2001).

Recent advances in genomics and molecular neurobiology have provided an unprecedented level of detail into how the adult *Drosophila* olfactory system is organized. Volatile odorants are sensed by two bilaterally symmetric olfactory sensory appendages, the third segment of the antenna and the maxillary palps, which, respectively, contain approximately 1200 and 120 olfactory receptor or sensory neurons (ORNs or OSNs) each. These ORNs express a divergent family of seven transmembrane domain odorant receptors (ORs) with no homology to vertebrate ORs, which determine the odor specificity of a given ORN.

Adults and larvae are anatomically and behaviorally much different, reflecting their different lifestyles. For example, adult *Drosophila* flies need to find food (as well as mates, egg-laying sites, etc.), which requires sophisticated odor-driven behavior. Fly larvae, in contrast, live on their food source and hence do not need long-range odor detection to find food. Although larvae respond to a variety of chemicals (Rodrigues *et al.*, 1980; Cobb *et al.*, 1999; Heimbeck *et al.*, 1999; Cobb and Domain 2000), one may expect the chemosensory systems of both developmental stages to display significant differences in terms of cell number, organization, and behavioral function. The cephalic chemosensory apparatus of the larva includes three external sense organs, dorsal organ (DO), terminal organ (TO), and ventral organ (VO), as well as three internal, pharyngeal organs (Singh and Singh, 1984; Singh, 1997; Python and Stocker, 2002a; Gendre *et al.*, 2004). Each of them consists of several sensilla, a sensillum comprising one to several sensory neurons and three accessory cells, all housed below a common cuticular structure or terminal pore. The DO is composed of the central "dome" and six peripheral sensilla. The dome, whose wall is perforated by thousands of pore tubules, is innervated by the profuse dendritic arbors of 21 olfactory receptor neurons (ORNs). The odorant receptor family in *Drosophila* consists of 62 members (Clyne, 1999; Vosshall, 1999; Robertson, 2003) compared to more than 1000 in rodents. At least 25 of the 62 receptors are expressed in the fly larva. Of these 25, 14 are larval-specific, while the rest are expressed in both adult and larval olfactory systems. As in the adult, the large majority of the 21 larval olfactory sensory neurons express one conventional odorant receptor, along with an atypical receptor, OR83b.

The evolution of studies on olfaction in *Drosophila* has come a long way, starting from the first odor attraction studies by Barrows (1907), establishment of role of antenna in odor response using genetic approaches (mutant antennaless) by Begg and Hogben (1946), further use of neurogenetics to study various neurological phenomenon by Benzer (1971, 1973), isolation of first single-gene olfactory mutations by Rodrigues and Siddiqi (1978, 1981), and since then many more single-gene mutations are isolated and are being studied in great detail by various researchers. A majority of these mutations have been identified using adult olfactory screens, but in several cases larvae of the mutants show abnormal responses as well. Rodrigues (1978) and Ayyub (1990) isolated olfA, olfB, olfC, olfD, and olfE mutants. olfD shows reduced response to all the odors while olfA, olfB, and olfE show reduction in response to benzaldehyde. olfC shows reduced response to acetate

esters. olfD was found to be allelic with smellblind (sbl, Aceves Pina and Quinn, 1979) and was found to be localed in the para, a class of Na+ channel gene (Lilly *et al.*, 1994). The mutant olf413 larvae show reduced response to only Ethyl acetate and it is normal for other chemicals tested (Tickoo, 2001). chsB is a chemosensory mutant (Trivedi, 2003). Defects in the olfactory behavior reflect lesions at any step of the pathway from odor detection, to transduction, processing, and motor response.

Isolation of *Drosophila* olfactory mutants is important in identifying the genes mediating the sense of smell. First single-gene olfactory mutations were isolated by Rodrigues and Siddiqi (1978, 1981). The P-element has been the workhorse of *Drosophila* genetics since it was developed as a tool for transgenesis in 1982; the subsequent development of a variety of systems based on the transposon have provided a range of powerful and flexible tools for genetics and genomics applications. P-element insertions are frequently used as starting-points for generating chromosomal deletions to remove flanking genes, either by screening for imprecise excision events or by selecting for male recombination events. One of the early uses for P-elements was in large-scale mutagenesis screens, the major advantage over traditional chemical or radiation methods being that mutants were molecularly tagged by virtue of the P-element sequence (Russell *et al.*, 2003). In addition to phenotypic screening, P-elements also can be used to study the pattern and timing of gene expression by enhancer trapping (Figure 3.1) (O'Kane *et al.*, 1987; Wilson *et al.*, 1989).

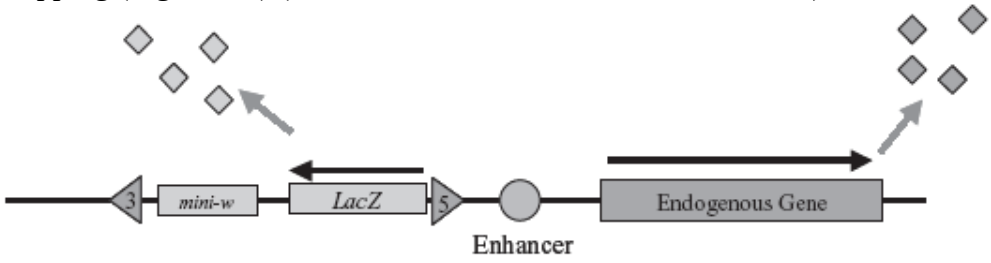



Figure 1. Enhancer trapping. A P-element construct containing a transformation marker, in this case a functional copy of the white gene (mini-w), and a LacZ reporter gene driven by a weak basal promoter inserts near a gene. An endogenous enhancer (grey circle) may then control the expression of the LacZ reporter in a similar pattern to the endogenous gene (black arrows). P-element ends are shown as triangles (5 and 3), and gene products are shown as squares.

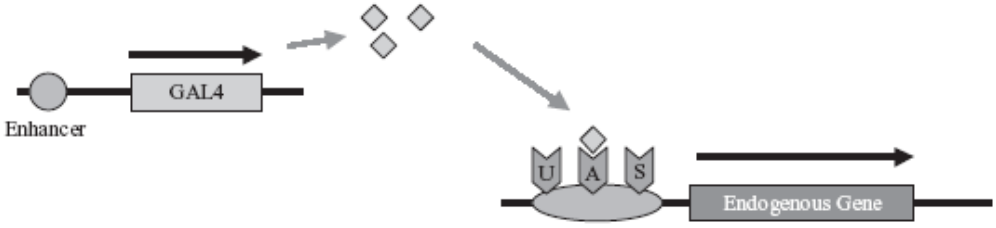



Figure 2. GAL4-activated gene expression. In the GAL4-UAS system, a construct containing the GAL4 gene is inserted randomly in the genome. As with the enhancer trap strategy shown in Figure 2, it may come under the influence of a genomic enhancer and express GAL4 in a pattern dictated by the enhancer. The GAL4 protein can then act at any UAS sites in the genome to activate expression of a gene of interest. Two scenarios are possible. In the first, a gene of interest is introduced into the genome in a P-element construct carrying UAS sites. In the second, a set of UAS sites in a P-element (an EP-element) are mobilised at random in the genome; if they insert in the vicinity of an endogenous gene, GAL4 can be used to activate the expression of that gene.

One widely-used variant of the enhancer trap strategy is the GAL4-UAS system developed by Brand and Perrimon (1993). This binary system utilizes enhancer trapping with a construct carrying the *Saccharomyces cerevisiae* transcriptional activator, GAL4, as a reporter gene and the activity of the GAL4 protein as a transcription factor can be detected by monitoring the expression of a second reporter gene under the control of a GAL4 responsive promoter, or upstream activation sequence (UAS) (Figure 3.2). On the one hand, reporter genes such as LacZ or GFP can be used to visualize the expression pattern of the enhancer. On the other hand, and far more importantly, any gene placed downstream of the UAS sequences in a construct can be activated by the GAL4 protein.

A set of P-element insertions containing P[lArB] (Figure 3.3) and P[GawB] (Figure 3.4) were screened in our lab on the basis of their LacZ and GFP reporter expression patterns in olfactory organs, their adult and larval behavior phenotypes were characterized by me, Nixon (2001), Sukant (2003), Bilal (2004), Shamsudeen (2005), Hisham (2005), Satyajit (2006), Amulya (2007), Deepitha (2007), Shobhana (2007), Shwetha (2007), and others.

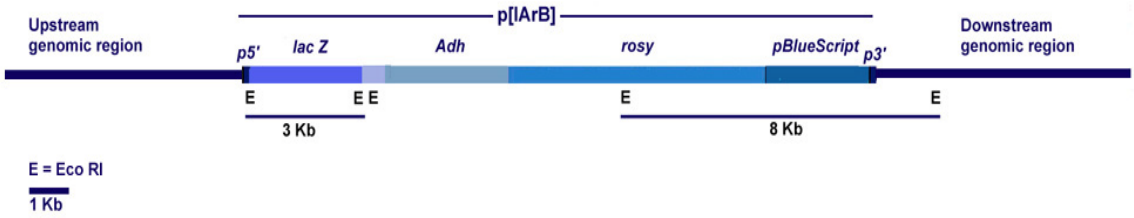



Figure 3. Schematic of the molecular structure of P[lArB]. It consists of enhancer trap *lacZ* and selectable markers *rosy* and *Adh*.

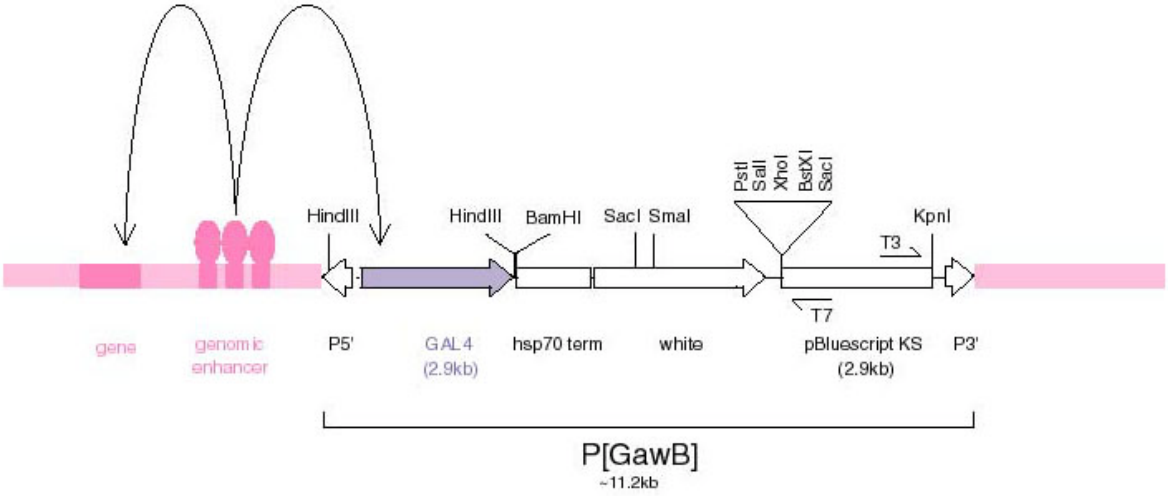



Figure 4. Schematic of the molecular structure of P[GawB]. It consists of enhancer trap *GAL4* (binary system) and selectable marker *white*.

## Materials and Methods

*Drosophila* stocks and maintenance

*Drosophila melanogaster* imagoes (all the strains tabulated below) were maintained at 24ºC in standard cornmeal agar medium with 14-hour light and 10-hour dark cycle. Standard procedures were used for handling the cultures (Roberts, 1986). Flies were allowed to lay eggs and transferred

every 24 hours for expansion. For larval work, flies were allowed to lay eggs in media bottles for 12 hours.

List of fly strains

(1) Canton Special Benzer (CSBz), Wild type stock, TIFR Stock Centre, Mumbai; (2) 003, 030, 191, 1110, OK66, OK140, OK284, OK294, OK301, OK309, Cahir O'Kane, University of Cambridge, Cambridge, UK; (3) 238Y, Josh Dubnau, CSHL.

## Results and Discussion

Adult olfactory responses of P[GawB] insertion lines

Adult flies of eight P[GawB] insertion lines were characterized behaviorally (after four days of conditioning) using the T-trap assay (Chakraborty *et al.*, 2011, 2009). There are different classes of insertion lines, which show varying degree of conditioned and unconditioned responses for the three chemicals tested (Ethyl acetate, Isoamyl acetate, and 1-Hexanol) when compared to the wild type response (Table 1). In the line 238Y, the unconditioned response is increased for both Ethyl acetate and Isoamyl acetate when compared to the wild type (the conditioned response is normal).

Table 1. Adult behavioral phenotype of P[GawB] insertion lines compared to wild type.

| Strains | Ethyl acetate | | Isoamyl acetate | | 1-Hexanol | |
|---|---|---|---|---|---|---|
| | CR | UCR | CR | UCR | CR | UCR |
| 003 | ↓ | ↓ | ↓ | N | ↓ | ↓ |
| 030 | ↓ | N | ↓ | ↓ | ↓ | ↓ |
| 191 | N | ↓ | ↑ | N | N | ↓ |
| OK66 | N | ↑ | ↑ | N | ↓ | ↓ |
| OK284 | N | N | ↓ | N | ↓ | ↓ |
| OK301 | ↓ | ↓ | ↑ | ↑ | ↓ | N |
| OK309 | ↓ | N | ↓ | ↓ | ↓ | ↓ |
| 238Y | N | ↑ | N | ↑ | ↓ | ↓ |

CR : Conditioned Response; UCR : Unconditioned Response; N : Normal;
↑ : Increased; ↓ : Decreased

Larval olfactory responses of P[GawB] insertion lines

The early third instar larval behavioral phenotype for the same eight insertion lines was measured using the larval plate test (Khurana *et al.*, 2009). The lines were tested for a range of log dilution ($10^{-1}$ to $10^{-9}$) of three chemicals (Ethyl acetate, Isoamyl acetate, and 1-Hexanol). There are again different classes of insertion lines showing varying responses across different chemicals tested. Interestingly, when compared to wild type, the responses of few insertion lines are different at certain log dilutions but normal at other log dilutions of the same chemical (Tables 2, 3 and 4) (Figures 5 to 12). The line 238Y shows increased response to all the three chemicals tested at lower dilutions.

The unconditioned response of 238Y line adult flies is increased for both Ethyl acetate and Isoamyl acetate when compared to the wild type and the conditioned response is normal. The third instar larvae show increased response to all the three chemicals tested at lower dilutions.

Isolation of *Drosophila* olfactory mutants is important in identifying the genes mediating the sense of smell. The P-element insertion (P[lArB] and P[GawB]) lines described in this thesis were screened in our lab on the basis of their LacZ and GFP reporter expression patterns in olfactory organs. Eight P[GawB] insertion lines were tested both in adult and larval stages using the T-trap

Table 2. Larval behavioral phenotype of wild type and P[GawB] insertion lines for Ethyl acetate.

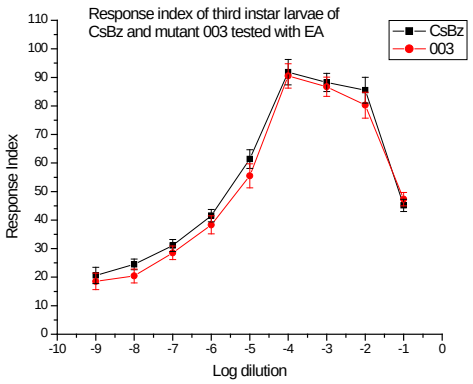
| Log dilution | CsBz | 003 | 030 | 191 | OK66 | OK284 | OK301 | OK309 | 238Y |
|---|---|---|---|---|---|---|---|---|---|
| $10^{-1}$ | 45.15 ± 2.12 | 47.32 ± 2.36 | 43.54 ± 3.28 | 43.19 ± 2.59 | 46.65 ± 3.19 | 46.88 ± 3.12 | 41.57 ± 3.21 | 46.17 ± 2.56 | 43.21 ± 3.24 |
| $10^{-2}$ | 85.53 ± 4.53 | 80.26 ± 4.54 | 86.89 ± 2.76 | 82.45 ± 4.23 | 88.74 ± 4.38 | 82.49 ± 4.23 | 80.92 ± 4.28 | 82.76 ± 3.42 | 80.12 ± 4.31 |
| $10^{-3}$ | 88.23 ± 3.17 | 86.72 ± 3.39 | 86.72 ± 4.23 | 90.73 ± 3.01 | 90.87 ± 4.52 | 85.31 ± 3.82 | 85.37 ± 3.54 | 87.39 ± 3.41 | 83.61 ± 4.64 |
| $10^{-4}$ | 91.83 ± 4.45 | 90.53 ± 4.27 | 92.1 ± 4.43 | 92.56 ± 3.29 | 91.95 ± 3.29 | 90.99 ± 4.21 | 80.53 ± 4.52 | 92.14 ± 4.21 | 85.9 ± 2.28 |
| $10^{-5}$ | 61.38 ± 3.27 | 55.54 ± 4.21 | 52.55 ± 3.21 | 65.01 ± 4.52 | 57.52 ± 2.49 | 65.32 ± 2.38 | 51.76 ± 3.16 | 55.26 ± 2.41 | 62.15 ± 3.5 |
| $10^{-6}$ | 41.56 ± 2.18 | 38.32 ± 3.15 | 45.02 ± 2.76 | 50.56 ± 2.35 | 44.67 ± 3.25 | 50.92 ± 2.73 | 32.47 ± 2.57 | 35.19 ± 2.53 | 56.43 ± 3.45 |
| $10^{-7}$ | 31.11 ± 2.1 | 28.52 ± 2.38 | 35.67 ± 2.91 | 38.42 ± 2.53 | 32.8 ± 3.2 | 40.42 ± 2.45 | 24.19 ± 2.45 | 24.42 ± 2.15 | 51.8 ± 2.76 |
| $10^{-8}$ | 24.5 ± 1.86 | 20.43 ± 2.43 | 25.32 ± 2.19 | 30.23 ± 2.19 | 25.98 ± 2.43 | 30.31 ± 1.99 | 20.32 ± 2.42 | 19.29 ± 2.1 | 49.1 ± 3.85 |
| $10^{-9}$ | 20.62 ± 2.85 | 18.52 ± 2.87 | 22.74 ± 2.84 | 25.65 ± 2.85 | 21.84 ± 2.79 | 24.67 ± 2.42 | 16.12 ± 1.67 | 17.15 ± 1.98 | 36.5 ± 2.19 |

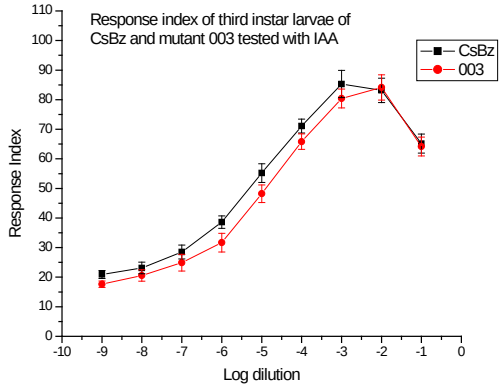
Table 3. Larval behavioral phenotype of wild type and P[GawB] insertion lines for Isoamyl acetate.

| Log dilution | CsBz | 003 | 030 | 191 | OK66 | OK284 | OK301 | OK309 | 238Y |
|---|---|---|---|---|---|---|---|---|---|
| $10^{-1}$ | 65.16 ± 3.21 | 64.18 ± 3.18 | 63.17 ± 2.43 | 69.79 ± 3.21 | 69.72 ± 3.19 | 69.72 ± 3.81 | 66.83 ± 2.45 | 67.91 ± 2.84 | 62.16 ± 3.16 |
| $10^{-2}$ | 83.15 ± 4.12 | 84.13 ± 4.28 | 77.32 ± 3.12 | 80.41 ± 2.56 | 81.83 ± 2.62 | 80.19 ± 4.12 | 82.35 ± 3.41 | 78.31 ± 2.87 | 85.35 ± 2.45 |
| $10^{-3}$ | 85.29 ± 4.6 | 80.42 ± 3.19 | 75.79 ± 2.51 | 82.94 ± 3.63 | 83.52 ± 4.17 | 84.39 ± 2.15 | 84.19 ± 3.67 | 80.74 ± 3.74 | 90.15 ± 4.23 |
| $10^{-4}$ | 71.09 ± 2.36 | 65.81 ± 2.63 | 63.61 ± 2.72 | 75.23 ± 2.69 | 70.94 ± 2.35 | 72.74 ± 3.51 | 75.78 ± 2.49 | 66.73 ± 2.69 | 75.32 ± 3.27 |
| $10^{-5}$ | 55.19 ± 3.14 | 48.23 ± 2.98 | 48.36 ± 3.17 | 60.35 ± 3.52 | 57.29 ± 3.15 | 60.86 ± 2.31 | 63.71 ± 2.68 | 55.81 ± 4.12 | 70.45 ± 2.48 |
| $10^{-6}$ | 38.61 ± 2.12 | 31.68 ± 3.15 | 29.16 ± 2.1 | 50.52 ± 3.19 | 41.82 ± 3.67 | 49.92 ± 3.27 | 50.18 ± 3.72 | 32.93 ± 2.73 | 47.85 ± 3.19 |
| $10^{-7}$ | 28.51 ± 2.34 | 24.92 ± 2.83 | 24.62 ± 2.19 | 38.1 ± 2.15 | 36.52 ± 2.13 | 36.61 ± 2.54 | 38.21 ± 2.81 | 24.76 ± 1.89 | 35.74 ± 2.31 |
| $10^{-8}$ | 23.12 ± 1.97 | 20.56 ± 1.92 | 20.14 ± 1.92 | 30.46 ± 1.98 | 27.42 ± 1.38 | 28.85 ± 1.96 | 32.98 ± 1.93 | 20.75 ± 1.67 | 30.98 ± 2.18 |
| $10^{-9}$ | 20.9 ± 1.35 | 17.63 ± 1.1 | 16.28 ± 1.31 | 24.91 ± 2.17 | 24.91 ± 1.42 | 24.96 ± 2.01 | 26.99 ± 2.14 | 17.82 ± 1.2 | 27.16 ± 1.97 |

Table 4. Larval behavioral phenotype of wild type and P[GawB] insertion lines for 1-Hexanol.

| Log dilution | CsBz | 003 | 030 | 191 | OK66 | OK284 | OK301 | OK309 | 238Y |
|---|---|---|---|---|---|---|---|---|---|
| $10^{-1}$ | 68.15 ± 3.21 | 70.28 ± 4.14 | 64.91 ± 3.16 | 70.8 ± 4.13 | 70.45 ± 2.91 | 67.82 ± 4.15 | 65.97 ± 2.31 | 66.93 ± 2.19 | 66.32 ± 2.13 |
| $10^{-2}$ | 79.12 ± 3.53 | 77.81 ± 2.21 | 78.45 ± 3.52 | 76.29 ± 3.61 | 81.19 ± 2.76 | 80.19 ± 4.21 | 76.12 ± 4.37 | 76.19 ± 4.27 | 78.94 ± 3.61 |
| $10^{-3}$ | 61.81 ± 2.47 | 62.94 ± 3.18 | 63.21 ± 4.81 | 60.57 ± 3.53 | 59.39 ± 3.27 | 62.76 ± 2.84 | 59.52 ± 3.19 | 60.65 ± 3.17 | 72.01 ± 3.72 |
| $10^{-4}$ | 57.5 ± 3.76 | 51.85 ± 2.74 | 50.81 ± 2.36 | 58.98 ± 4.01 | 51.98 ± 4.1 | 54.86 ± 3.91 | 54.87 ± 3.26 | 51.87 ± 3.32 | 70.61 ± 2.78 |
| $10^{-5}$ | 43.61 ± 2.14 | 36.82 ± 2.85 | 34.17 ± 3.19 | 47.78 ± 2.91 | 45.83 ± 2.98 | 44.29 ± 2.38 | 37.19 ± 2.16 | 35.62 ± 2.91 | 53.82 ± 2.19 |
| $10^{-6}$ | 31.18 ± 2.41 | 25.91 ± 1.47 | 24.31 ± 2.64 | 36.47 ± 2.1 | 30.98 ± 2.13 | 36.19 ± 2.13 | 21.91 ± 1.31 | 25.78 ± 2.45 | 44.87 ± 3.52 |
| $10^{-7}$ | 24.52 ± 1.91 | 20.1 ± 2.15 | 20.67 ± 1.69 | 29.32 ± 1.48 | 23.16 ± 1.38 | 30.28 ± 2.87 | 16.35 ± 1.2 | 18.32 ± 1.25 | 40.62 ± 1.98 |
| $10^{-8}$ | 17.19 ± 1.01 | 14.13 ± 1.24 | 13.16 ± 1.03 | 22.91 ± 1.31 | 13.31 ± 1.12 | 20.91 ± 1.25 | 12.81 ± 1.91 | 11.53 ± 1.73 | 30.84 ± 2.01 |
| $10^{-9}$ | 10.12 ± 1.23 | 8.27 ± 1.09 | 8.23 ± 1.14 | 17.78 ± 1.3 | 10.19 ± 1.41 | 14.21 ± 1.01 | 7.06 ± 1.41 | 7.32 ± 1.05 | 20.41 ± 1.57 |



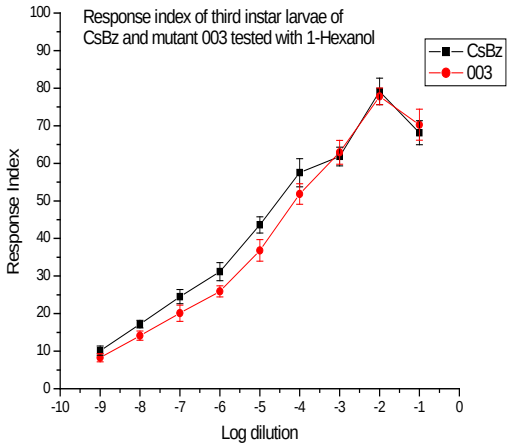



Figure 5 (previous page). Olfactory response of third instar larvae of wild type and 003 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate, and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.

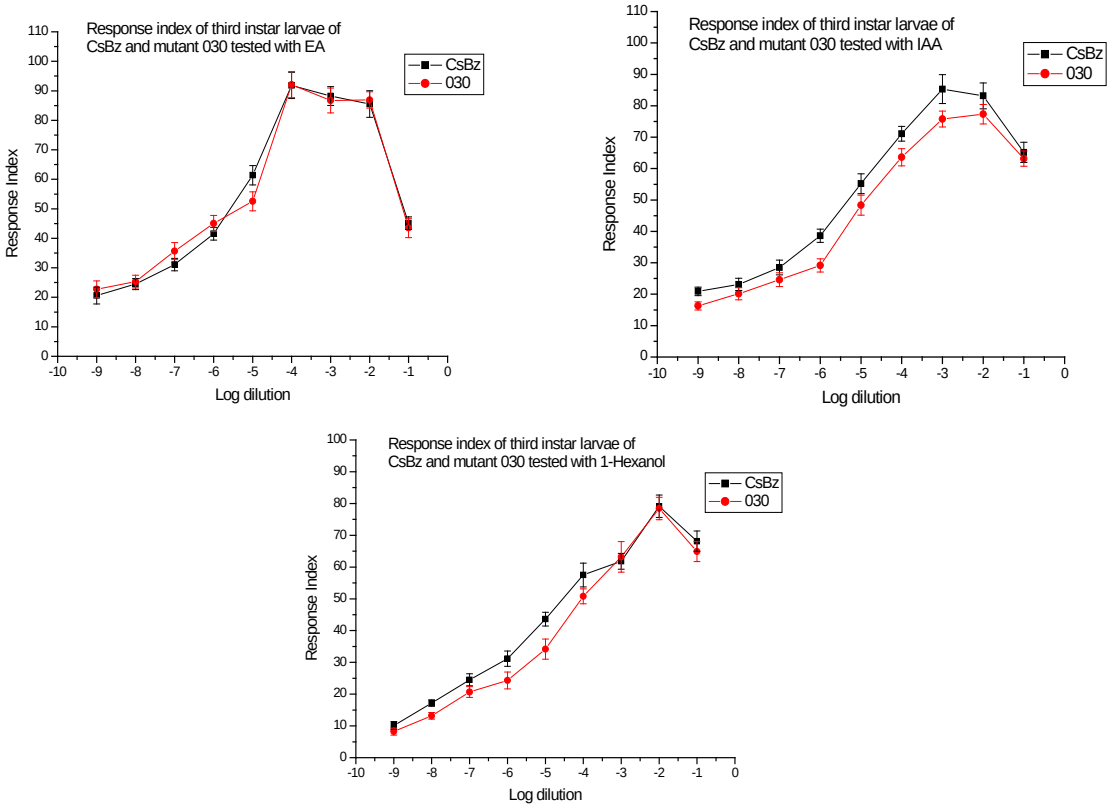



Figure 6. Olfactory response of third instar larvae of wild type and 030 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate, and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.

and larval plate assay, respectively, for three chemicals (Ethyl acetate, Isoamyl acetate and 1-Hexanol). In adults, both conditioned and unconditioned responses were measured and compared with that of wild type. The conditioning was done in Thorpe's media in presence of one of the three odors at standardized concentration. For Ethyl acetate, conditioning at $10^{-4}$ dilution and testing at $10^{-5}$ dilution gives maximum difference between conditioned and unconditioned response, means maximum amount of conditioning. These concentrations were used to screen mutants. Different classes of insertion lines were found with varying responses to the chemicals at different concentrations both in adult and larval stages. In larval stages, the difference with wild type was mainly at lower concentrations of the odor. When the larval response was compared with that of adult unconditioned response (the innate response), larvae of most of the lines show no carryover of the response to adults, meaning the specific response at larval stage does not go to adult necessarily. This indicates toward the brain re-arrangement during metamorphosis (Stocker, 2008). Adult flies need to find food (as well as mates, egg-laying sites, etc.), which requires sophisticated odor-driven

behavior. Fly larvae live on their food source and hence do not need long-range odor detection to find food. Although larvae respond to a variety of chemicals (Rodrigues *et al.*, 1980; Cobb *et al.*, 1999; Heimbeck *et al.*, 1999; Cobb and Domain, 2000), one may expect the chemosensory systems of both developmental stages to display significant differences in terms of cell number, organization, and behavioral function. There are few odorant receptors that are specific to adult and larvae and few are common. For example, larvae of the *003* line show normal response to all the concentrations of the three odors tested, but adults show decreased response to Ethyl acetate and 1-Hexanol and normal response to only Isoamyl acetate. The larvae of *191* line show decreased response to Ethyl acetate and Isoamyl acetate and normal response to 1-Hexanol, but adults show decreased response to Ethyl acetate and 1-Hexanol and increased response to Isoamyl acetate. Thus, the two systems, larvae and adults, behave differently for most of the lines tested. The pleiotropic mutations like these affecting responses to so many chemicals probably exemplify genes whose products are used downstream. In contrast, ligand specific mutations are expected to be in the genes whose products are closer to the receptor end of the pathway. Thus, these P-element insertion lines are important reagents to study the mechanism of olfactory response at the molecular level. And the first crucial step would be to localize the P-element insertions in these lines at base pair level.

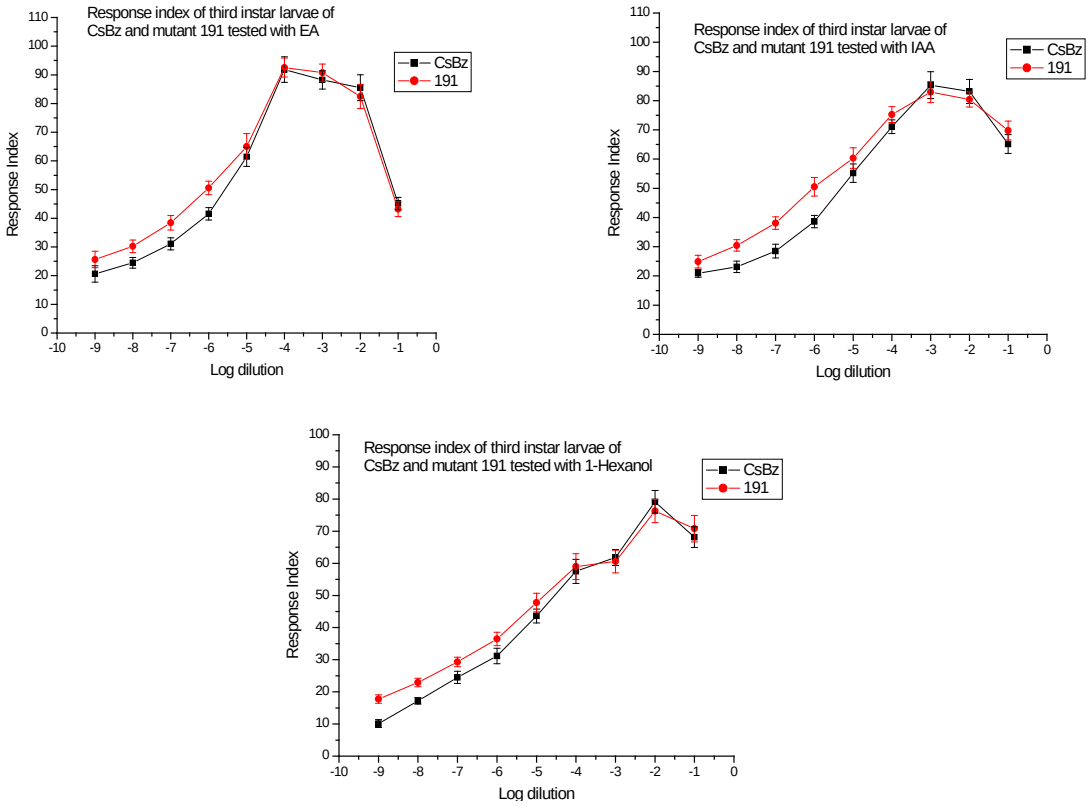



Figure 7. Olfactory response of third instar larvae of wild type and 191 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate, and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.

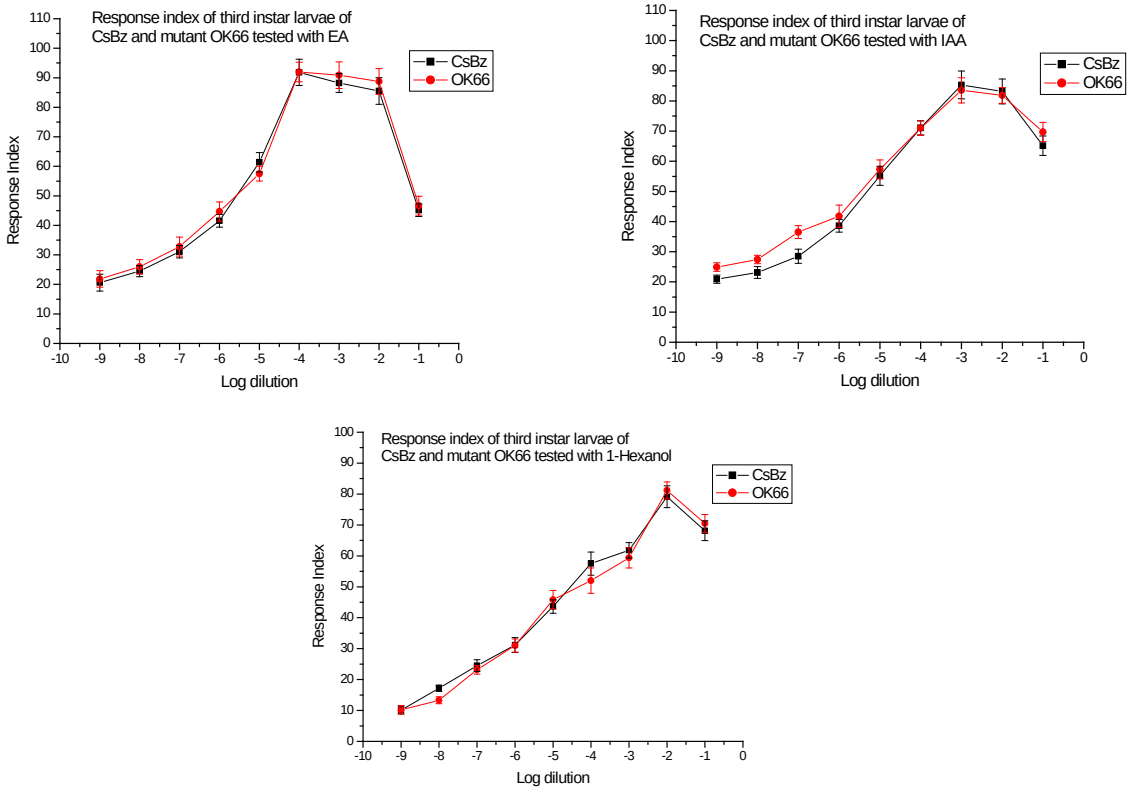



Figure 8. Olfactory response of third instar larvae of wild type and OK66 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate, and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.

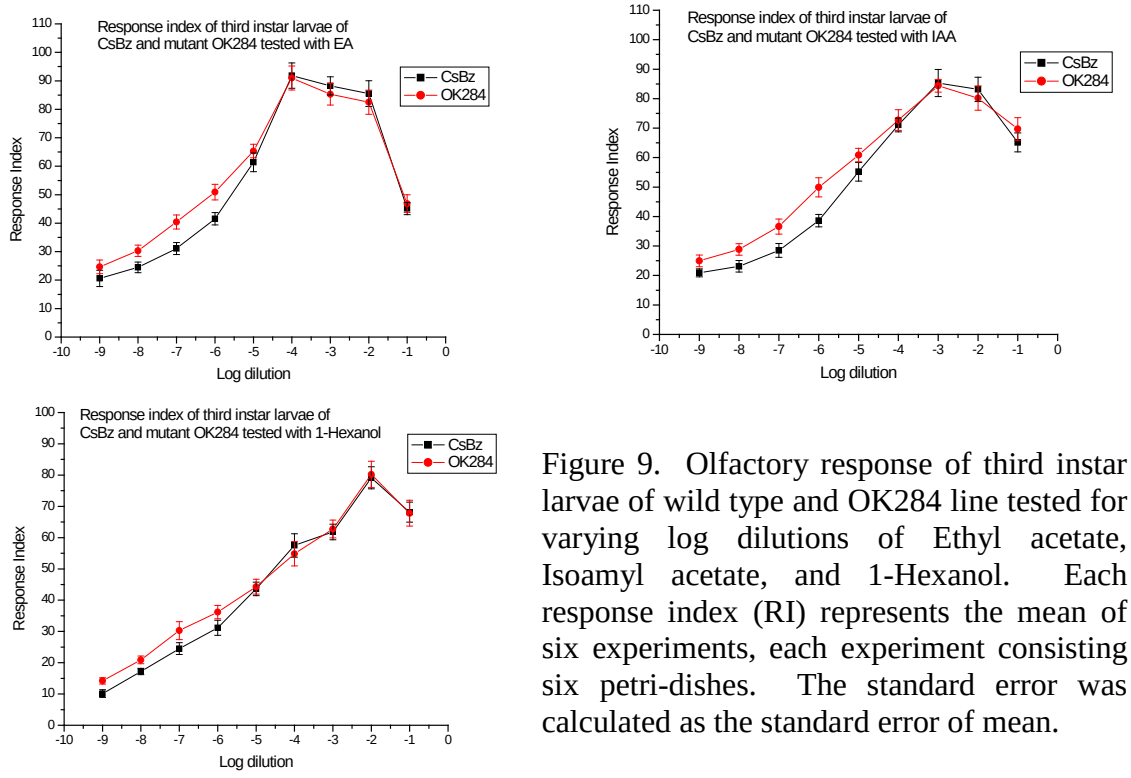



Figure 9. Olfactory response of third instar larvae of wild type and OK284 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate, and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.

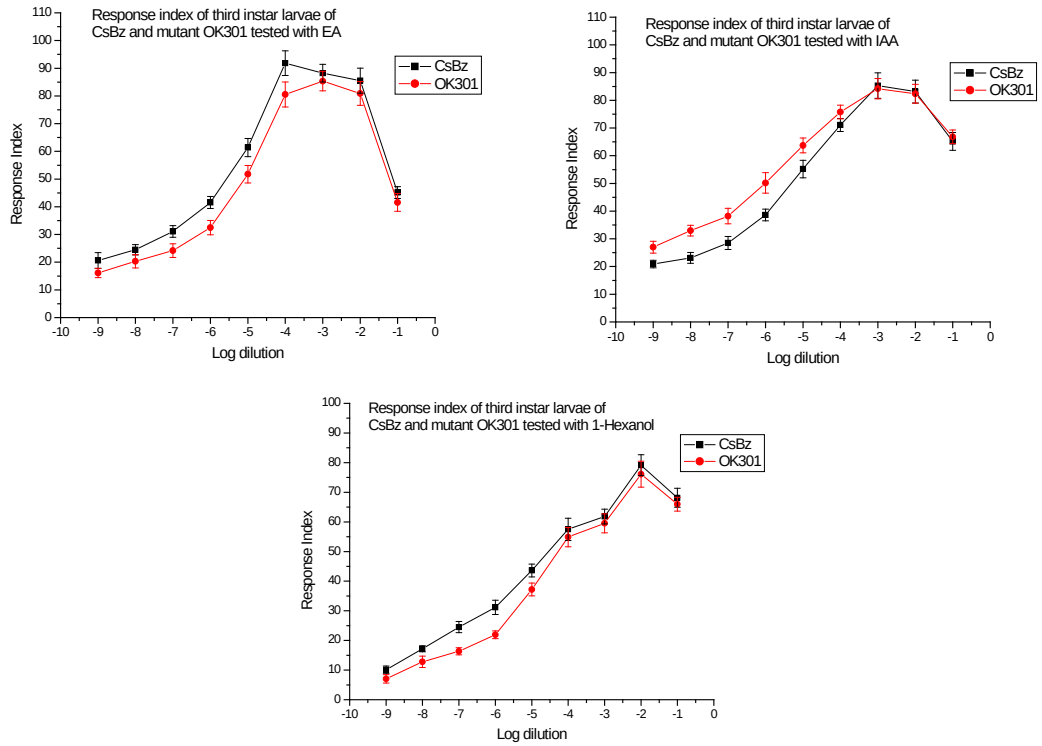



Figure 10. Olfactory response of third instar larvae of wild type and OK301 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate, and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.

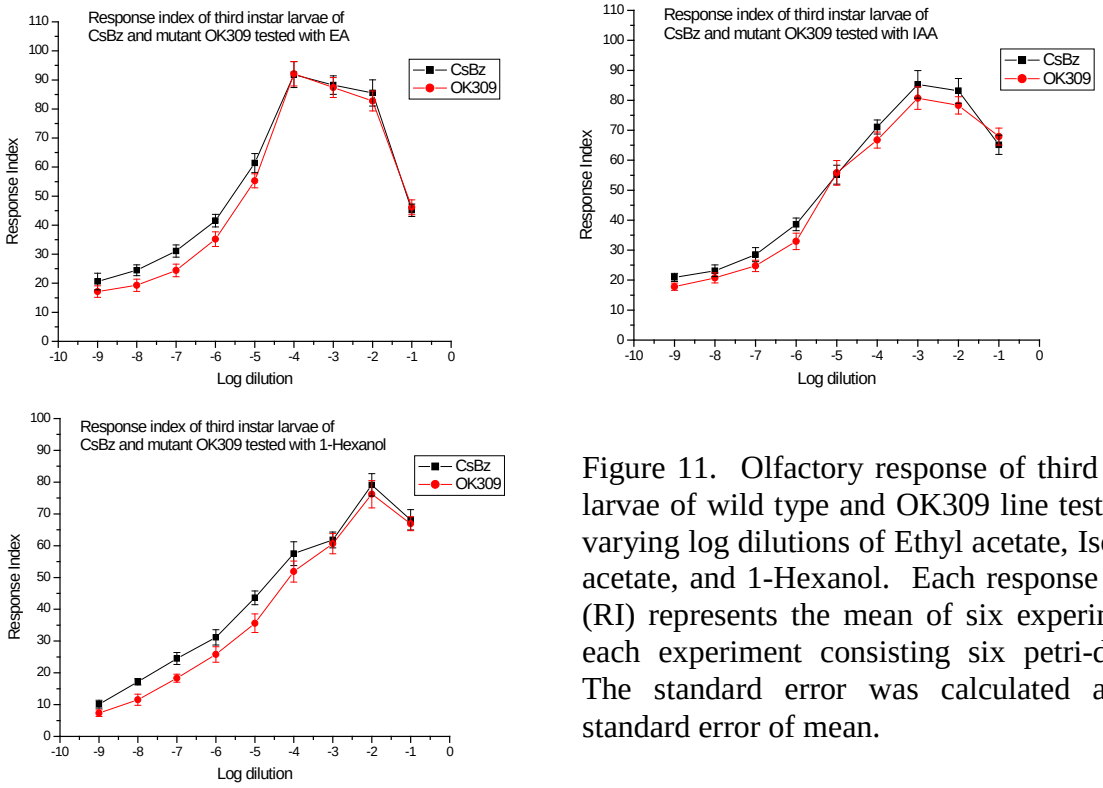



Figure 11. Olfactory response of third instar larvae of wild type and OK309 line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate, and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.

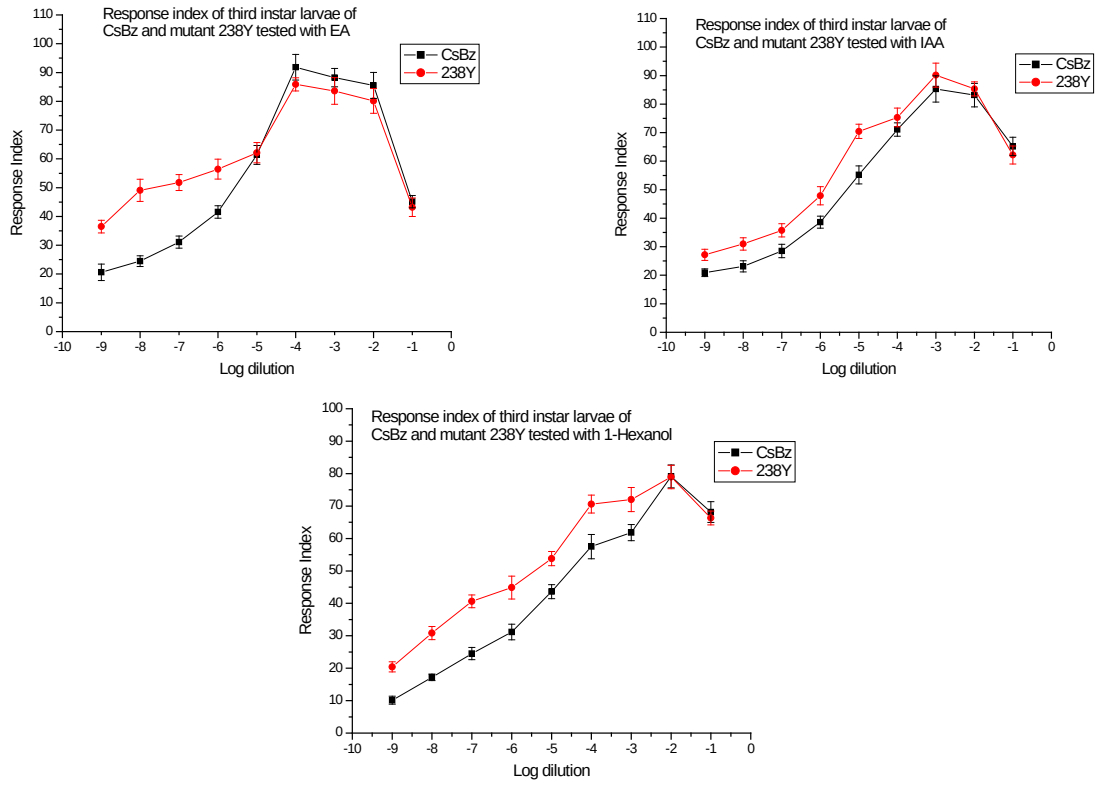



Figure 12. Olfactory response of third instar larvae of wild type and 238Y line tested for varying log dilutions of Ethyl acetate, Isoamyl acetate, and 1-Hexanol. Each response index (RI) represents the mean of six experiments, each experiment consisting six petri-dishes. The standard error was calculated as the standard error of mean.

In our previous and this paper, nine P-element insertion lines have been localized at base pair level. We have olfactory behavioral data for many of these lines at both larval and adult stages, and few of them show interesting phenotypes and few show interesting GFP expression patterns in olfactory organs. The molecular localization of P-element in these lines gave us a list of candidate genes, and the roles of these genes could be established in mediating those interesting olfactory behavioral phenotypes. Few insertions are in intron regions of genes, and they show mutant phenotype and would be really worth investigating. Electrophysiological recordings (EAG and single unit) would tell more about these lines and the mechanism of sense of smell at peripheral level. The plasmid rescue and inverse PCR protocol have been standardized, and using these methods more P-element insertions could be localized and studied if required.

The line *238Y* shows increased olfactory response to certain chemicals at larval and adult stages, and also GFP is expressed specifically in mushroom bodies. It is established that this altered behavioral phenotype in this line is indeed due to the presence of P-element insertion. The molecular and behavioral characterization of this line show that this altered phenotype is not due to *frizzled* gene. So, there could be other possibilities like this insertion might be affecting some gene in trans or enhancer elements of some gene. These possibilities need to be investigated further to understand the molecular mechanism of this altered olfactory behavior in *238Y* line. The larvae of this line show increased initial learning in electroshock paradigm, which is due to the presence of P-element in the

genome. Further characterization of this line at the molecular level and knowing which gene products (if not *frizzled*) are affected in this line should give answers to all these questions.

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## P-element disruption of the *Drosophila melanogaster* homolog of human cancer susceptibility gene does not increase fertility of female heterozygotes.

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### Abstract

BRCA1 and BRCA2 function in homologous meiotic recombination, and mutations in these genes cause human breast and ovarian cancer. Nevertheless, disruptive mutations in these genes are present at an unexpectedly high frequency in human populations. A recent study suggested that BRCA1/2 mutations are maintained because they increase the early-life fertility of their carriers (Smith *et al*., 2011). However, further study of this unexpected fitness advantage in humans is impeded by family planning strategies. Just like its human counterpart, *Drosophila* BRCA2 (*dmbrca2*) interacts with RAD51 and functions in DNA repair by homologous recombination (Klovstad *et al*., 2008) and thus may experience similar selective pressures. We produced flies heterozygous for a BRCA2 knockout and compared fecundity to flies not bearing the mutation in the first two days postcopulation to test for a fitness advantage similar to that observed in humans. In contrast to humans, *Drosophila* heterozygous for a disruption of BRCA2 do not show increased

numbers of eggs compared to their wild-type counterparts, indicating that disruption of this gene does not confer a similar fecundity advantage across species.

## Introduction

Human germline mutation carriers of BRCA1 and BRCA2 mutations have up to an 80% lifetime risk of developing breast cancer (Thorlacius *et al*., 1998). The protein products of tumor-suppressor genes BRCA1/2 are ubiquitously expressed and function in transcription, DNA repair and recombination, and checkpoint control of cell cycle (Venkitaraman, 2002). Women with BRCA1/2 mutations may develop cancer before menopause, resulting in moderate negative selection against these alleles (Smith *et al*., 2011; da Silva, 2012). In spite of this selective pressure, these mutations remain curiously prevalent in human populations.

A recent study suggested that BRCA1/2 mutations are maintained in human populations because they increase the fertility of their carriers (Smith *et al*., 2011). BRCA1/2 mutations correlated with increased fertility in human populations. However, family planning makes further examination of this unexpected fitness advantage in humans challenging (Smith *et al*., 2011). Understanding whether BRCA1/2 mutations have the same effect in model species would facilitate the use of these model species to understand better the genetics that control human cancer susceptibility and possibly treatment.

*Drosophila melanogaster* is an ideal model for the study of reproductive advantages conferred by BRCA2 mutations. The *Drosophila* transcript corresponding to CG30169, hereafter known as *dmbrca2*, is a homolog for human BRCA2 (Lo *et al*., 2003; Klovstad *et al*., 2008). *Drosophila dmbrca2* is known to function in DNA repair by homologous recombination (Klovstad *et al*., 2008) and interact with Rad51 just like its human counterpart (Brough *et al*., 2008). Also similar to humans, *D. melanogaster* exhibit naturally occurring nonsynonymous polymorphisms in *dmbrca2* that appear to have been maintained by balancing selection (Langley *et al*., 2012). However, *Drosophila* are easy to rear and can be subjected to controlled mating to study the impact of BRCA2 mutations. Identifying a reproductive advantage associated with BRCA2 mutations would not only support the result found in human populations, but would serve as the foundation for the use of *D. melanogaster* as a model species for the study of the evolutionary forces acting to maintain variation at this disease-relevant gene in humans.

In the present study we tested whether disruption of *dmbrca2* increased the fecundity of heterozygous females.

## Methods

Three strains of *Drosophila melanogaster* were used in this study. We used two wild type strains, Zimbabwe 29 and Zimbabwe 30 (kindly provided by C. Aquadro and C.-I Wu), and Bloomington Drosophila Stock Center stock number 13272. Stock number 13272 has a P-element inserted immediately upstream of the coding region of *dmbrca2* that prevents expression of the protein product (*y*[1] *w*[67c23]; P{y[+mDint2] *w* [BR.E.BR]=SUPor-P}CG30169[KG03961]). Barnwell *et al.* (2008) showed this P-element insertion disrupts expression of the transcript. For simplicity, the stocks used will be referred to as "Z29", "Z30", and "P-*dmbrca2*".

Several crosses were employed to measure the fertility of *dmbrca2* heterozygotes without subjecting the lines to a potential loss in fertility due to inbreeding depression. First, P-*dmbrca2* was crossed to Z30. This cross produced two classes of progeny, half with the P-element disruption

upstream of *dmbrca2*, and half with the balancer. The female progeny of this cross that did not bear the balancer were individually pair mated to a Z29 male. The second cross ensured that the resultant progeny would not be homozygous for segments derived from any single strain.

To assay the fecundity of *dmbrca2* heterozygotes, female progeny of the Z29 male cross were individually paired with two Z30 males for one day. The females were then transferred to a grape agar petri dish. We scored the number of eggs and larvae produced by each female one and two days after the female flies were transferred to the petri dish. To confirm fertilization, a subset of females that did not produce larvae were dissected, and presence of sperm was assayed by microscopy. We performed PCR to determine whether these mothers were heterozygous for the P-element disruption of *dmbrca2* (F: 5'-TGGGATAGCCTGTTCGGATGAC-3'; R: 5'-GCAACGCTTACCAACAC-TGCA-3'; P-element: 5'-GCTATCGACGGGACCACCTTATG-3'). This three-primer combination yielded a 130 bp band for chromosomes bearing the P-element insertion and a 152 bp band for chromosomes without a P-element. PCR products were visualized on 3% TBE agarose gels. We scored 177 mothers for fecundity and genotype. The fecundity difference between mutant and wild type mothers was tested with a nonparametric Mann-Whitney U-test.

**Results and Discussion**

We estimated an overall fertilization rate of 91% based on the combined percentage of females that had eggs hatch to larvae or bore sperm upon dissection (N = 111). Despite this high fertilization rate, there was extensive day-to-day variation in the number of eggs laid by both *dmbrca2* heterozygotes and wild type females (Figure 1). A Kruskal-Wallis test found that this variation in egg laying was predominantly explained by the day that the flies were allowed to lay eggs ($N = 176$, $p < 0.0001$).

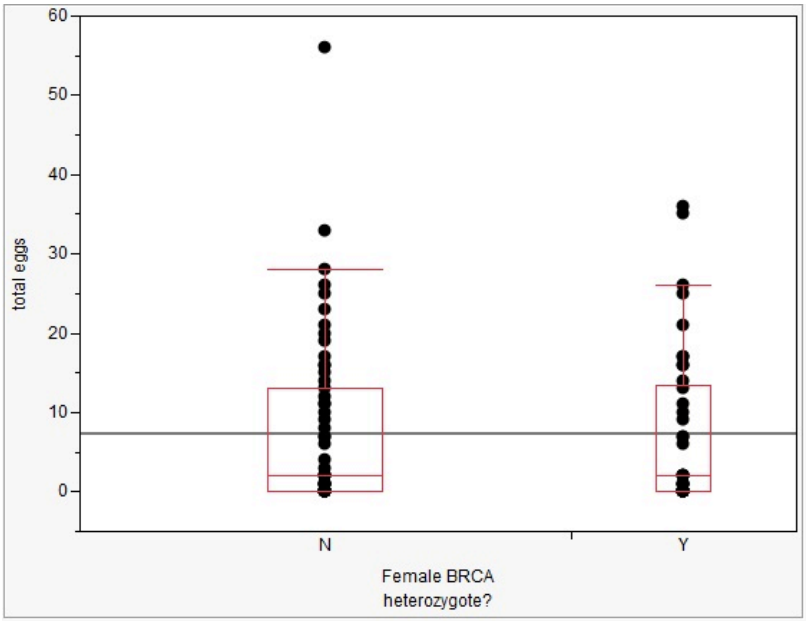


Figure 1. Both *dmbrca2* heterozygotes and wild type females showed large variation in the number of eggs they laid.

Both with and without controlling for the daily variance, females heterozygous for the P-element disruption of *dmbrca2* did not lay significantly more eggs than wild type females (see Figure

1; Mann-Whitney U-test, N = 155, p = 0.55). The lack of difference in fecundity between *dmbrca2* heterozygotes and wild type extended to the number of larvae found on the second day (Mann-Whitney U-test, N = 155, p = 0.28). In fact, wild type females had slightly more larvae on average than the *dmbrca2* heterozygotes.

Overall, we fail to see a fecundity advantage associated with heterozygosity of a *dmbrca2* disruptive mutation. In contrast, flies homozygous for this disruption laid few eggs and exhibited reduced meiotic recombination (2008). Hence, the high nonsynonymous polymorphism in *dmbrca2* across *D. melanogaster* natural populations (Langley *et al*., 2012) is not readily explained by overdominant effects on fecundity, at least in the first two days after mating and for this particular mutation. We cannot exclude the possibility that fitness differences do exist but were not apparent because of specific conditions in which the flies were raised and/ or fecundity differences that may have occurred later after mating.

Furthermore, our result contrasts the previous finding that human BRCA2 disease-causing mutation heterozygotes have significantly more children (Smith *et al*., 2011). This contrast may represent interspecies differences in the function of BRCA2, or it may be a result of the nature of the specific mutation used to disrupt *dmbrca2* in our *Drosophila* study. Like humans, *D. melanogaster* show naturally occurring nonsynonymous polymorphisms in *dmbrca2* across different populations that appear have been maintained by balancing selection possibly due to antagonistic pleiotropy (Langley *et al*., 2012). Indeed, a human BRCA2 variant has been associated both with increased breast cancer risk and increased fetal viability (Healey *et al.*, 2000). This contrast lends itself to the hypothesis that it is not the disruption of BRCA2 that might cause a fecundity advantage in heterozygotes, but instead the *type* of disruption, its interaction with other species-specific factors, or both.

Acknowledgments: We thank C. Aquadro and C.-I Wu for generously providing us with fly stocks. The National Institutes of Health grant GM086445 provided financial support for this project.

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## Electrophoretic variants of xanthine dehydrogenase enzyme in natural populations of *Drosophila ananassae.*

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Allozymes are allelic variants of enzymes which are encoded by structural genes. Polymorphism at enzyme loci arise when mutation occurs followed by the action of evolutionary

forces, such as natural selection, genetic drift or migration, which spread the mutant allele through the population in which it arose. It has been observed that allelic frequency changes at a particular allozyme locus in natural as well as laboratory populations and are often considered as evidence for the occurrence of selection (Ayala *et al.* 1972; Prakash *et al* 1969). Xanthine dehydrogenase (*Xdh*) locus in *Drosophila pseudoobscura* is highly polymorphic for electrophoretic alleles. The allele $Xdh^{1.00}$ is reported to be the most frequent allele, and slower mobility alleles occur in low frequency (Prakash *et al.*, 1969; Singh *et al.*, 1976). Prakash *et al*. (1969) reported eight alleles of this locus in their study by simple native gel electrophoresis. Singh *et al*. (1976) did exhaustive work to determine genetic variation at structural gene locus of xanthine dehydrogenase by taking 146 isochromosomal lines from 12 geographic populations of *Drosophila pseudoobscura*. By using 4 electrophoretic conditions and a heat stability test, they were able to find 37 allelic classes out of 146 genomes examined where only 6 had been previously revealed by usual methods of gel electrophoresis.

*Drosophila ananassae*, a cosmopolitan and domestic species, is known to possess many unique genetic properties among the genus *Drosophila*, and it is one of the most prevalent species in our country (Singh and Singh, 1988; Singh, 2010; Tobari, 1993). Genetic polymorphism in this species has extensively been studied by using three cosmopolitan inversions in its polytene chromosomes (Singh and Singh, 2007, 2010). Genetic polymorphism owing to allozyme variation has not been substantially studied in this species. In the present study we have observed xanthine dehydrogenase polymorphism in two natural populations (Varanasi and Lucknow) of *Drosophila ananassae* by using native polyacrylamide gel electrophoresis of enzymes. Figure 1 shows the in gel assay of Xdh variants.

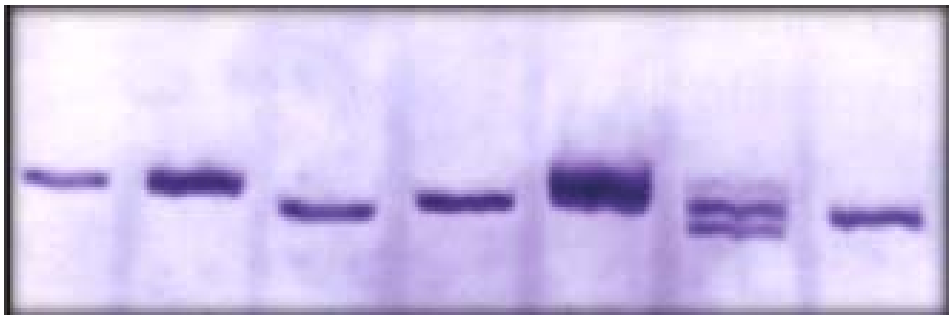

Figure 1. Electrophoretic variants of xanthine dehydrogenase in *Drosophila ananassae.*

Table 1. Allelic frequencies of Xanthine dehydrgenase in two natural populations of *Drosophila ananassae*

| Populations | N | 0.98 | 0.99 | 1.00 | 1.02 | $H_o$ | $H_e$ | $\chi^2$ |
|---|---|---|---|---|---|---|---|---|
| Varanasi | 90 | 0.32 | 0.22 | 0.33 | 0.13 | 0.06 | 0.72 | 225.2* |
| Lucknow | 32 | 0.23 | 0.23 | 0.43 | 0.11 | 0.06 | 0.70 | 76.57* |

*P< 0.001

*Xdh* locus in this species is represented by four alleles and, therefore, we expected ten genotypes to be expressed. Table 1 shows the number of individuals analyzed from Varanasi and Lucknow populations, the frequency of *Xdh* alleles, and observed and expected heterozygotes in these populations. In total we could score only seven genotypes from both the populations. The four alleles marked as *0.98, 0.99, 1.00,* and *1.02* and their frequencies in Varanasi population were 0.32, 0.22, 0.33, and 0.13, respectively. In Lucknow population, the frequencies of these alleles were

found to be 0.23, 0.23, 0.43, and 0.11, respectively. The observed and expected numbers of heterozygotes were also computed. The chi square analysis reveals that the two populations do not follow Hardy–Weinberg equilibrium ($p < 0.001$) indicating the role of some evolutionary forces on this locus.

Acknowledgments: The authors gratefully acknowledge to the University Grant Commission (UGC) for financial support in the form of a major research project.

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## Larval competition among *D. melanogaster* and *D. ananassae.*

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**Abstract:**

Intra- and interspecific preadult competition observations were made using two commonly available *Drosophila* species, namely *D. melanogaster* and *D. ananassae*. Rate of development and viability were taken as fitness parameters in both observations at selected densities. There is an effect of density on developmental time and viability of *Drosophila* when observed at the larval stage. Key words: *Drosophila*, Competition, Rate of development, Viability, Fitness

### Introduction

Any population is made up of interacting individuals with other organisms also (Emmel, 1976). Two species which vie for the same resource co-exist in the nature (Ayala, 1969). It is also true that two species depending on one niche cannot co-exist and results in competitive exclusion (Gause, 1934). To know the intra and interspecific competitive relation the present observation was carried out.

### Materials and Methods

*D. melanogaster* and *D. ananassae* were collected from Jogimatti forest area of Chitradurga (Karnataka-India) and used in the present studies. In the experiments, rate of development and viability have been recorded in intra and interspecific competition. In intraspecific competition the larvae of same age (48 ± 4 hr) were distributed in four different densities of 50, 100, 150, and 200 per vial (3" × 1"). Similarly, in interspecific competition, the larvae of same age (48 ± 4 hr) of both the

species in equal numbers were distributed in four above mentioned densities. For each density, ten replicates were maintained. The emergence of flies was recorded in both the cases at different densities. The experiment was carried at 25 ± 1°C and RH 60%. The obtained data were analyzed using chi-square test for statistical significance.

## Results

The mean developmental rate and viability of *D. melanogaster* and *D. ananassae* at four different densities in intraspecific competition are shown in Table 1. It is observed that there is an effect of density on selected parameters. The developmental time is significantly prolonged along with decreased viability at densities of 150 and 200 larvae per vial. Similarly, in interspecific competition (Table.2) the same result is obtained. There are no significant differences in developmental time and viability between *D. melanogaster* and *D. ananassae* larvae at selected densities in both the cases.

Table 1. Mean developmental time and viability of *D. melanogaster* and *D. ananassae* larvae (48 ± 4hr) at different densities (Intraspecific competition).

| Larvae density (Per each vial) | *D. melanogaster* (In days) | *D. ananassae* (In days) | % Viability of *D. melanogaster* | % Viability of *D. ananassae* |
|---|---|---|---|---|
| 50 | 10.16 ± 0.02 | 9.86 ± 0.07 | 96 | 94 |
| 100 | 10.87 ± 0.06 | 10.14 ± 0.01 | 97 | 98 |
| 150 | 16.15 ± 0.03* | 15.23 ± 0.08* | 81* | 83* |
| 200 | 19.28 ± 0.02* | 17.15 ± 0.06* | 79* | 82* |

- Significant at 5% level.

Table 2. Mean developmental time and viability of *D. melanogaster* and *D. ananassae* larvae (48 ± 4hr) at different densities (Interspecific competition).

| Larvae density (Per each vial) | *D. melanogaster* (In days) | *D. ananassae* (In days) | % Viability of *D. melanogaster* | % Viability of *D. ananassae* |
|---|---|---|---|---|
| 50 | 9.03 ± 0.08 | 9.54 ± 0.03 | 98 | 97 |
| 100 | 9.14 ± 0.02 | 9.32 ± 0.05 | 95 | 93 |
| 150 | 18.22 ± 0.04* | 19.10 ± 0.03* | 85* | 87* |
| 200 | 25.16 ± 0.07* | 23.59 ± 0.02* | 82* | 83* |

- Significant at 5% level.

## Discussion

The competitive principle has been tested in *Drosophila* (Merrell, 1951; Miller, 1954; Budnik and Brncic, 1972; Sevenster and Van Alphen, 1993; Joshi and Thompson, 1995; and Yadav and Singh, 2005). Coexistence of species is regulated by different components of the environment, and species co-exist if the competition is weak (Hardin 1960). In the present experiment it is shown that both species of *Drosophila* are co-existing, and there is an effect of high density on developmental time and viability of the same organisms when observed at 48 ± 4 hr larval stage.

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**Variation in the induction of sexual isolation among populations of *Drosophila ananassae* subjected to different temperature and diet regimes.**

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## Abstract

Sexual isolation results from different mating success among individuals within a population and is recognized as a driving force behind intra-population evolution and speciation. Experiments were conducted to test behavioral isolation among populations of *Drosophila ananassae* raised in different temperature and diet regimes for fifteen generations to test the effect of these environmental variables on the induction of sexual isolation. Multiple choice technique was used, and matings were observed in Elens Wattiaux mating chamber. Results showed non-random mating between populations kept in different temperature and diet regimes though the effect of temperature on the induction of behavioural isolation was more pronounced. This study indicates that the populations have adapted to the different rearing conditions and that the genes involved in some aspects and mating behavior may be involved in the adaptation leading to the development and non-random mating among the populations adapted to the different environmental variables. Temperature may have a more pronounced effect on the mating behavior of the flies, which resulted in the development of sexual isolation among the populations.

## Introduction

The early steps of animal speciation are thought to be the development of reproductive isolating mechanisms that act as barriers to gene flow between incipient species or populations. To gain insight about the early stages of species formation, we need to understand the basis of ethological isolating mechanisms causing speciation. Premating ethological isolating mechanisms are thought to precede the evolution of post-zygotic isolating mechanisms and is of more importance as occurrence of inferior hybrids is minimized or avoided. The evolution of reproductive isolation and speciation has recently received a great deal of attention, and one of the most pervasive conclusions is that speciation becomes more probable in allopatry as geographically distant populations are more likely to experience divergent selective conditions as well as a reduction in homogenizing gene flow, which is one of the greatest impediments to the evolution of isolating mechanisms (Dobzhansky 1937; Mayr 1942, 1963; Coyne and Orr 2004; Gavrilets 2004). The influence of rearing temperature on mating propensity was reported in *D. melanogaster* (Casares *et al*., 2005). The effect of diet on reproductive isolation was studied by Dodd (1989).

A number of studies on sexual behavior have been carried out in *D. ananassae*. Singh and Chatterjee (1985a, b) reported sexual isolation using mass culture and isofemale lines. Recently, sexual isolation was observed between karyotypically different homozygous strains as well as among drift lines (Nanda and Singh, 2011a, b). However, many questions still remain about the exact biological conditions and the basis of incipient reproductive isolation among populations. The present work was carried out to study the induction of sexual isolation by two environmental variables, temperature and diet.

## Materials and Methods

*Drosophila Stocks*

Three mass culture stocks of *Drosophila ananassae* were established from flies collected from three geographical localities: (a) : AG : established from Agra, UP, India; (b) : MT : established from Mathura, UP, India; and (c) : AL : established from Aligarh, UP, India.

The six stocks from each locality were established from naturally impregnated females (number given in experimental design). The stocks were maintained on agar corn meal sugar medium under normal laboratory conditions by transferring 50 flies (females and males in equal number) to fresh culture bottles in each generation. The tests for sexual isolation and sexual selection were conducted among the three stocks after a generation of culture prior to the experiment, and random mating was observed among the stocks. The stocks were then subjected to different temperatures and diets for fifteen generations of culture, and multiple choice tests were run.

The codes for the stocks are as follows :

*Stocks reared in different temperatures*

AG1, MT1, AL1 - 18°C ; AG2, MT2, AL2 – 20°C; AG3, MT3, AL3 – 24°C

*Stocks reared in different diets*

AG4, MT4, AL4 – Ethanol; AG5, MT5, AL5 – Lactic acid; AG6, MT6, AL6 – Normal

*Experimental Design*

Crosses were conducted for all the 36 possible combinations, five replicates for each cross, after fifteen generations of culture. One day before the experiment, the flies were transferred to fresh food vials with either red or green food color for identification of the flies. The food coloring had no effect on the mating behavior of the flies. Fifteen flies of each sex were transferred without etherization in Elens Wattiaux (1964) mating chamber and observed for 60 minutes. The mated pairs were aspirated out and observed under the binocular for identification.

*Statistical Analysis*

Data were recorded and analyzed using JMATING (Carjaval Rodriguwz and Rolan Alvarez, 2006) software for mating frequency. Sexual isolation was tested by calculating $I_{PSI}$ index and G test. Oneway ANOVA was used to calculate the level of sexual isolation between the crosses. SPSS 17.0 for MS Windows was used to perform statistical analysis.

## Results

Table 1 shows the $I_{PSI}$ index and heterogeneity G test for sexual isolation and sexual selection effects between the stocks of *D. ananassae.* Significant values were observed in some combinations,

showing evidence of sexual isolation among the crosses. Stocks from all the three locations showed nonrandom mating when reared under different temperature regimes, the most significat being crosses from Agra reared in 18°C and 20°C. Figure 1 shows the average IPSI index between the different crosses reared in same and different temperatures and diets. The results show that sexual isolation is greater between stocks reared under different temperatures than stocks reared under same temperature. Similarly, isolation is greater between crosses reared in different diets than between those reared in same diet. ANOVA used to test the deviation from random mating (data not given) among the stocks showed statistically significant results suggesting nonrandom matings in these pairwise combinations.

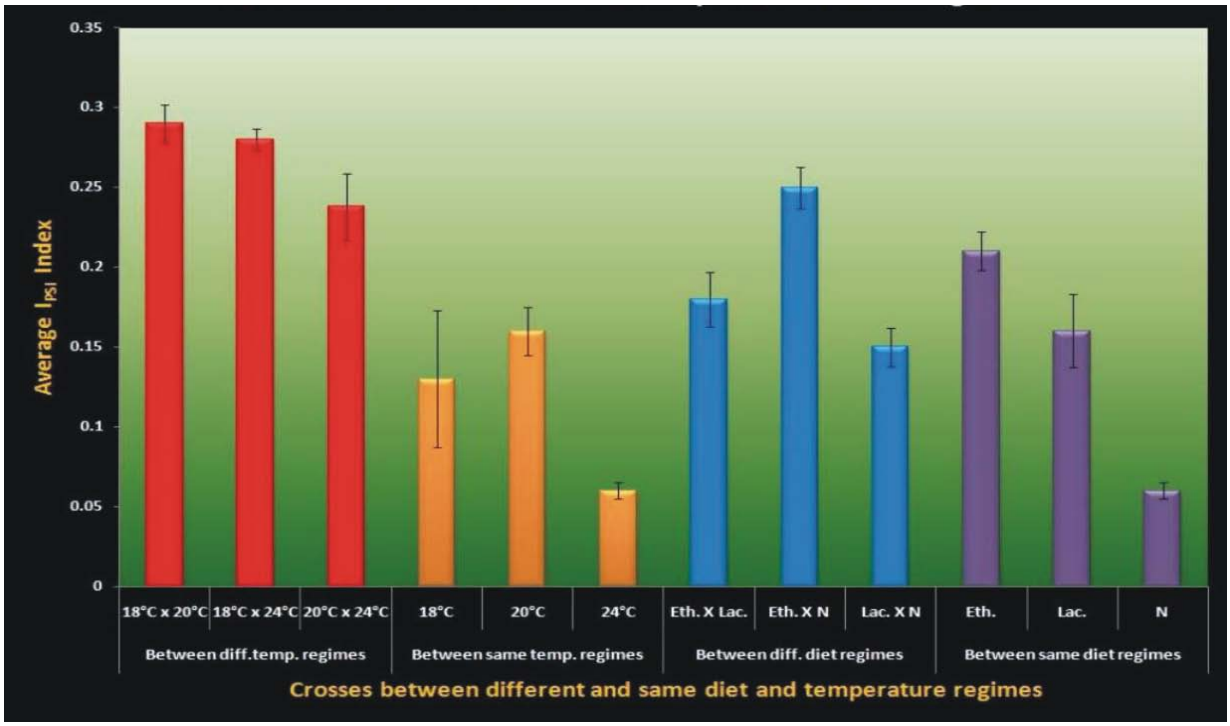


Figure 1. Multiple choice mating tests results between populations of *D. ananassae* raised in different temperature and diet regines.

**Discussion**

Our results provide evidence of significant sexual isolation between populations reared in different temperature and diet regimes. Crosses between populations kept in different temperature regimes showed higher sexual isolation than that between populations reared on different diets, suggesting that temperature may have a more profound effect on the genes affecting the mating behavior of the flies leading to behavioral isolation.

The study indicates that the populations have adapted to the different temperature and diet regimes and that genes involved in some aspects of mating behavior may be involved in the adaptation leading to the development of non-random mating among the populations. Earlier studies done on stocks reared in different temperatures showed the induction of sexual isolation among the strains (Yadav and Yadav, 2012).

Table 1. IPSI coefficients along with their standard deviations and heterogeneity G test for sexual isolation and sexual selection effects between the stocks of *D. ananassae*.

| Crosses | IPSI | SD | P value | GI | GS | GT |
|---|---|---|---|---|---|---|
| AG1 vs AG2 | 0.3027 | 0.0962 | **0.0024** | 9.14 | 0.04 | **9.18** |
| AG1 vs AG3 | 0.2779 | 0.0962 | **0.0042** | 7.79 | 0.04 | **7.83** |
| AG2 vs AG3 | 0.2161 | 0.0973 | 0.0322 | 4.77 | 0.08 | 4.85 |
| MT1 vs MT2 | 0.2962 | 0.0969 | **0.0034** | 8.96 | 0 | **8.96** |
| MT1 vs MT3 | 0.292 | 0.103 | **0.005** | 7.6 | 0.09 | **7.69** |
| MT2 vs MT3 | 0.2419 | 0.0982 | 0.0164 | 5.84 | 0.08 | 5.92 |
| AL1 vs AL2 | 0.2785 | 0.0933 | **0.0032** | 8.14 | 0.1 | **8.24** |
| AL1 vs AL3 | 0.2902 | 0.1086 | **0.0078** | 6.71 | 0.22 | **6.93** |
| AL2 vs AL3 | 0.258 | 0.0999 | 0.0124 | 6.2 | 0.04 | 6.24 |
| AG1 vs MT1 | 0.1254 | 0.0968 | 0.197 | 1.62 | 0.1 | 1.72 |
| AG1 vs AL1 | 0.1797 | 0.0994 | 0.0796 | 3.21 | 0.08 | 3.29 |
| MT1 vs AL1 | 0.0947 | 0.0966 | 0.3344 | 0.94 | 0.19 | 1.13 |
| AG2 vs MT2 | 0.1484 | 0.0998 | 0.1336 | 2.2 | 0.1 | 2.3 |
| AG2 vs AL2 | 0.1776 | 0.0984 | 0.076 | 3.21 | 0.08 | 3.29 |
| MT2 vs AL2 | 0.1712 | 0.1008 | 0.092 | 2.87 | 0.02 | 2.89 |
| AG3 vs MT3 | 0.0657 | 0.0974 | 0.503 | 0.46 | 0.02 | 0.48 |
| AG3 vs AL3 | 0.0643 | 0.0963 | 0.503 | 0.45 | 0.09 | 0.54 |
| MT3 vs AL3 | 0.0737 | 0.0958 | 0.4604 | 0.59 | 0.07 | 0.66 |
| AG4 vs AG5 | 0.1701 | 0.0969 | 0.0824 | 3.07 | 0 | 3.07 |
| AG4 vs AG6 | 0.2369 | 0.0974 | **0.0074** | 6.84 | 0.04 | **6.88** |
| AG5 vs AG6 | 0.138 | 0.0953 | 0.1502 | 2.06 | 0.09 | 2.15 |
| MT4 vs MT5 | 0.1746 | 0.097 | 0.074 | 3.13 | 0.04 | 3.17 |
| MT4 vs MT6 | 0.2591 | 0.097 | 0.0098 | 6.73 | 0.08 | 6.81 |
| MT5 vs MT6 | 0.1602 | 0.1062 | 0.1342 | 2.24 | 0.05 | 2.29 |
| AL4 vs AL5 | 0.2018 | 0.1079 | 0.062 | 3.44 | 0.12 | 3.56 |
| AL4 vs AL6 | 0.2606 | 0.1 | **0.0096** | 6.51 | 0.02 | **6.53** |
| AL5 vs AL6 | 0.1585 | 0.1057 | 0.1444 | 2.19 | 0.18 | 2.37 |
| AG4 vs MT4 | 0.2109 | 0.1019 | 0.0414 | 4.2 | 0.17 | 4.37 |
| AG4 vs AL4 | 0.1986 | 0.1002 | 0.05 | 3.74 | 0.1 | 3.84 |
| MT4 vs AL4 | 0.2237 | 0.1012 | 0.0284 | 4.72 | 0.27 | 4.99 |
| AG5 vs MT5 | 0.1678 | 0.0976 | 0.0856 | 2.85 | 0.17 | 3.03 |
| AG5 vs AL5 | 0.1366 | 0.0996 | 0.1722 | 1.89 | 0.04 | 1.93 |
| MT5 vs AL5 | 0.1823 | 0.0996 | 0.0706 | 3.29 | 0.2 | 3.49 |
| AG6 vs MT6 | 0.0657 | 0.0974 | 0.503 | 0.46 | 0.02 | 0.48 |
| AG6 vs AL6 | 0.0643 | 0.0963 | 0.503 | 0.45 | 0.09 | 0.54 |
| MT6 vs AL6 | 0.0737 | 0.0958 | 0.4604 | 0.59 | 0.07 | 0.66 |

The results are in accordance to those of Dodd (1989), which demonstrates that reinforcement of the premating isolating mechanisms through selection is not necessary for the development or

induction of significant levels of behavioural isolation. The sexual isolation observed developed in complete allopatry and is solely due to the process of adaptation to the different regimes.

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## Role of inversion system on morphometric and fitness traits in *Drosophila ananassae*.

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### Introduction

The role of inversions on the fitness characters have been well demonstrated (Dobzhansky and Wallace, 1953). Many experiments have demonstrated the superiority of heterokaryotypes over homokaryotypes (Singh, 1989a; Singh and Som, 2001). *Drosophila ananassae* is one such species which exhibits high level of inversion polymorphism. It is a cosmopolitan domestic species having a unique status among *Drosophila*. Due to certain peculiarities such as male crossing over, high mutability, high level of chromosomal polymorphism it has been used for many genetic studies. This species harbors a large number of inversions. Influence of different chromosomes on sternopleural bristle number have been detected, and different genetic factors controlling sternopleural bristle number have been located in different chromosomes by using marker strains (Shrimpton and Robertson, 1988a). Genetic heterogeneity for sternopleural bristle number has been found in Indian populations of *D. melanogaster* (Singh and Das 1991; Griffiths *et al*., 2005; Yadav and Singh, 2006).

In certain cases, association between chromosomal inversion polymorphism and morphometric characters has been reported (David *et al*., 2003; Griffiths *et al*., 2005; Singh and Das, 1991; Yadav and Singh, 2006). The earlier studies on inversion polymorphism in *D. ananassae* (Da Cunha, 1960; Singh, 1998) have demonstrated that most of the inversions are distributed either on second or third chromosome. Two hypotheses have been proposed to account for the concentration of inversions on single chromosome, the co-adaptation hypothesis and mechanical hypothesis.

The author in the present studies has made analysis of inversion polymorphism, comparison of morphometric traits and fitness are studied in an inversion free strain and strains carrying 2LA, 3LA and 2LA+3LA inversions of Dharwad population of *D. ananassae*.

## Materials and Methods

In the present study inversion strains were established from *D. ananassae* flies collected from wild locality of Dharwad using the procedure of Hegde *et al.* (1999). These flies were maintained in the vivarium at constant temperature of 22 ± 1°C and relative humidity of 70%. Preparations of polytene chromosomes were made to screen for the presence or absence of inversions following the procedure of Jayaramu (2009). Progenies of these inversion strains were isolated and aged for 5-6 days analyze morphometric traits (sternopleural bristles, scutellar bristles of the left side of the body, head width and wing length of the female), and fitness character fecundity and ovarioles have been measured using the procedure of Jayaramu (2009) and Prathibha (2011).

Table 4a. Morphometric traits in different inversion strains in *D. ananassae* (Values are Mean ± SE)

| Strain → ↓Parameters | Inversion free | 2LA inversion | 3LA inversion | 2LA+3LA inversion |
|---|---|---|---|---|
| Sternopleural bristles | 6.26 ± 0.102[a] | 6.70 ± 0.086[b] | 6.84 ± 0.082[b] | 7.74 ± 0.14[c] |
| Scutellar bristles | 4.30 ± 0.06[a] | 4.48 ± 0.05[b] | 4.56 ± 0.07[b] | 4.64 ± 0.06[b] |
| Head width | 0.72 ± 0.007[a] | 0.75 ± 0.009[b] | 0.77 ± 0.011[b] | 0.82 ± 0.003[c] |
| Wing length | 1.82 ± 0.02[a] | 1.83 ± 0.02[a] | 1.84 ± 0.03[a] | 1.89 ± 0.02[a] |

Same superscript in each row indicates that the value is non significant by DMRT.

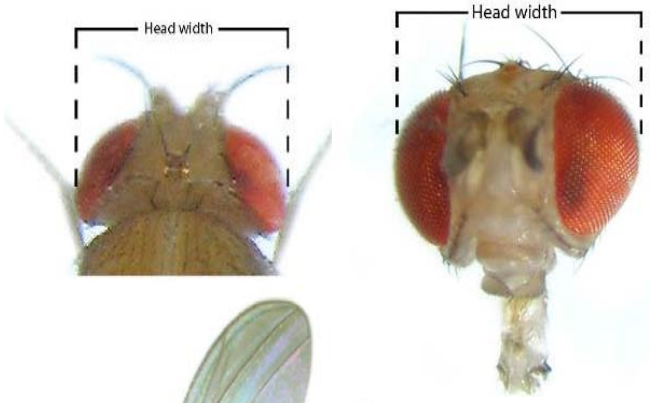


Figure 1. Points of head width of *D. ananassae* (a = Dorsal view, b = Front view).

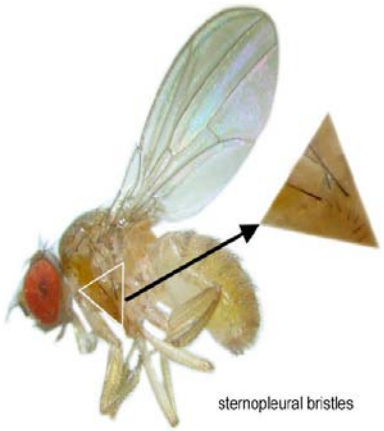


Figure 2. Counting sternopleural measurement of bristles.

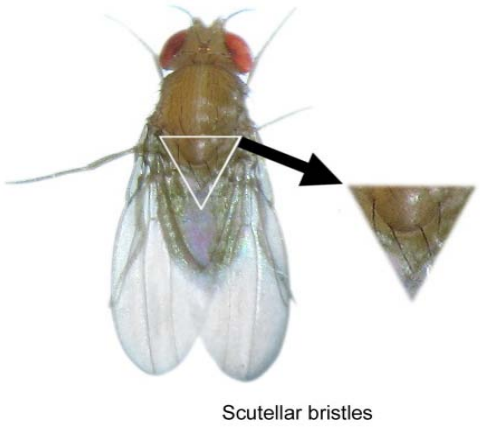


Figure 3. Counting scutellar bristles.

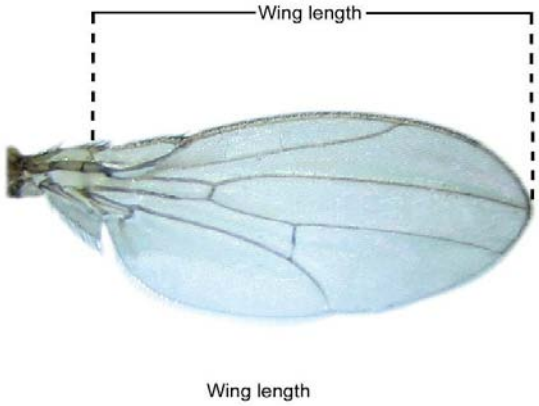


Figure 4. Points of measurement of wing length.

Table 4b. One way ANOVA for morphometric traits in different inversion strains of *D. ananassae*

| Source | | Sum of squares | df | Mean squares | F value | P value |
|---|---|---|---|---|---|---|
| Sternopleural bristles | Between groups<br>Within groups<br>Total | 57.895<br>110.460<br>168.355 | 3<br>196<br>199 | 19.298<br>0.564 | 24.243 | 0.000** |
| Scutellar bristles | Between groups<br>Within groups<br>Total | 03.295<br>46.660<br>49.955 | 3<br>196<br>199 | 1.098<br>0.238 | 4.614 | 0.004* |
| Head width | Between groups<br>Within groups<br>Total | 0.289<br>0.896<br>1.186 | 3<br>196<br>199 | 0.096<br>0.004 | 21.082 | 0.000** |
| Wing length | Between groups<br>Within groups<br>Total | 0.141<br>5.951<br>6.092 | 3<br>196<br>199 | 0.046<br>0.036 | 1.544 | $0.204^{NS}$ |

NS- non significant *P<0.05; **P< 0.001.

## Results and Discussion

In the present study the relationship between different inversions with some morphometric traits such as sternopleural bristles, scutellar bristles, head width and wing length was studied (Table 4a, and Figures 1, 2, 3, and 4). Significant difference found in such an analysis suggests differential genotypic influences on these traits (Table 4b). In the present study the author has analyzed the differences in four morphometric traits in four different genetic strains of *D. ananassae* viz., inversion free strain, the second with a sub-terminal inversion on the left arm of second chromosome 2LA strain, third with a terminal inversion on the third chromosome 3LA strain and the fourth with two inversions, one on second chromosome and another on the third chromosome 2LA+3LA strain. Among the four morphometric traits analyzed, sternopleural and scutellar bristles are the polygenic traits whose expression is under the influence of the environmental conditions (Mather, 1943; Whittle, 1969), while the head width and wing lengths are polygenic traits that determine the body size of the flies (Capy *et al*., 1994; Gracia-Vazuez *et al*., 1989; Sokoloff, 1965, 1966; Yadav and

Singh, 2003, 2006). This study thus permits the analysis of relationship between these morphometric traits and inversions strains.

The number of sternopleural and scutellar bristles was highest in the strain with 2LA+ 3LA (double inversion strain) and lowest in the strain with inversion free. The number of sternopleural bristles was intermediate in the strain which carried 2LA and 3LA inversions. But the inversion free strain had lower number of sternopleural and scutellar bristles than inversion strains. This shows that the presence of inversion 2LA+ 3LA (double inversion strain) produces extra bristles on the sternopleural, scutellar plate. The presence of extra bristles in the inversion karyotypes of *D. melanogaster* has also been noticed by Das and Singh (1992b). Thus the study of the author confirms with the observation of these authors. The association of inversion with individuals having extra dorso-central and scutellar bristles has also been reported in certain other studies on *Drosophila* (Garcia-Vazquez and Sanchez -Refusta, 1989).

Table 5a. Ovarioles and fecundity of different inversion strains of *D. ananassae* (Values are Mean ± SE)

| Strain → ↓Parameters | Inversion free | 2LA inversion | 3LA inversion | 2LA+3LA inversion |
|---|---|---|---|---|
| Ovarioles | 14.05 ± 0.65[a] | 18.00 ± 0.88[b] | 20.50 ± 1.10[b] | 24.95 ± 1.52[c] |
| Fecundity | 172.06 ± 3.21[a] | 187.84 ± 4.57[b] | 189.58 ± 2.87[b] | 210.82 ± 6.02[c] |

Same superscript in each row indicates that the value is non significant by DMRT.

Table 5b. One way ANOVA for ovarioles and fecundity of different inversion strains of *D. ananassae*

| Source | | Sum of squares | df | Mean squares | F value | P value |
|---|---|---|---|---|---|---|
| Ovarioles | Between groups | 1251.850 | 3 | 417.283 | 22.225 | 0.000** |
| | Within groups | 1426.900 | 196 | 18.775 | | |
| | Total | 2678.750 | 199 | | | |
| Fecundity | Between groups | 38006.77 | 3 | 12668.92 | 13.352 | 0.000** |
| | Within groups | 185977.10 | 196 | 948.86 | | |
| | Total | 223983.87 | 199 | | | |

**P< 0.001.

Scrutiny of Table 4a and b shows that the mean head width differs in flies carrying different inversions. It was noticed that head width was larger in strains carrying both 2LA + 3LA inversion (Double inversion strain). The head width of strains carrying single inversion (either 2LA or 3LA) was lesser than the strains carrying double inversion or without inversions. Furthermore, the head width of strain without inversion was significantly different from that of the strain with double inversion. This observation on head width, on the other hand, contradicts the observations of Dobzhansky and his associates (1960) who in *D. pseudoobscura* demonstrated superiority of inversion heterozygotes over homozygotes. Thus the heterotic effect of inversions may be limited to only certain genes particularly to those concerned with fitness and need not be found in all characters of a given species.

The wing length of double inversion strain (2LA+3LA) was highest and it was not significantly different from other strains. Thus the heterotic effect of inversion could be seen with regard to this trait also. As wing length is an index of body size (Monclus and Prevosti, 1971;

Sisodia and Singh, 2001, 2004), the present study indicates that the flies of the strain carrying double inversion are larger than the others.

The maintenance of inversion polymorphism in natural populations of *D. ananassae* seems to be associated with many other adaptive functions in terms of sexual behavior, fitness and morphometric traits. This observation thus confirms earlier studies on *Drosophila* with regard to paracentric inversions (Da Cunha, 1955; Ray Chaudhuri and Jha, 1966; Singh, 1988, 1991; Singh and Das, 1990). Ovarioles and fecundity are fitness characters which determine the reproductive success of a species. In the present study (Table 5a, b) the author has noticed highest ovarioles and fecundity in the double inversion strain (2LA+3LA) and lowest in inversion free strain of *D. ananassae.* Ovarioles and fecundity is one of the fitness characters which has relevance to the reproductive success and survival of a given species (Sisodia and Singh 2004; Prathibha, 2011). The relationship between ovarioles number and fecundity were significantly stronger as compared to relationship between fecundity and female size (David and Le nobre'd, 1970; Branquart and Hemptinne, 2000). As more species are studied the number of examples where female size and fecundity (Chenoweth *et al*, 2007) and female size and ovarioles number are uncorrelated is increasing (Togashi and Life, 2007). In contrast to this, there is a strong positive relation between ovarioles number and fecundity (Branquart and Hemptinne, 2000; Wayne *et al*, 1997).

In the present study the persistence of inversion polymorphism in these populations could be explained by an advantage of inversion heterozygotes over corresponding homozygotes. The author in the present study has noticed more heterokaryotypes than homokaryotypes. This confirms the fact that the inversion polymorphism is adaptive and balanced due to higher Darwinian fitness on inversion heterozygotes (Dobzhansky, 1951).

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**A preliminary survey report of the family Drosophilidae in the Garhwal hills, Uttarakhand, India**.

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The members of family Drosophilidae, particularly the Genus *Drosophila*, occupy a very important position among the organisms that on regular basis are used as raw material for genetic studies. Extensively in recent years, *Drosophila* has become a remarkably versatile experimental tool organism in nearly every discipline of biology. Especially the *Drosophila melanogaster* continues to be one of the greatest sources of information regarding the principles of heredity that apply to all animals including human beings. The family Drosophilidae is relatively large, consisting of 76 genera with more than 3800 described species from the world (Wheeler, 1981, 1987; Bächli *et al*., 2004). The family is divided into two subfamilies, Steganinae and Drosophilinae, which include approximately 16 and 84% of the total species, respectively.

In 1920 the studies on *Drosophila* systematics laid its foundation in the subcontinent of India. Bezzi was the first man who reported *Drosophila repleta* from Calcutta. The Indian Drosophilid fauna, which is widely distributed throughout the Indian subcontinent, has been studied considerably in recent years and in most areas they have been collected and studied extensively by Prof. J.P. Gupta and his students, at Banaras Hindu University, Uttar Pradesh, India. But it was suggested by Prof. S.P. Ray-Chaudhuri, a pioneer *Drosophila* geneticist, surveying studies of Indian Drosophilidae were initiated. Recently, Prof. J.P. Gupta published a book *"A Monograph on Indian Drosophilidae"* 2005, in which he summarized all scattered information and results of Indian Drosophilids.

The Kumaun and Garhwal regions of central Himalayas comprises a vast diversity of fauna and flora, but Drosophilid fauna of this region were mainly explored from the Kumaun region. The cytogenetics laboratory in the Department of Zoology of the Kumaun University, Nainital, India, has actively explored the Drosophilid fauna of Kumaun region since 1984, which was earlier completely unknown for its Drosophilid fauna. Since then a number of new species of Drosophilidae have been reported from Kumaun (Singh and Dash, 1993, 1998; Singh, Dash, and Fartyal, 2000, 2004; Singh and Fartyal 2002; Fartyal, Singh, and Toda, 2005; Joshi, Fartyal, and Singh, 2005; Upadhyay and Singh, 2006; Fartyal and Singh, 2007). However, Garhwal region which is located in the northern part of India still remains a virgin field and awaits exploration. This paper summarizes the result of several surveys undertaken at Agastyamuni and Srinagar in districts Rudraprayag and Pauri, respectively, of the Garhwal region.

Material for the present study was collected from Srinagar, Pauri district (30.22 N lat. and 78.78 E long.) and Agastyamuni, Rudraprayag district (30.39 N lat. and 79.02 E long). These sampling sites are characterized by having evergreen forests of oak, conifers, and mixed forests with median to very steep slopes and some areas of extremely moist condition due to rainfall.

Collection of the study material was achieved using different methods, which included net sweeping over natural substrates, using fermenting fruit baits, and the use of Toda's Retainer Box (banana bait trap box) designed by Prof. M.J. Toda (1977).

Table 1. A total of 3090 flies belonging to different genera of the family Drosophilidae were collected during the period of present survey.

| S. No. | Genus / Subgenus | Srinagar (Pauri) | | Agastyamuni (Rudraprayag) | |
|---|---|---|---|---|---|
| | Genus- *Drosophila* | Male | Female | Male | Female |
| | Subgenus- *Sophophora* Sturtevant | | | | |
| 1 | *Drosophila melanogaster* Meigen | 45 | 65 | 46 | 50 |
| 2 | *Drosophila nepalensis* Okada | 25 | 45 | 16 | 35 |
| 3 | *Drosophila kikkawai* Burla | 5 | 5 | 6 | 2 |
| 4 | *Drosophila bifasciata* Pomini | 1 | 5 | 2 | 15 |
| 5 | *Drosophila jambulina* Prasad & Paika | 1 | 5 | 2 | 15 |
| 6 | *Drosophila suzukii indicus* Prasad & Paika | 156 | 190 | 44 | 75 |
| 7 | *Drosophila takahashii* Sturtevant | 16 | 18 | 2 | 3 |
| 8 | *Drosophila punjabiensis* Prasad & Paika | 123 | 110 | 23 | 25 |
| 9 | *Drosophila* sp. K1* | 8 | 5 | — | — |
| | Subgenus- *Dorsilopha* Sturtevant | | | | |
| 10 | *Drosophila busckii* Coquillett | 207 | 290 | 144 | 175 |
| | Subgenus-*Drosophila* Fallén | | | | |
| 11 | *Drosophila paunii* Singh & Negi | 1 | 1 | 4 | — |
| 12 | *Drosophila immigrans* Sturtevant | 271 | 300 | 44 | 75 |
| 13 | *Drosophila lacertosa* Okada | 8 | 5 | 3 | 5 |
| 14 | *Drosophila repleta* Wollaston | 26 | 31 | 2 | 5 |
| | Genus-*Leucophenga* Mik | | | | |
| 15 | *Leucophenga bellula* (Bergroth) | 1 | 2 | — | — |
| 16 | *Leucophenga* sp. A1* | — | — | 2 | 2 |
| 17 | *Leucophenga albiceps* de-Meijere | 20 | 26 | — | — |
| 18 | *Leucophenga* sp.K1* | 1 | 2 | — | — |
| 19 | *Leucophenga* spZ1* | 1 | 2 | — | — |
| | Genus *Paraleucophenga* Hendel | | | | |
| 20 | *Paraleucophenga todai* Fartyal & Singh | 1 | — | — | — |
| 21 | *Paraleucophenga neojavanaii* Singh & Negi | 1 | 1 | — | — |
| | Genus *Dettopsomyia* Lamb | | | | |
| 22 | *Dettopsomyia nigrovittata* (Malloch) | — | — | 2 | 5 |
| | Genus *Scaptomyza* Hardy | | | | |
| 23 | *Scaptomyza himalayana* Takada | 29 | 33 | 2 | 5 |
| | Genus *Scaptodrosophila* Duda | | | | |
| 24 | *Scaptodrosophila* sp.K1* | — | — | 1 | 1 |
| | Genus *Gitona* Meigen | | | | |
| 25 | *Gitona distigma* Meigen | 1 | 1 | — | — |
| | Genus *Zaprionus* Coquillett | | | | |
| 26 | *Zaprionus indianus* Gupta | 56 | 81 | 10 | 15 |
| | Total flies | 1004 | 1223 | 355 | 508 |

Note: The species marked with * were not identified and are supposed to be new species.

The specimens studied were preserved in 70% ethanol. External morphology of adult flies was examined under a stereomicroscope and metric characters were measured with an ocular micrometer. To observe the detailed structures of male and female terminalia, respective organs were dissected from the adult body or whole body was cleared by warming in 10% KOH around 100°C for several minutes and then observed in a droplet of glycerol under a light microscope.

The morphological terminology and the definitions of measurements and indices mostly follow McAlpine (1981), Zhang and Toda (1992), and Hu and Toda (2001). A total of 3090 flies belonging to different genera of the family Drosophilidae were collected during the period of present survey. (Table 1).

Altogether 26 species representing 8 genera of the family Drosophilidae were collected during preliminary surveys started from 2008 to 2010. Out of 26 species, 5 species are supposed to be new species. Among genus *Drosophila* altogether 14 species, in which nine species of subgenus *Sophophora*, one species of subgenus *Dorsilopha* and four species of subgenus *Drosophila*, were collected. Out of them one species of subgenus *Sophophora (Sophophora* sp. K1) was unidentified and are supposed to be new species. Genus *Leucophenga* (*Leucophenga* sp. A1, K1 and Z1) was represented by five species, out of which three species were unidentified and are supposed to be new species. Genus *Scaptodrosophila* was represented by only one species. K1 was unidentified and is supposed to be new species. Genus *Dettopsomyia, Scaptomyza, Gitona,* and *Zaprionus* were represented by one species each. The two Indian species of *Paraleucophenga, P. todai and P. neojavanai*, were also present in this area. The identification of yet unidentified species will be completed very soon.

The above table indicates the prominence of *D. melanogaster, D. immigrans, D. busckii, D. nepalensis, D. takahashi*, and *D. punjabiensis* in both the sampling sites of Srinagar, Pauri and Agastyamuni, Rudraprayag accompanied by *Scaptomyza himalayana* and *Zaprionus indianus.*

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## Correlated changes in seasonal acquisition of desiccation resistance and cold-tolerance in *Drosophila simulans* along an altitudinal gradient in western Himalayas.

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### Abstract

Correlated changes in seasonal increases in desiccation resistance and cold-tolerance were examined in field-collected adult females of *D. simulans*. Low temperature and desiccation stress are

thought to be mechanistically similar in insects, and several studies indicate that there is a degree of cross-tolerance between them, such that increased cold tolerance results in greater desiccation tolerance and vice versa. From autumn to winter, *D. simulans* flies exhibited an increase in cold-tolerance and desiccation resistance along an altitudinal gradient. The increase in cold-tolerance was probably due to a concomitant increase in trehalose level that acts as cryoprotectant in insects. In contrast to the gradual increase in cold-tolerance, flies exhibited reduced rates of water loss. The reductions in rates of water loss during seasonal changes were probably the result of reduced cuticular water loss as flies increased the amount of their cuticular hydrocarbons. The correlation between seasonal increases in trehalose content and reduction in rates of water loss may represent a link between desiccation resistance and cold-tolerance in this species. These findings provide little support for cross-tolerance between survival to cold stress and desiccation stress in *D. simulans*.
Keywords: *Drosophila simulans*, desiccation resistance, cold tolerance, altitude

## Introduction

Many insects that overwinter in temperate and polar regions must tolerate not only extreme cold but desiccation stress. Recently, several reviews suggested that certain behavioral and physiological adaptations promoting cold-tolerance may also influence, or were originally adaptations for, desiccation resistance (Ring and Danks, 1994; Block, 1996; Danks, 2000). There are a remarkable number of overlaps in the tolerances of insects to differing environmental stresses. For example, mild desiccation elicits increased cold tolerance in the springtail *Folsomia candida* (Bayley et *al.*, 2001), and a period of anoxia induces increased cold tolerance in the house fly *Musca domestica* (Coulson and Bale, 1991). Cross-tolerance is thought to be a useful way to approach complex traits, and selection experiments in *Drosophila* have been frequently used to examine the relationships between environmental stressors, including high and low temperatures, desiccation and starvation (Nghiem *et al.*, 2000; Hoffmann *et al.*, 2003; Bubliy and Loeschcke, 2005). Freeze-tolerant insects use glycerol and other low-molecular-mass polyols and sugars, termed cryoprotectants, to decrease the amount of body water that freezes at a given temperature, thereby preventing excessive cellular dehydration (Baust and Lee, 1981; Storey and Storey, 1992; Zachariassen, 1991). Increased cryoprotectant concentrations may also lower water loss rates by collectively reducing the vapor pressure deficit between the insect's hemolymph and environmental water vapor (Ring and Danks, 1994; Bayley and Holmstrup, 1999; Sjursen *et al.*, 2001). However, most studies have focused on how adaptations promote low temperature survival with little attention to the possible effects these adaptations may have on water conservation.

Previous studies examined several parameters of cold-tolerance and their possible link to desiccation resistance in cold-hardy insects collected in mid-winter (Williams *et al*., 2002). Of the many environmental stresses that insects face regularly, desiccation and cold are thought to be particularly closely related (Ring and Danks, 1994). Many overwintering insects consequently show reduced water content, and many of the biochemical adaptations to cold also serve to protect against desiccation (Ring and Danks, 1994). This relationship is well-supported in insects that are highly permeable (Holmstrup *et al.*, 2002), exposed to very cold, desiccating conditions (Ramløv and Lee, 2000), or are freeze tolerant (Sinclair and Wharton, 1997). However, in *Drosophila simulans*, the link between cold and desiccation is less well-explored. To survive the low temperatures and desiccating conditions of winter, *D. simulans* increase their cold-tolerance during the autumn and have extremely low rates of water loss. The seasonal increase in cold-tolerance is correlated with the accumulation of the cryoprotectant trehalose. However, it is unknown if seasonal changes in the rate of water loss exist, are these reductions in rate of water loss linked to physiological process of

increasing cold-tolerance? The purpose of the present study was to characterize seasonal changes in cold-tolerance and resistance to water loss in adult females of *D. simulans* to determine if the acquisition of desiccation resistance is linked to increases in cold-tolerance. To investigate this question we measured survival after exposure to low temperatures, trehalose levels as a measure of cryoprotectant, and rate to water loss of field collected flies from autumn to winter. In conjunction with the field study, we also examined the effect of mild desiccation stress on rates of water loss and cold-tolerance.

## Materials and Methods

*Collection and cultures*

Wild living *D. simulans* individuals (n = 320–400 flies from each collection site) were collected by net sweeping and banana bait-trap methods in autumn as well as winter season of 2010 from six altitudinal sites (600–2226 m). For both the seasons, multiple collections over a week were made from each of the six localities. In the laboratory, after mild anesthesia with solvent ether, the wild caught females were sorted out to initiate isofemale lines (20 lines per population). All cultures were initiated with 6–8 h egg laying period and were maintained at low density (60–70 eggs per vial of 37 mm × 100 mm size) on cornmeal-agar medium at 21ºC. For seasonally varying as well as altitudinal populations, the laboratory progeny of wild-caught individuals were analyzed for all the traits. Analyses were made for each season at different times of the year, *i.e*. there was a time gap of 3 months corresponding to two different seasons. However, the experimental conditions were made uniform with the use of a temperature controlled room set at 21°C. Further, for each season, $G_1$ and $G_2$ (Generations 1 and 2) of the wild caught flies were analyzed in order to avoid possible effects of laboratory adaptation (which may result due to laboratory cultures maintained for months and years before analysis). However, there were no changes in cold tolerance and desiccation resistance in *D. simulans* cultures maintained for 10–12 generations for all the populations. Climatic data for the sites of origin of populations were obtained from Indian Institute of Tropical Meteorology (IITM; www.tropmet.res.in) and the data are shown in Table 1.

Table 1. Geographical and seasonal climatic data of the sites of origin of *Drosophila simulans* populations.

| Populations/Alt. (m) | Lat. (°N) | Autumn | | | Winter | | |
|---|---|---|---|---|---|---|---|
| | | $T_{min}$ (°C) | $T_{max}$ (°C) | RH (%) | $T_{min}$ (°C) | $T_{max}$ (°C) | RH (%) |
| Kalka (600) | 30.51 | 21.7 | 31.3 | 68.6 | 12.2 | 22.0 | 64.0 |
| Bhuntur (1096) | 30.20 | 20.5 | 29.5 | 63.2 | 10.0 | 18.0 | 60.0 |
| D.shala (1211) | 32.00 | 17.8 | 28.6 | 59.3 | 7.3 | 17.1 | 55.2 |
| Solan (1440) | 32.58 | 15.4 | 23.6 | 56.4 | 7.1 | 16.2 | 50.0 |
| Kasauli (1951) | 30.17 | 13.2 | 20.8 | 51.1 | 6.2 | 15.4 | 45.0 |
| Shimla (2202) | 31.06 | 10.2 | 18.6 | 50.2 | 5.1 | 13.2 | 42.0 |

*Measurement of cold-tolerance*

Cold resistance was measured individually on ten males or females from each isofemale line (n = 20 per population). Adult flies were aspirated and placed individually in 42 ml glass vials which were placed in 10% glycol solution at 0ºC for 8 hours (Hoffmann, Anderson and Hallas, 2002). This was followed by transfer of flies to petri-plates (9 cm diameter) in a temperature controlled room at 21°C, and chill coma recovery period (in minutes) was recorded. Recovery to chill coma is inversely related to cold resistance, *i.e*. longer recovery period to cold stress means lesser resistance level. For each population, recovery time in minutes was subtracted out of 100 to denote relative resistance level to cold stress. This helped in comparing the data on resistance to heat and cold stress.

*Measurement of desiccation resistance related traits*

*2.4.1. Desiccation resistance*

To measure desiccation resistance, five wild caught or 10 laboratory reared individuals from each line (six replicates per isofemale line) were isolated in a dry plastic vial, which contained 2 g of silica gel at the bottom and was covered with a piece of foam. Such vials with muslin wraps were placed in a desiccation chamber (Secador electronic desiccator cabinet) which maintained 4–5% relative humidity at 21 ± 0.5ºC. The vials were inspected every hour and the numbers of dead flies (completely immobile) were recorded. The desiccation survival hours were based on $LT_{50}$ values.

*2.4.2. Cuticular water loss*

For individual flies, total body water content was estimated from the difference between wet and dry weights (dried overnight at 60ºC) and divided by initial wet weight × 100. Cuticular water loss (under dehydration) was analyzed by giving short-term desiccation stress (8 h) in groups of five wild-caught or ten laboratory reared flies. After mild anesthesia with solvent ether, flies were weighed on a Sartorius microbalance (Model-CPA26P; with precision 0.001 mg) both before and after desiccation, and the cuticular water loss was calculated: (initial body weight – body weight after 8 h desiccation stress)/initial body weight × 8; and the values were given in mg $h^{-1}$.

*2.4.3. Cuticular lipids*

For cuticular lipid estimation, each individual fly was dried overnight at 60ºC to get constant dry mass, *i.e*. devoid of body water. Each dried fly was kept in HPLC-grade hexane for 1 h and thereafter it was removed from the solvent and was again dried at room temperature and finally reweighed. For each individual fly, cuticular lipid mass was estimated as: difference between initial dry weight and dry weight after solvent treatment/initial dry weight × surface area (where area was expressed in $cm^2$ and wet body mass in mg; and surface area scaled to 2/3 power of the wet body mass). Finally, the data on cuticular lipid mass per fly were shown as mg $cm^{-2}$ $fly^{-1}$. According to scaling relationships (Schmidt-Nielsen, 1984; Brown *et al.*, 2000; Gibbs, 2002), changes in surface area due to increase in body mass were represented by the equation: $y = xb$; where y = dependent variable; x = independent variable (body mass), b = scaling exponent (*i.e*., ratio of surface in squares to volume in cubes = 2/3).

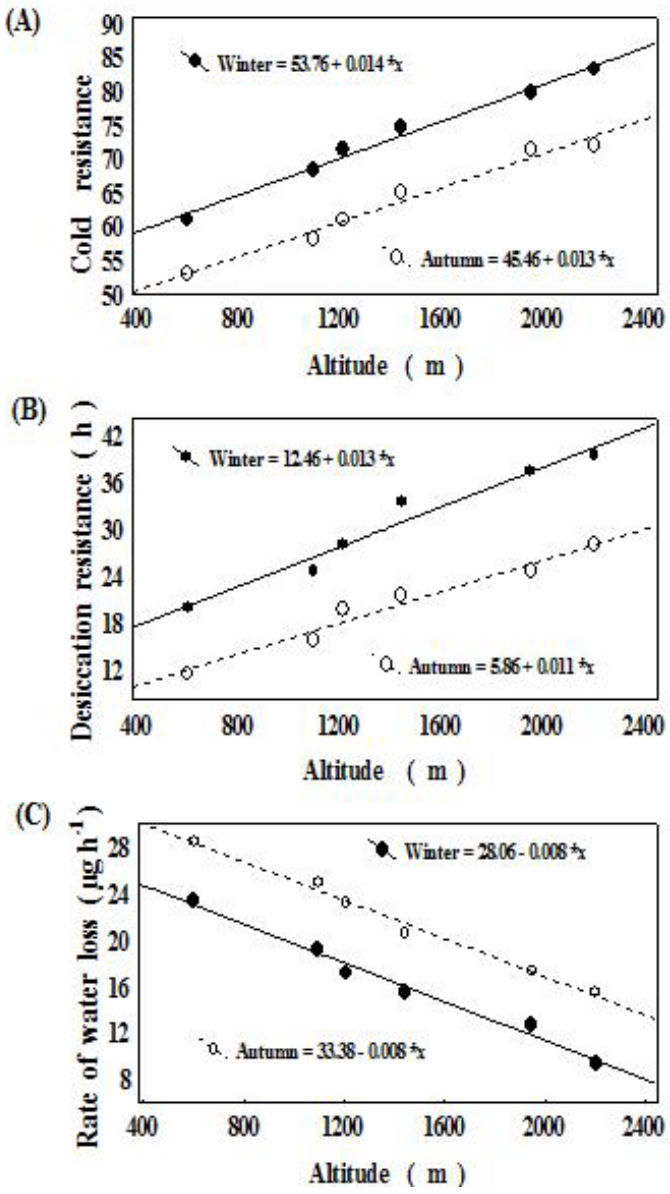
*Trehalose estimation*

For trehalose estimation, aliquots (200 µl) were placed in two different tubes; one was taken as a blank while the other was digested with trehalase at 37ºC (Megazyme trehalose assay kit; K-Treh 10/10; www.megazyme.com). In this assay, released D-glucose was phosphorylated by hexokinase and ATP to glucose-6-phosphate and ADP, which was further coupled with glucose-6-phosphate dehydrogenase and resulted in the reduction of nicotinamide adenine dinucleotide (NAD). The absorbance by NADH was measured at 340nm (UV-2450-VIS; USA). Pre-existing glucose level in the sample was determined in a control reaction lacking trehalase and subtracted from total glucose concentration.

*Effect of moderate desiccation stress on cold-tolerance and desiccation resistance*

Flies collected in late autumn were used to determine the effects of mild water stress on cold-tolerance and desiccation resistance. To measure pretreatment time duration, 10 female individuals of each replicate (20 I.F lines × 10 replicates each) were subjected to desiccation stress at ~0 - 5%

relative humidity. The initial body water content in replicate groups was recorded. The time period in which flies lost ~ 15-17% body water was assessed as pre-treatment time duration. Further, for recovery period, individuals were placed on non-nutritive agar and tested at hourly intervals for increase in body water till the lost body weight was regained. Such flies were subjected to desiccation stress until death in order to test the increased desiccation resistance due to acclimation. Increased desiccation survival hours were calculated after subtracting the desiccation resistance hours of non-acclimated (control) from acclimated individuals. Control and treatment experiments were run simultaneously under identical experimental conditions.




*Statistical analyses*

Seasonal data were analyzed using a one-way analysis of variance (ANOVA). Variance (%) due to populations, seasons, and their interactions were calculated as proportion of MS × degree of freedom out of the total sum of such values. Correlation values (r) were calculated for flies from each season. For climatic data analysis, we used multiple regressions of trait values as a simultaneous function of Taverage ($T_{ave.}$) and relative humidity (RH) of the sites of origin of populations. Statistica (Statsoft Inc., Release 5.0, Tulsa, OK, USA) was used for calculations as well as illustrations.

Figure 1. Comparison of altitudinal slope values for cold resistance (A); desiccation resistance (B); and rate of water loss (C) flies collected during autumn and winter seasons from six altitudinal populations of *D. simulans*. Values for linear regression analysis (y = a + bx) are shown where 'a' = intercept and 'b' = slope value.

## Results

*Seasonal acquisition of cold-tolerance and desiccation resistance*

For wild-caught flies, there is significant population divergence for all the traits as a function of altitude of origin (Figure 1A– D). The clinal trends were compared as a ratio of trait values of lowest *vs*. highest altitudinal populations. Based on this criterion, cold resistance and desiccation

related traits showed a 1.40-fold divergence (Figure 1). Although trait values differed across seasons, the magnitude of clinal increase was identical across seasons. By contrast, there were no changes in the total body water content (69.4 ± 0.13%) across seasons as well as along altitude. For assessing the effects due to seasons, we analyzed different quantitative traits in wild-caught individuals of *D. simulans* and the data are given in Table 2. For the quantitative traits (cold resistance, desiccation resistance, cuticular water loss, and trehalose content), there were similar magnitudes of % variance due to populations; seasons, and their interactions (Table 2). Thus, there were significant parallel effects of seasonal changes for desiccation related traits.

Table 2. ANOVA for wild caught individuals trait variability due to population (P), seasons (S) and their interactions for six altitudinal populations of *D. simulans*.

| Traits | df | Populations (5) | Seasons (1) | P x S (5) | Error (1188) |
|---|---|---|---|---|---|
| 1. Cold survival | *MS* | 91.23 | 1126.25 | 72.32 | 0.12 |
| | *F* | 12.32** | 9445.14*** | 8.43* | |
| 2. Desiccation resistance | *MS* | 84.01 | 996.85 | 23.36 | 0.03 |
| | *F* | 36.23*** | 1236.02*** | 11.23** | |
| 3. Rate of water loss | *MS* | 6.23 | 112.69 | 3.23 | 0.0001 |
| | *F* | 11.23** | 6532.21*** | 9.36** | |

*Trait correlations*

Associations of desiccation resistance with cuticular lipids as well as cuticular water loss are illustrated in Figure 2 (for within population level). Analyses of seasonal wild caught samples clearly indicated positive associations between desiccation resistance and cuticular lipids ($r = 0.90 \pm 0.010$, $p < 0.001$) but negative with cuticular water loss ($r = -0.97 \pm 0.007$, $p < 0.001$; Figure 2A–B). Correlation analysis evidenced positive association of trehalose content with cold resistance and of cold resistance with desiccation resistance ($r = 0.90 \pm 0.012$, $p < 0.001$; Fig. 2C - D). Thus, for a given population, cold resistant flies during winter were more desiccation resistant and showed reduced cuticular water loss. By contrast, flies with lower cold resistance during autumn showed lower desiccation resistance but higher cuticular water loss. Further, the gradual increase in cold-tolerance of *D. simulans* was mirrored by increases in trehalose content (Figure 2C). Values for trehalose content increased significantly ($P < 0.05$) at successive testing season. In contrast to the increase in cold-tolerance, water loss rates decreased during the winter.

*Cold-tolerance and desiccation resistance after moderate desiccation stress*

To determine whether desiccation stress could induce changes in rates of water loss and cold-tolerance, autumn collected flies from one highland locality were subjected to desiccating conditions in the laboratory. Flies were subjected to either 0-5% RH at 21°C for 2 days prior to assessing their cold-tolerance, desiccation resistance, rate of water loss and changes in trehalose level, if any. There was an apparent trend toward increased resistance at 0°C after prior treatment to desiccation stress. Moderate desiccation stress enhanced desiccation resistance as rates of water loss were significantly lower ($P < 0.05$) for flies subjected to lower humidity conditions, than the field group (Figure 3A-B). Further, desiccation pre- treated flies had higher levels of trehalose content as compared to control groups. Cuticular lipid amount did not change in control and desiccation treated flies. These data suggest that mild desiccation stress induced an enhanced cold by increasing levels of cryoprotectant

(trehalose) and desiccation resistance by decreasing rate of water loss in *D. simulans*. Further, results of ANOVA explaining the trait variability are shown in Table 3. We observed significant *F* values for increase in desiccation resistance as well as cold resistance (desiccation resistance: $F = 3923.23$; $P < 0.001$; cold resistance: $F = 2196.47$; $P < 0.001$) due to prior exposure to stress in *D. simulans.*

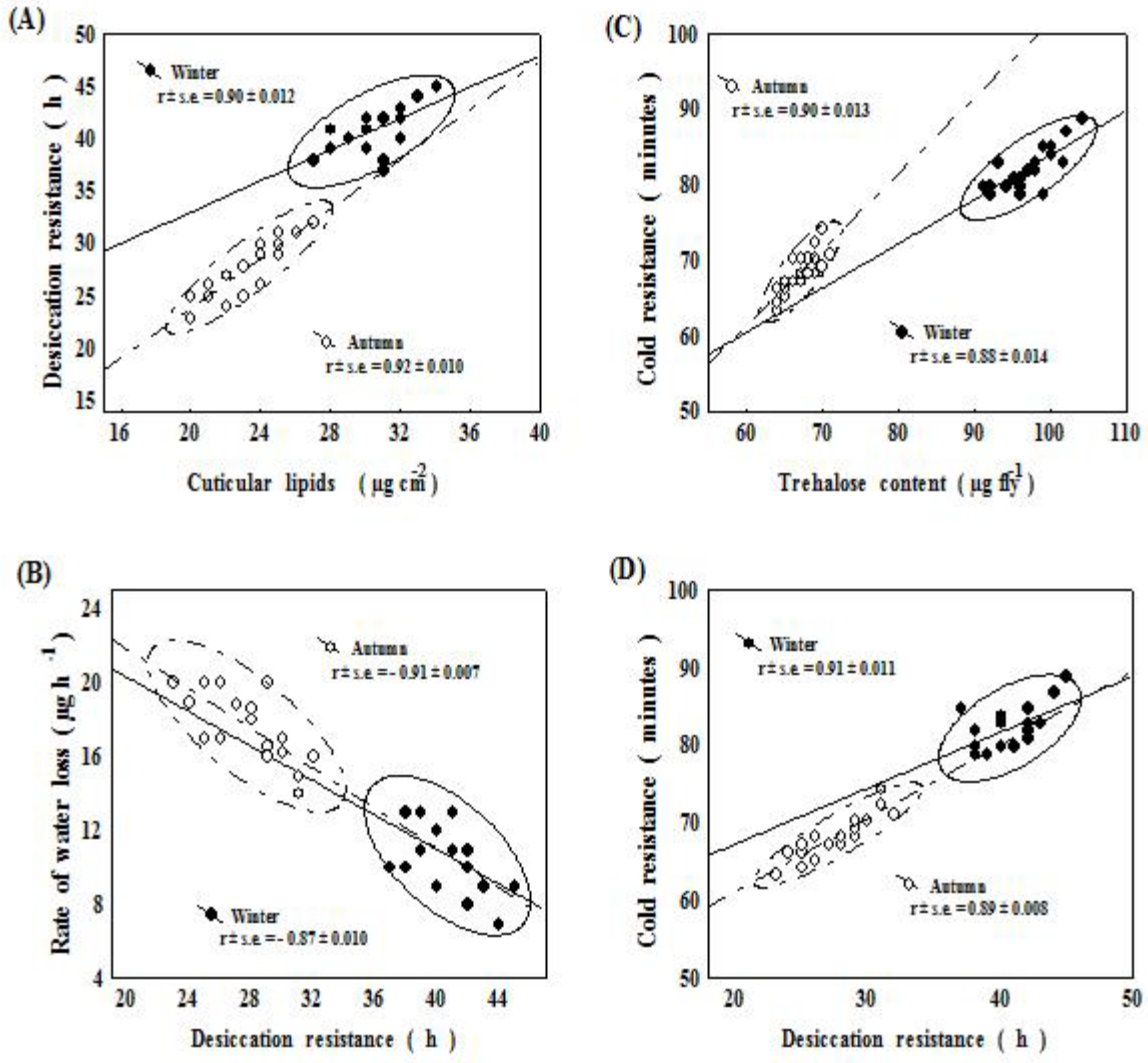



Figure 2. Trait correlations based on within population analysis. (A) Correlations between desiccation resistance with cuticular lipids; (B) negative correlation of desiccation resistance with cuticular water loss mg $h^{-1}$; (C) significant positive correlation of trehalose content per fly with cold resistance; and (D) positive correlation of desiccation resistance with cold resistance in individuals from autumn *vs*. winter populations of *D. simulans* from a highland locality. All the correlation values are highly significant ($p < 0.001$), but the signs are different.

## Discussion

For ectothermic insects, spatial variations in quantitative traits are well documented, but temporal variations have received lesser attention (Bijlsma and Loeschcke, 1997; Hoffmann and

Table 3. Results of ANOVA (isofemale lines were nested in treatment group) for explaining trait variability due to desiccation acclimation on cold resistance and trehalose content in *D. simulans.*

| Traits | df | MS | *F* |
|---|---|---|---|
| 1. Desiccation resistance | | | |
| Treatment | 1 | 47912.21 | 3923.23*** |
| IF lines | 38 | 203.25 | 14.27*** |
| Error | 360 | 10.23 | |
| 2. Cold resistance | | | |
| Treatment | 1 | 62306.32 | 2196.47*** |
| IF lines | 38 | 458.45 | 22.36*** |
| Error | 360 | 17.36 | |

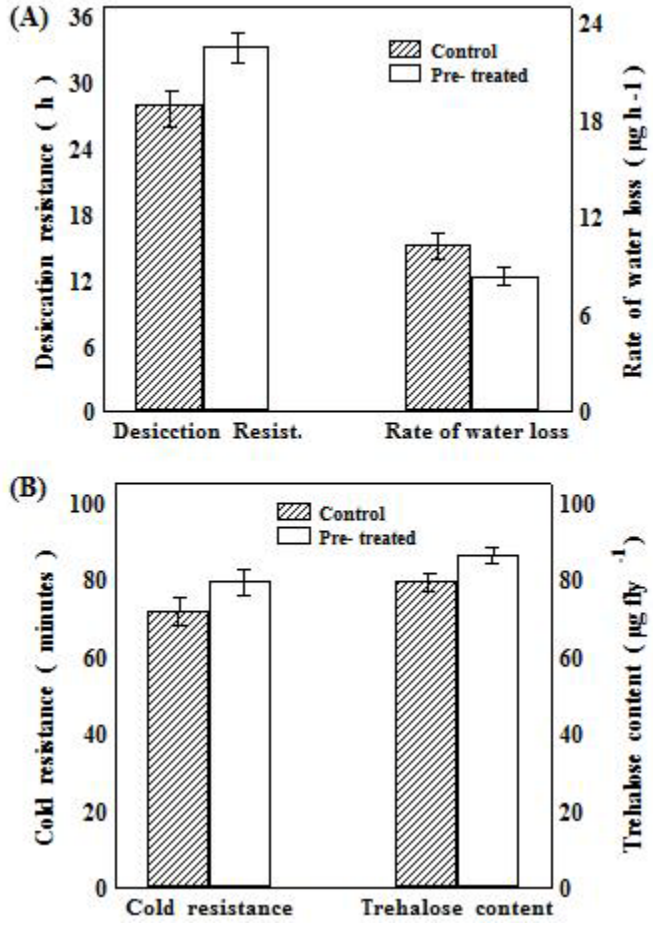



Figure 3. Bars represent changes in desiccation resistance and rate of water loss (A) and cold resistance and trehalose content (B) in control and desiccation pre-treated flies of *D. simulans* from a highland locality (20 I.F lines × 10 replicates each).

Weeks, 2007). In temperate regions, several studies have shown huge variations in body size for different *Drosophila* species. However, a large part of such heterogeneity results due to thermal and nutritional stress under wild conditions and accordingly the heritability in the field is much reduced (Coyne and Beecham, 1987; Imasheva *et al.*, 1994). By contrast, there are few studies on the analysis of desiccation related traits (which show high heritability) in wild populations living under seasonally varying conditions. A single study on Australian populations of *D. melanogaster* has shown lack of temporal variation in desiccation resistance (Mckenzie and Parsons, 1974). However, on the Indian subcontinent, there are significant seasonal variations in the montane localities (Table 1). Our data have evidenced considerable changes in desiccation and cold stress related traits across two seasons (autumn and winter). However, seasonal plastic effects for body size are not correlated with desiccation resistance. Further, there are correlated changes in cuticular lipids, which account for variations in desiccation resistance across seasons. To the best of our knowledge, adaptive significance of cuticular lipid plasticity to cope with seasonally varying desiccation stress has not been previously analyzed.

The mechanistic basis of desiccation resistance involves reduction of cuticular water loss due to changes in cuticular lipids (Zachariassen *et al.*, 1987; Hadley, 1994; Gibbs, 1998; Addo- Bediako

*et al.*, 2001). Several studies on thermal acclimation for cuticular lipids have shown correlated changes in cuticular water loss (Hadley, 1977; Toolson and Kuper-Simbron, 1989). However, there are reports which contradict such associations, *e.g*., in *D. mojavensis*, there are no effects of thermal acclimation on the rate of cuticular water loss (Gibbs *et al.*, 1998). In *D. melanogaster*, analysis of latitudinal populations has previously shown lack of geographical variations in the amount of cuticular lipids per fly (Parkash *et al.*, 2008). Laboratory selection for desiccation resistance has also shown a lack of difference in the amount of cuticular lipids per fly between resistant and sensitive strains (Gibbs *et al.*, 1997). By contrast, in the present study, we observed plastic effects in the amount of cuticular lipids per fly across seasons as well as in altitudinal populations. Thus, cuticular lipids do confer desiccation resistance in seasonally varying population of *D. simulans*.

Previous studies have created discrepancy on the association between desiccation resistance and basal cold tolerance. Selection for desiccation (and the starvation control) does not affect the basal tolerance of adult *D. melanogaster* to acute cold exposure, nor does selection affect the ability of *D. melanogaster* to exhibit the RCH response (Sinclair *et al*., 2007). The present study appears to be the first to examine the correlated changes of desiccation resistance on another stress resistance trait (cold resistance). The correlated change in cold tolerance observed here matches with the results of a study by Bubliy and Loeschcke (2005), who show that desiccation selection confers increased tolerance to a long exposure at 0°C; with Telonis-Scott *et al.* (2006), who show a decrease in cold tolerance after selection for desiccation resistance in one of their two selected lines; and with Hoffmann *et al.* (2005), who show that *D. melanogaster* selected for starvation tolerance have decreased survival of an acute cold shock. Telonis-Scott *et al.* (2006) employ an acute cold exposure (2.5 h at – 2°C) as their assay, so their metric is likely directly comparable with that used in the present study. Moreover, these three studies combined with the present contrasts with a laboratory-evolved desiccation resistance and basal cold tolerance study in *D. melanogaster* (Sinclair *et al.*, 2007). It is unclear whether this divergence in relationship is due to chance variation in trajectories of selection lines, or due to genetic differences between the founding populations, other studies perform cold tolerance experiments on mated females (Bubliy and Loeschcke, 2005; Hoffmann *et al.*, 2005; Telonis-Scott *et al.*, 2006 ). However, Telonis-Scott *et al.* (2006) perform many of their stress assays (but not cold tolerance) on both males and females and demonstrate that the direction of response is consistent between genders for other traits.

In the present study increased desiccation resistance as a result of prior treatment to desiccating conditions was correlated closely with increases in trehalose levels and suggests a link between desiccation resistance and cold-tolerance in *D. simulans* (Figure 3). Prior desiccation stress increased cold resistance in *D. simulans*. Several studies have shown that dehydration pretreatment followed by rehydration can equip insects or terrestrial arthropods to tolerate a subsequent exposure to dehydration (Holmstrup and Somme, 1998; Sjursen *et al.*, 2001; Benoit *et al.*, 2007). However, a single study has analyzed acclimation to desiccation stress in four *Drosophila* species from Australia (Hoffmann, 1991). This study has shown lack of acclimatory response in tropical rainforest *D. birchii* but improved desiccation resistance due to acclimation in *D. serrata*, *D. simulans*, and *D. melanogaster*. Further, *D. birchii* and *D. serrata* are sibling species of montium species subgroup and have shown divergence in acclimation response to dehydration stress, and such observations are in agreement with divergence in their desiccation resistance (Hoffmann, 1991). Subsequently, another study on a single population of *D. melanogaster* has provided indirect evidence that acclimation to desiccation resistance may be affected by reduction in cuticular water loss rates but not through changes in respiratory water loss (Bazinet *et al.*, 2010). Our results have shown an increase in desiccation resistance as well as cold tolerance in *D. simulans* due to desiccation pretreatment, and such changes are consistent with their stress resistance level under field conditions. In summary, desiccation resistance and cold-tolerance of *D. simulans* increased during the winter season as

compared with autumn. An initial rapid decrease in seasonal rates of water loss was correlated with decreased water loss as increased cuticular lipids. Later in the autumn, cryoprotectant accumulation may have affected cold tolerance in *D. simulans*.

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## ***In-vivo* testing for genotoxicity of ethyl methanesulfonate (EMS) using *Drosophila melanogaster.***

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**Abstract**

*Drosophila melanogaster* is one among the heavily exploited model organisms, used to study genetics and more commonly used in the field of genetic toxicology due to the primary factor of

easiness in culturing. The objective of this study was to evaluate the genotoxic potential of ethyl methanesulfonate (EMS) that has been reasonably anticipated to be a carcinogen based on animal studies. Two endpoints namely, DNA fragmentation assay and SMART (Somatic mutation and Recombination test) were chosen. The DNA fragmentation assay allows determining the amount of DNA that is degraded upon exposure of the fruit fly to genotoxic agents. This assay involved exposure of Canton flies to 0.5 M, 0.05 M, and 0.005 M of EMS for 24 hrs and 48 hrs. Extensive shearing was observed in the DNA samples of both parent and progeny, whereas intact band formation was seen only in progeny. The Wing spot assay provides a rapid means to assess the potential of a chemical to induce loss of heterozygosity (LOH) resulting from gene mutation, chromosomal rearrangement, chromosome breakage, or chromosome loss. Analysis of wings obtained from the flies, emerged from the trans-heterozygous larvae of *mwh/flr3* cross-over, exposed as in the previous end-point, revealed spot formation characteristic of both the recessive markers. Single spots representing mutated *flr3* clone and trichomes with twin spots per cell representing mutated *mwh* clone was observed. This indicates the occurrence of either mitotic recombination or mutation, in the form of point mutation, deletion, or translocation. Keywords: *Drosophila*, DNA fragmentation assay, Ethyl methanesulfonate, Gentotoxicity, SMART.

## Introduction

*Drosophila melanogaster*, commonly referred to as "fruit fly", is regarded as the most ideal eukaryotic model organism for the purpose of screening chemical compounds for mutagenic and genotoxic activity. It posesses several advantages like short generation time, easy to culture and maintain, less number of chromosomes for analysis, and high degree homology to the human genome that makes it very useful in genotoxic assays (Graf and Singer, 1992).

Genotoxic agents are abundant in the environment and are often linked to human activity. The effects these agents have on humans include many common diseases, such as cancer. The response to genetic damage interfaces closely with many aspects of cellular metabolism, such as DNA repair and recombination, replication, transcription, cell cycle regulation, and cell death. These genotoxic agents fall into three categories, such as carcinogens, mutagens, and teratogens. Several types of chemical compounds have been tested for their genotoxic potential. One among them is ethyl methanesulfonate (EMS). EMS is a direct- acting mono-functional ethylating agent that is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals (Report on Carcinogens, 12$^{th}$ Edition, 2011). Alkylation of cellular, nucleophilic sites by EMS occurs via a mixed SN1/SN2 reaction mechanism. EMS produces significant levels of alkylation at oxygens, such as the O6 of guanine and in the DNA phosphate groups. Genetic data obtained using microorganisms suggest that EMS may produce both GC to AT and AT to GC transition mutations. There is also some evidence that EMS can cause base-pair insertions or deletions as well as more extensive intragenic deletions (Sega, 1984). The National Occupational Exposure Survey (conducted from 1981 to 1983) estimated that 971 workers, including 448 women, potentially were exposed to ethyl methanesulfonate (Report on Carcinogens, 12$^{th}$ Edition, 2011).

The mutagenicity tests that have been performed on *Drosophila* have adopted several assay methods, and the majority of the mutations have been reported in the regions of its eye, abdomen, bristles, and wings. DNA fragmentation assay is one among the standard tests that help in identifying genotoxicity at the molecular level. Damages induced by a compound on the DNA are observed in the form of fragments that clearly are indicative of a genotoxic incident. The Wing Spot Assay, on

the other hand, has been considered as one of the gold standard assays for mutagenicity testing in *Drosophila*.

The wing somatic mutation and recombination test is more sensitive in terms of detecting a vast range of genetic abnormalities, such as mutations, deletions, and somatic recombinations (Graf *et al.*, 1984; Würgler *et al*., 1984). This bioassay makes use of the wing-cell recessive markers multiple wing hairs (*mwh*, 3–0.3) and flare (*flr3*, 3–38.8) in transheterozygous *mwh* +/+ *flr3* animals (Henderson, 2004). During the embryonic development of *D. melanogaster*, imaginal disc-cell groups proliferate mitotically during larval growth until reaching the point of differentiating during metamorphosis of body structures of the adult insect. If genetic alteration occurs in any one of the imaginal disc cells, these changes will be present in all the following cells, subsequently forming a mutant cell clone. Induced mutations are detected as single mosaic spots on the wing blade of surviving adults that show either the multiple wing hairs or flare phenotype. Induced recombination leads to *mwh* and *flr3* twin spots and also to a certain extent, to *mwh* single spots. Recording of the frequency and the size of the different spots allows for a quantitative determination of the mutagenic and recombinogenic effects. The rapidity and ease of performance as well as the low costs of the test necessitate a high priority for validation of this promising *Drosophila* short-term test (Guzmán-Rincón, 1995). The present study is aimed at utilizing the two assays formerly described for evaluating the toxicity of EMS.

**Materials and Methods**

The alkylating agent, EMS that is to be tested for its genotoxic potential was supplied by Sigma Aldrich (M0880, CAS 62-50-0). Prior to drug exposure, the compound was dissolved in ethanol to the different concentrations used. Canton-S flies were bred for two generations on corn meal agar. Drug exposure studies were performed in Carolina Formula 4 instant medium. The different concentrations used were control,100 µl, and 500 µl of 0.5 M, 0.05 M, and 0.005 M of EMS, mixed with 3 g of instant food for 24 hrs and 48 hrs. Test and duplicates were set up for each concentration. The food was labeled with appropriate drug concentrations, and foiled, and allowed to set for two hours. For phenotypic analysis, 15 male and 15 female flies were exposed to each of the drug concentrations and analyzed microscopically after 24 hours and 48 hours of exposure, respectively. F1 generation analysis was performed on emergence, microscopically. Post-phenotypic analysis and DNA isolation of both parent and F1 flies was performed after 24 hours and 48 hours of exposure, respectively, which was followed by fragmentation assay. 100 trans-heterozygous larvae of the *mwh*/*flr3* cross were exposed to each of the four concentrations, and adult flies were allowed to emerge. The emerged flies of the *mwh*/*flr3* cross were etherized using 500 µl ether. The wings of the flies were dissected and placed on a glass slide. The wings were observed microscopically for any mutations.

**Results**

The results are tabulated in Tables 1, 2, 3, and Images 1, 2, and 3.

**Discussion**

Evaluation of genotoxicity of EMS was performed using two major gold-standard assays, namely the DNA fragmentation assay and wing spot assay. For the fragmentation assay, 30 wild type Canton flies (15 male and 15 female) were exposed to 100 µl and 500 µl of 0.5 M, 0.05 M, and 0.005 M each of EMS for 24 hours and 48 hours. The viability status was 100% at all doses. The breeding

efficiency, on the other hand, reduced to 50% for 0.5 M, and to 25% for 0.05 M and 0.005 (100 µL-48hrs). Analysis of the F1 flies prior to DNA isolation, under the stereo zoom microscope, revealed significant changes in phenotype in terms of discoloration of thorax and body, mild curling of wings, and stout appearance. At all concentrations, there was protracted delay in the emergence of F1 with reduction in size of larvae and pupae (Table 1).

Table 1. Results of phenotypic analysis of F1.

| Sample name and concentration (µl) | Rate of viability (%) | Rate of breeding efficiency (%) | Changes observed |
|---|---|---|---|
| Control | 100 | 100 | No changes observed |
| 0.5M-100µL-24hrs | 100 | Reduced to 50 | Reduction in the size of larvae and pupae; delay in emergence of F1. |
| 0.5M-100µL-48hrs | 100 | Reduced to 50 | Reduction in the size of larvae and pupae; delay in emergence of F1; discoloration of body color to pale yellow seen in both males and females. |
| 0.5M-500µL-24hrs | 100 | Reduced to 50 | Reduction in the size of larvae and pupae; delay in emergence of F1; discoloration of thorax to orange. |
| 0.5M-500µL-48hrs | 100 | Reduced to 50 | Reduction in the size of larvae and pupae; delay in emergence of F1; discoloration of thorax to deep orange seen prominently in males. |
| 0.05M-100µL-24hrs | 100 | Reduced to 25 | Protracted delay in the emergence of F1; discoloration of thorax to orange; males appear short and females appear stout. |
| 0.05M-100µL-48hrs | 100 | Reduced to 25 | Protracted delay in emergence of F1; discoloration of thorax to deep orange; males appear short and shrunken and females appear stout. |
| 0.05M-500µL-24hrs | 100 | Reduced to 25 | Protracted delay in emergence of F1; discoloration of thorax to orange; pale appearance of females. |
| 0.05M-500µL-48hrs | 100 | Reduced to 25 | Protracted delay in emergence of F1; discoloration of thorax to deep orange; mild curling of wings seen at the posterior end. |
| 0.005M-100µL-24hrs | 100 | 100 | Protracted delay in emergence of F1; discoloration of thorax to orange; males appear stout and females appear short and stout. |
| 0.005M-100µL-48hrs | 100 | Reduced to 25 | Protracted delay in emergence of F1; discoloration of thorax to orange; males appear stout and females appear short and stout. |
| 0.005M-500µL-24hrs | 100 | 100 | Protracted delay in the emergence of F1; discoloration of thorax to deep orange. |
| 0.005M-500µL-48hrs | 100 | 100 | Protracted delay in the emergence of F1; discoloration of thorax to deep orange. |

DNA was isolated from both parent and progeny flies and was followed by gel electrophoresis. Parent DNA showed intense shearing and intact band formation (Figure 1). The area of band formation was restricted to maximum 0.1 kb-0.3 kb (Table 2). However, fragmentation assay performed in F1 DNA showed only shearing and no band formation (Figure 2). There was no shearing documented in the DNA of F1 whose parent flies were exposed to 100 µL of 0.005 M EMS for 48 hrs. This could be attributed to low DNA yield (419.3 ng/ µL), due to poor breeding efficiency at that concentration, *i.e.* the number of flies obtained was significantly reduced. It is thereby clear that the progeny showed pronounced genetic damage than the parent, thus proving that the damage been inherited. However, to identify the type of inheritance followed, a self-fertilization between the

F1 flies and a test-cross between the F1 flies obtained from the exposed cultures and the F1 of control shall be helpful.

Table 2. Results of DNA fragmentation assay.

| Concentration (µl) | Sample name and nanodrop value(ng/µl) | Description of band formation |
|---|---|---|
| Control | Parent - 1509.8<br>Progeny - 4874.9 | Nil |
| 0.5M-100µL-24hrs | Parent - 1999.2<br>Progeny - 2642.3 | Two distinct bands below 0.1kb and one distinct band approx. at 0.2kb. |
| 0.5M-100µL-48hrs | Parent - 1922.4<br>Progeny - 4780.6 | Two distinct bands below 0.1kb and one distinct band approx. at 0.2kb. |
| 0.5M-500µL-24hrs | Parent - 2175.5<br>Progeny - 2371.9 | Two distinct bands below 0.1kb and one distinct band approx. at 0.2kb. |
| 0.5M-500µL-48hrs | Parent - 2522.6<br>Progeny - 2638.8 | Two distinct bands, one below 0.1kb and other approx. at 0.2kb, and one faint band below 0.1kb. |
| 0.05M-100µL-24hrs | Parent - 5228.6<br>Progeny - 2425.4 | Two distinct bands, one below 0.1kb and other approx. at 0.2kb, and one faint band below 0.1kb. |
| 0.05M-100µL-48hrs | Parent - 5070.6<br>Progeny - 1194.1 | Two distinct bands, one below 0.1kb and other approx. at 0.2kb, and one faint band below 0.1kb. |
| 0.05M-500µL-24hrs | Parent - 3236.8<br>Progeny - 2615.7 | Two distinct bands, one below 0.1kb and other approx. at 0.2kb, and two faint band below 0.1kb. |
| 0.05M-500µL-48hrs | Parent - 2830.4<br>Progeny - 2005.1 | Two distinct bands, one below 0.1kb and other approx. at 0.2kb, and one faint band below 0.1kb. |
| 0.005M-100µL-24hrs | Parent - 3437.8<br>Progeny - 1851.4 | One band at 0.1kb and two bands below 0.1kb |
| 0.005M-100µL-48hrs | Parent - 3944.4<br>Progeny - 419.3 | First band between 0.2 and 0.3kb, second band at 0.2kb and third band below 0.1kb. |
| 0.005M-500µL-24hrs | Parent - 2602.6<br>Progeny - 3565.2 | One distinct band at 0.2kb and one faint band below 0.1kb. |
| 0.005M-500µL-48hrs | Parent - 2945.1<br>Progeny - 2953.9 | One distinct band at 0.1kb. |

Table 3. Wing Spot assay.

| Concentration of EMS (µL) | *flr3* | *mwh* | *Mwh / flr3* | Observations |
|---|---|---|---|---|
| Control | - | - | - | Absence of spots |
| 0.5M-100 | + | - | - | Presence of large single spots – *flr3* |
| 0.5M-500 | + | + | - | Single large spot -*flr3*; Multiple trichomes per cell - *mwh* |
| 0.05M-100 | + | + | - | Multiple trichomes &2 hair per cell - *mwh*; Multiple single spots- *flr3* |
| 0.05M-500 | + | + | + | Multiple trichomes per cell - *mwh*; 6 single spots - *flr3* and cluster of trichomes - *mwh* |
| 0.005M-100 | + | + | + | Large single spots- *flr3* and multiple trichomes per cell – *mwh*; single spots-*flr3* |
| 0.005M-500 | + | + | + | 8 single spots -*flr3* and cluster of trichomes per cell – *mwh*; Multiple single spots - *flr3* |

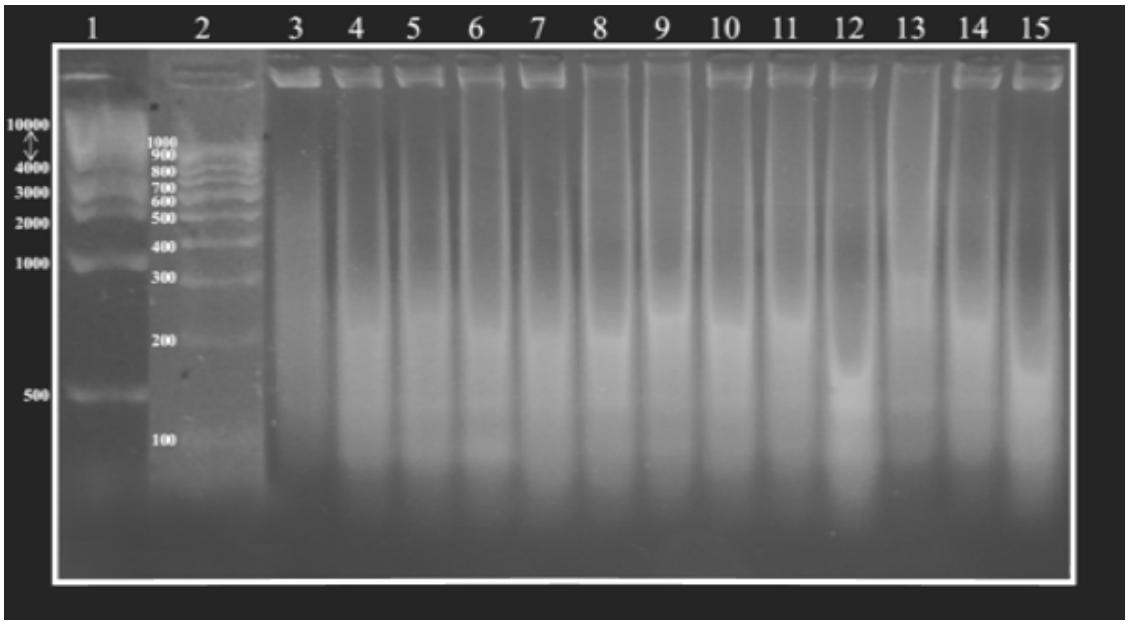


Figure 1. Agarose gel electrophoresis showing DNA fragmentation of Parent DNA samples. Description: Wells 1,2 – 100 and 500 bp ladder; 3 – Control; 4,5 – 100 µl of 0.5 M EMS for 24 hrs and 48 hrs; 6,7 – 500 µl of 0.5 M EMS for 24 hrs and 48 hrs; 8,9 – 100 µl of 0.05 M EMS for 24 hrs and 48 hrs; 10,11 – 500 µl of 0.05 M EMS for 24 hrs and 48 hrs; 12,13 – 100 µl of 0.005 M EMS for 24 hrs and 48 hrs; 14,15 – 500 µl of 0.005 M EMS for 24 hrs and 48 hrs.

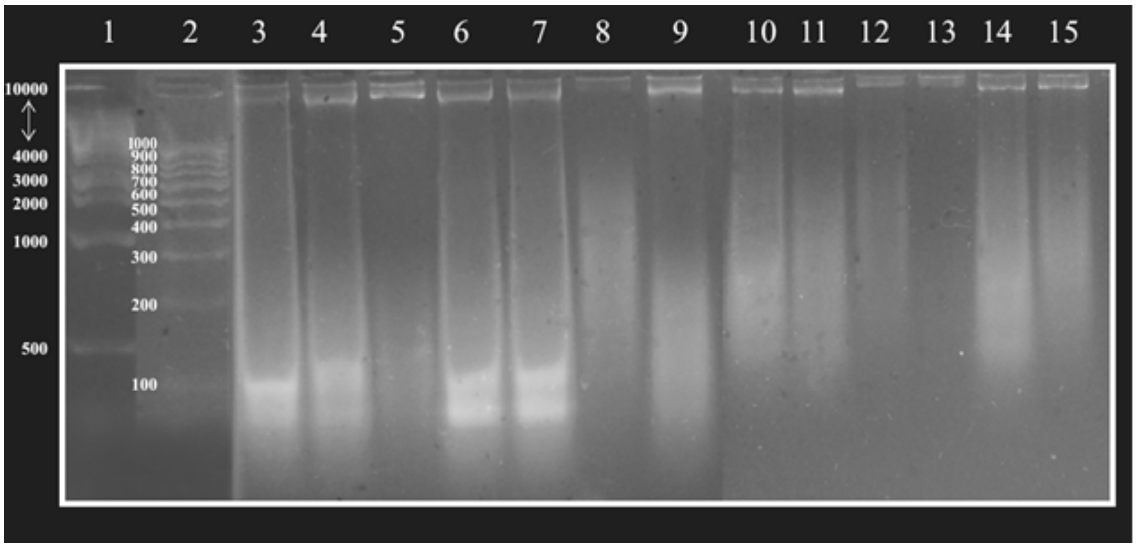


Figure 2. Agarose gel electrophoresis showing DNA fragmentation of F1 DNA samples. Description: Wells 1,2 – 100 and 500 bp ladder; 3 – Control; 4,5 – 100 µl of 0.5 M EMS for 24 hrs and 48 hrs; 6,7 – 500 µl of 0.5 M EMS for 24 hrs and 48 hrs; 8,9 – 100 µl of 0.05 M EMS for 24 hrs and 48 hrs; 10,11 – 500 µl of 0.05 M EMS for 24 hrs and 48 hrs; 12,13 – 100 µl of 0.005 M EMS for 24 hrs and 48 hrs; 14,15 – 500 µl of 0.005 M EMS for 24 hrs and 48 hrs.

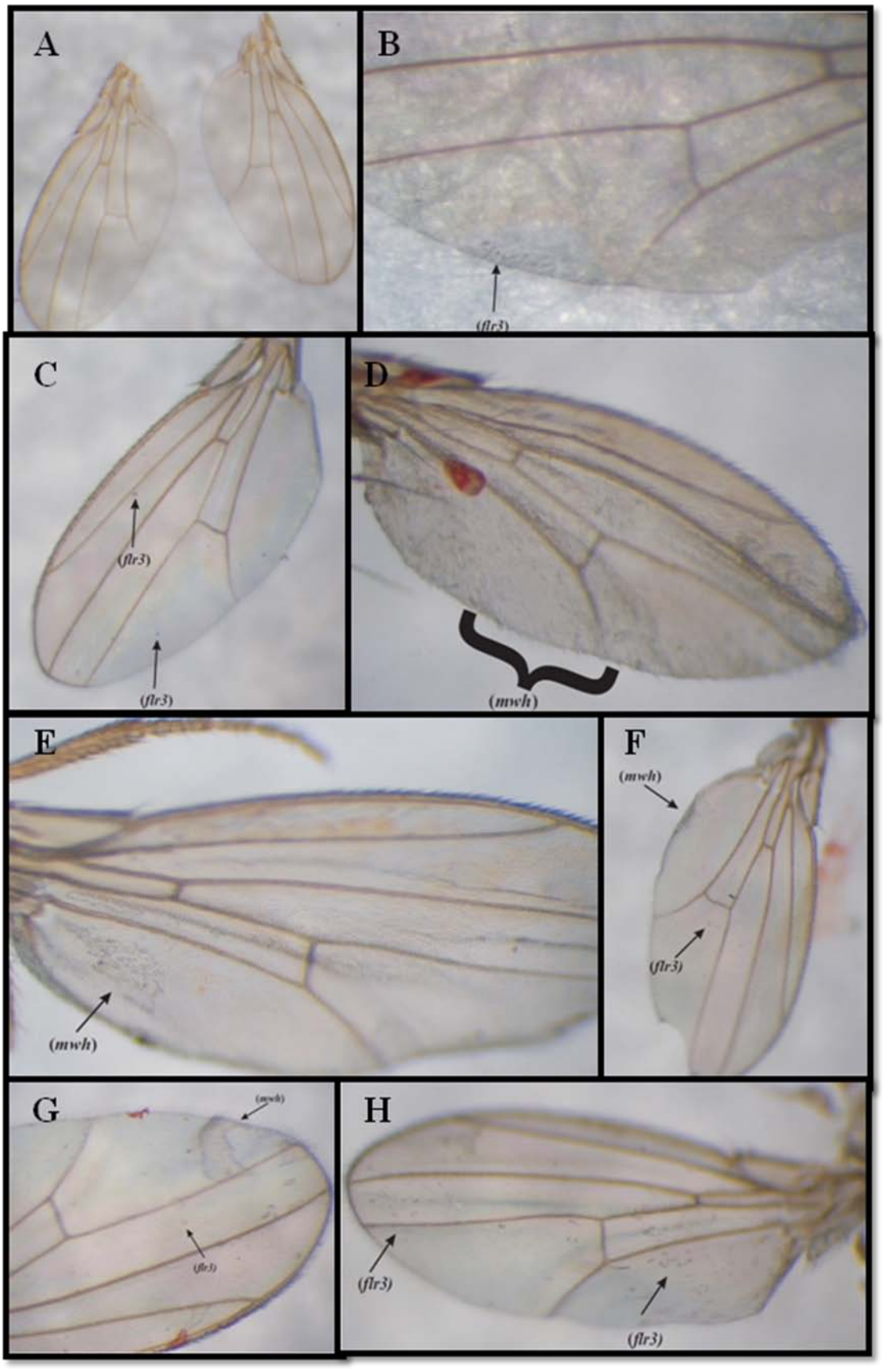
A
B
(flr3)
C
(flr3)
(flr3)
D
(mwh)
E
(mwh)
F
(mwh)
(flr3)
G
(mwh)
(flr3)
H
(flr3)
(flr3)

Figure 3 (previous page). SMART analysis – A: Control; B: Exposure to 100 µl of 0.5 M EMS - Presence of large single spots -*flr3;* C and D: Exposure to 500 µl of 0.5 M EMS showing single large spot –*flr3* and multiple trichomes per cell – *mwh*, respectively; E and F: Exposure to 500 µl of 0.05 M EMS showing multiple trichomes per cell – *mwh* and single spots - *flr3* with cluster of trichomes – *mwh*, respectively; G and H: Exposure to 500 µl of 0.005 M EMS showing 8 single spots – *flr3* along with cluster of trichomes per cell – *mwh* and multiple single spots - *flr3*, respectively.

Further understanding of the exact mechanism and the type of mutation that influences the expression of phenotypic changes involved the second assay, *i.e.,* SMART test. Third instar larvae trans-heterozygous for two genetic markers *mwh* and *flr3,* were treated with 100 µl and 500 µl of 0.5 M, 0.05 M, and 0.005 M of EMS. Wings of the emerging adult flies were scored for the presence of spots of mutant cells, which result from either somatic mutation or mitotic recombination. The appearance of single spots clearly indicated the contribution of *flr3* clone and the twin spots with trichomes per cell represented the *mwh* clone. The exact type of genotoxic effect was further evaluated by comparing the frequencies of spots per wing in the treated series to the control group (Table 3).

The results of the wing spot assay showed that all the doses gave rise to single spots corresponding to *flr3* clone (approx. 75%) and the rest showed a combination with *mwh* clone, depicted by clustering of trichomes per cell (Figure 3). The SMART test also yielded dose-dependent results. It has been understood from previous data obtained that *flr3* clone arises due to point mutation, interstitial deletion and double crossing-over, and twin spots (*mwh/flr3*) arise due to mitotic recombination. The presence of twin spots confirms the recombinagenic action of a compound. From the results obtained, the experimental study clearly depicts that EMS is a potent genotoxic agent.

Based on these findings, a dose-assessment analysis could be conducted, in-order to define a threshold dose that is considered “safe” for human exposure, since EMS is a well known pharmaceutical contaminant.

Acknowledgment: The authors sincerely acknowledge the grant received as a part of intra mural funding from Sri Ramachandra University.

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## Call for Papers

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# A comparison between polytene chromosomes of two sibling species of *Drosophila*: *D. ananassae* and *D. pallidosa*.

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## Abstract

Polytene chromosomes which occur in certain tissues of dipteran larvae have great utility in cytogenetic studies in *Drosophila.* A preliminary study on comparison between polytene chromosomes of two sibling species of *Drosophila*, *D. ananassae* and *D. pallidosa*, have revealed that they look similar. Interestingly, no asynapsis was observed in the polytene chromosomes of $F_1$ hybrids indicating that there is normal pairing between homologous chromosomes and homology between the banding patterns of the two species. However, the presence of heterozygous loops in $F_1$ hybrids in certain regions of autosomes demonstrates that these two sibling species differ in the order of gene arrangements. *D. ananassae* and *D. pallidosa* are sibling species. *D. pallidosa* is endemic to South Pacific regions where it coexists with *D. ananassae*, a cosmopolitan species, which is reproductively isolated by behavioral isolation. Presence of heterozygous loops in $F_1$ suggests that change in the order of genes in certain regions of chromosomes might have played role in speciation.

## Introduction

Polytene chromosomes, which were discovered by Balbiani in *Chironomus*, have been extensively utilized for various kinds of studies in *Drosophila*, particularly preparation of chromosome maps, detection of chromosomal aberrations, population genetics of inversions, gene activity, deletion and cytological mapping, chromosome replication, molecular biology, mapping of any DNA segment to specific chromosome loci, and so forth (Zhimulev, 1996). Further, polytene chromosomes have also been used in hybridization studies to understand speciation genetics and divergence (Dobzhansky and Tan, 1936; Bock, 1971; Naveira and Fontdevila, 1986). In hybrids between *D. pseudoobscura* and *D. miranda*, pairing of homologous arms was found to be highly variable and generally poor. Gene arrangements were found to be very different in these two species, indicating phylogenetic divergence between them (Dobzhansky and Tan, 1936). Bock (1971) studied the polytene chromosomes of all the four species of the *Drosophila bipectinata* complex and their hybrids. All the species have similar polytene chromosome complements. In the interspecific hybrids, there was excellent synapsis of homologous chromosomes when *D. bipectinata*, *D. parabipectinata*, and *D. malerkotliana* were hybridized suggesting their close phylogenetic relationship. Heterozygous inversions were also observed in the species and their hybrids. Synapsis between homologous chromosomes in hybrids between *D. pseudoananassae* and each of the remaining three species of the complex is weak, which provides evidence that *D. pseudoananassae* may be remotely related with the other three species (Bock, 1971). This has also been confirmed by the studies on genetic interactions underlying hybrid male sterility in this complex (Mishra and Singh, 2006). Naveira and Fontdevila (1986) observed strong asynapsis in all the chromosomes of hybrids between two sibling species of *Drosophila*: *D. buzzatii* and *D. serido*.

*D. ananassae* Doleschall (1858) and *D. pallidosa* Bock and Wheeler (1972) are sibling species. *D. ananassae* is a cosmopolitan and domestic species whereas its sibling *D. pallidosa* is endemic to the Islands of the South Pacific Oceans. In spite of their sympatric distribution, these two species are reproductively isolated by strong sexual isolation, although post mating barriers such as hybrid inviabilty and sterility are absent (Futch, 1973; Doi *et al*., 2001; Vishalakshi and Singh, 2006). Further, the interspecific hybrids of *D. ananassae* and *D. pallidosa* are developmentally as stable as their parents (Vishalakshi and Singh, 2009).

Chromosomal polymorphism has been extensively studied in *D. ananassae* (Singh, 2010) following the chromosome map constructed by Ray-Chaudhuri and Jha (1966). Futch (1966) detected chromosomal heterozygous inversions in sympatric light and dark forms of *D. ananassae* and their hybrids. During the present investigation, we have compared the polytene chromosomes of *D. ananassae* and *D. pallidosa* and also analyzed the chromosomes in their hybrids to know pairing between homologous chromosomes and the presence of heterozygous loops. The results of these investigations are reported in this communication.

## Materials and Methods

During the present study, the following stocks of these two species were used:

(i) *D. ananassae* GL 10: it is a mass culture stock established from flies collected from Gwalior, M.P in 2010.

(ii) *D. pallidosa* NAN 57: Stock has been provided by Prof. M. Matsuda, Kyorin University, Tokyo, Japan (origin: Fiji, isofemale line).

These stocks were maintained on simple yeast-agar medium by transferring 15 pairs of flies to fresh culture bottles in every generation in the *Drosophila* culture laboratory maintained at approximately 24$^{o}$C. For getting interspecific hybrids, two species were hybridized by making reciprocal crosses.

Temporary squash preparations of salivary glands were made from third instar larvae of *D. ananassae*, *D. pallidosa*, and their hybrids using lacto-aceto-orcein stain. The slides were observed under the microscope, at different magnifications.

## Results and Discussion

Both the species have the same chromosome karyotype and six arms in polytene chromosomes with identical tip morphology (XL, XR, 2L, 2R, 3L, and 3R; Figures 1, I and II) radiating from common chromocentre. In *D. ananassae*, two inversions, *i.e*., alpha (AL) in 2L (Figure 1, III) and delta (DE) in 3L (Figure 1, IV) were found to be present in heterozygous condition. Thirty larvae were analyzed, out of which 23 were found to have homozygous arrangement in 2L and 7 were found to be alpha heterozygotes. Sixteen larvae were found to have homozygous arrangement in 3L, and 14 were delta heterozygotes. The mean number of heterozygous inversions per individual was found to be 0.70. In *D. pallidosa*, one median inversion was detected in 2R (Figure 1, V). Out of 30 larvae examined, 26 were found to be inversion heterozygotes. Interestingly, no asynapsis was observed in the polytene chromosomes of $F_1$ hybrids indicating that there is normal pairing between homologous chromosomes and there is homology between the banding patterns of the two species (Figure 1, VI).

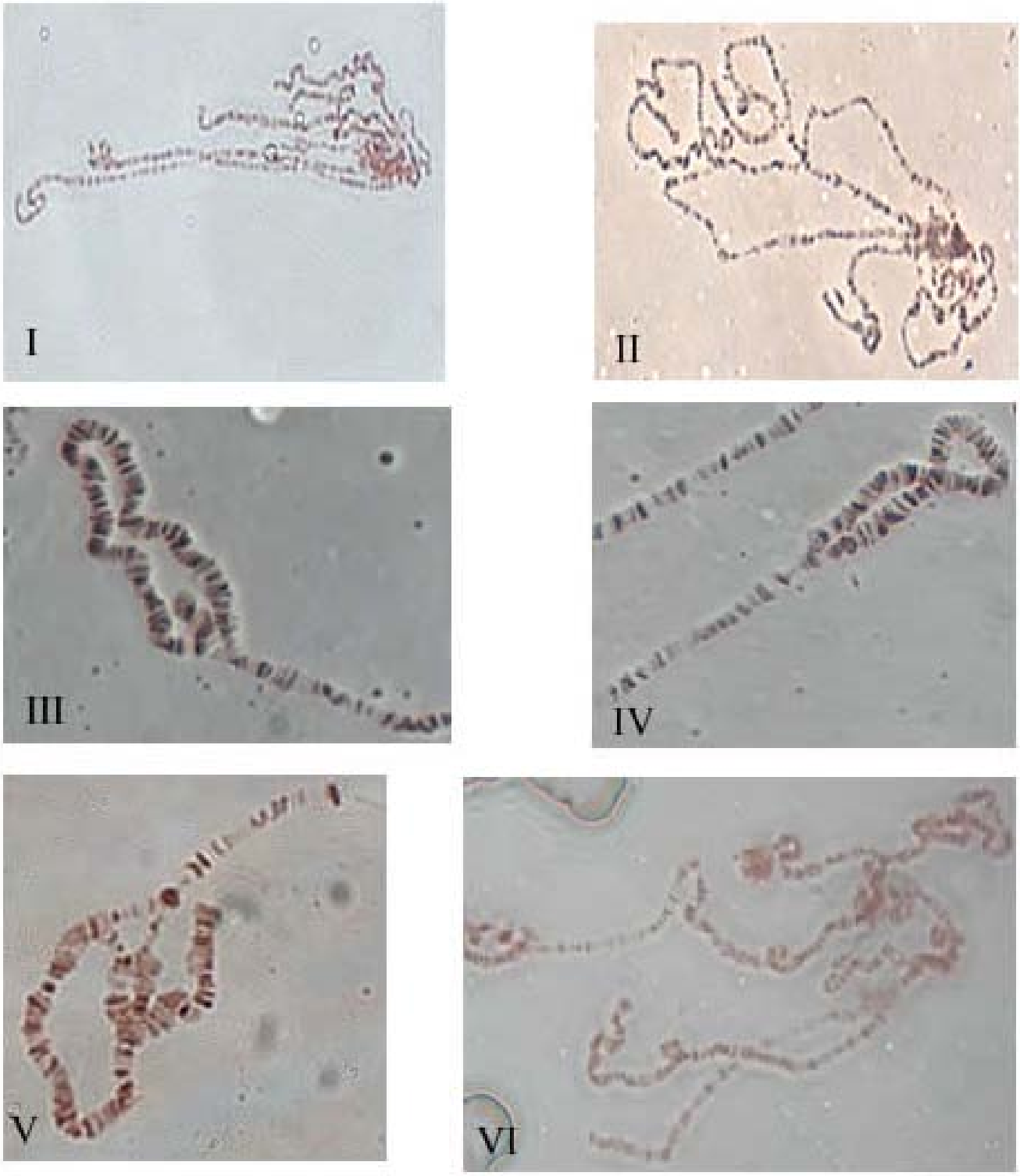


Figure 1, Part 1.

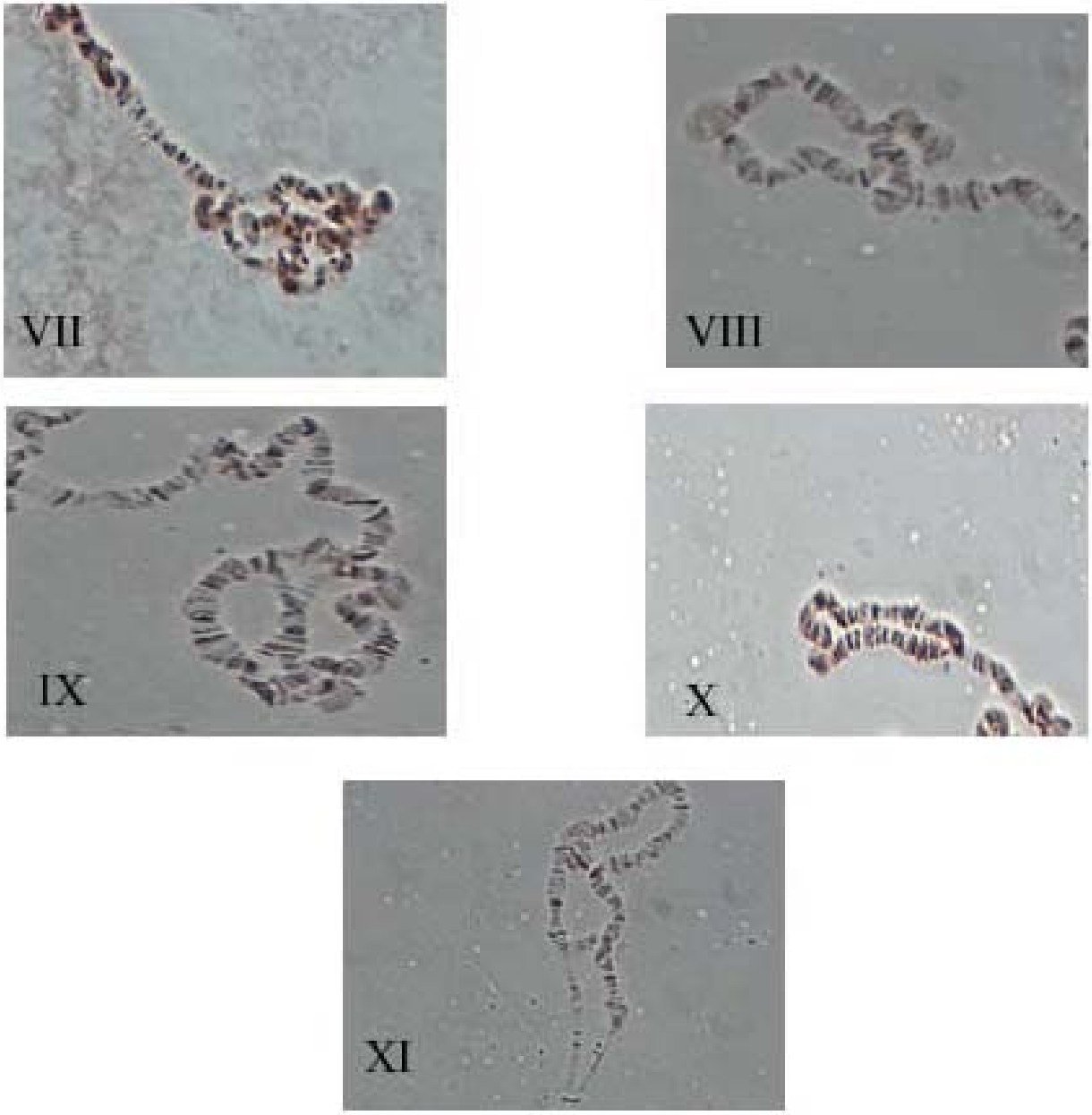


Figure 1. Microphotographs of polytene chromosomes of *Drosophila ananassae, D. pallidosa,* and their hybrids I, *D. ananassae* (25X); II, *D. pallidosa* (25X); III, AL (2L) inversion in *D. ananassae* (100X); IV, DE (3L) inversion in *D. ananassae* (100X); V, A median inversion in 2R in *D. pallidosa* (100X); VI, Hybrid polytene chromosomes showing no asynapsis (25X); VII, Complex configuration in 2L in hybrids (100X); VIII, A subterminal inversion in 2R in hybrids (100X); IX, Complex configuration in 2R in hybrids (100X); X, DE like loop in 3L in hybrids(100X); and XI, Complex configuration in 3R in hybrids (100X).

However, a number of paracentric inversion heterozygous loops were found in the autosomes of F1 hybrids. In 2L, complex configuration in sub-terminal region was detected (Figure 1, VII). In 2R two kinds of loops were detected, one was a simple sub- terminal loop (Figure 1, VIII) and the other a complex one (Figure 1, IX). In 3L, a loop similar to delta heterozygous loop could be detected (Figure 1, X). In 3R, a large sub-terminal complex configuration was found (Figure 1, XI). In XL and XR, no heterozygous loops were detected.

Based on these observations, it is concluded that: (i) these two sibling species, which belong to the *ananassae* subgroup of the *melanogaster* species group, have the same chromosome number, same number of arms in polytene chromosomes, homology and normal pairing between homologous chromosomes as there is lack of asynapsis in chromosomes of interspecific hybrids, (ii) *D. ananassae* stock has two heterozygous paracentric inversions (AL and DE) and *D. pallidosa* has one paracentric heterozygous inversion (a median inversion in 2R), (iii) Presence of heterozygous inversions and complex configurations in interspecific hybrids indicate that that these two sibling species differ in the order of gene arrangements in certain regions of autosomes.

These two sibling species show strong sexual isolation, which prevents gene flow between them in sympatric situations although post mating barriers such as hybrid inviabilty and sterility are absent (Futch, 1973; Doi *et al*., 2001; Vishalakshi and Singh 2006). Sawamura *et al.* (2008) have demonstrated that genes causing sexual isolation between the two species may be clustered in certain regions of chromosomes rather than being distributed randomly, and the presence of heterozygous loops and complex configurations in interspecific hybrids validate this. This indicates that certain segments in these regions differ in order of gene arrangements between the two species which might have played crucial role in speciation. Reversion in order has perhaps affected regulation. Indeed as it is known that it is not so much the divergence in sequences of genes as alterations in their regulation that can be attributed to speciation (De and Babu, 2010).

Acknowledgment: The financial assistance in the form of Meritorious Fellowship to PB from the UGC, New Delhi is gratefully acknowledged.

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**Effect of genetic variation on locomotion in laboratory stocks of *Drosophila melanogaster*.**

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## Abstract

Locomotory behavior was studied in five different stocks of *Drosophila melanogaster*. Out of these stocks, one was wild type and the remaining were mutants for different phenotypic traits. Individual flies of either sex were introduced in the locomotor chamber to observe its behavior and locomotory speed in one minute period of time. The observations of each group of flies with respect to their sex were also recorded. The results obtained indicate that there is significant variation in the locomotory speed of the flies belonging to different stocks (tested through one way ANOVA). Out of all these stocks, flies having curled wings showed maximum speed than other types, which may be due to their specific genetic quality. Further it was also observed that the two sexes show insignificant difference in their locomotory behavior, in all the stocks studied (as analyzed through student t- Test). The varying speed of locomotion observed in the stocks could be due to genetic variations.

## Introduction

*Drosophila* is one of the useful animal models to study behavioral aspects. This insect can be cultured in laboratory conditions and can be employed in a number of behavior studies, both sexual and non sexual. Sexual behavior comprises courtship and mating behavior, and non sexual behavior includes feeding behavior, interactions with other members of the group as well as members of other groups, larval feeding, locomotion of adults and their larvae, pupation site preference, movements based on light source, effect of geotaxis, and so forth. Behavior genetic analysis is an approach to the study of organisms and their behavior that combines the concepts and methods of behavioral analysis from psychology and ethology (Sisodia and Singh, 2004).

The relationship between genes and behavior, however, is less straight-forward. Although genes can influence behavioral function at all these levels, they do not specify which behavior occurs where, when, and why. Behavioral phenotypes in *Drosophila* are associated with a large number of mutants belonging to more than 100 different genes. Many of them play a role during nervous system development and/or affect aspects of functioning of the nervous system, such as membrane excitability, synaptic transmission, or muscle contraction.

Several mutations have been isolated on the basis of aberrant locomotor activity. The "inactive" mutation was described by Kaplan (1977). O'Dell and Burnet (1988) reported that the locomotor activity is reduced in adult flies by the mutant genes inactive, inactive$^2$, hypoactive-C, and hypoactive E. The frequency of jumping is greatly reduced in all four mutants, and the threshold for the jumping response appears to be related to speed of locomotion. Differences in the expression of reactivity in lines selected for changes in locomotor activity have indicated that spontaneous activity and reactivity are at least partially under the control of different genes (Connolly, 1967; Van Dijken, 1982). Seven non-allelic hypoactive mutations, described by Honyk and Sheppard (1977) and Honyk

*et al*. (1980) were isolated using a screen for mutants of reduced flight abilities. Burnet *et al*. (1988) reported that the amounts and speed of locomotion are largely under independent genetic control. Diagana *et al*. (2002) created a mutation called *homer* and showed that flies homozygous for this mutation are viable and show coordinated locomotion, suggesting that *Homer* is not essential for basic neurotransmission. However, they also found that the *homer* mutant displays defects in behavioral plasticity and the control of locomotor activity. Mutations which have been found to cause abnormalities of the jumping response are *bendless* (Thomas, 1980; Thomas and Wyman, 1982), *jumpless* (Hall, 1982), and *non-jumper* (Thomas, 1980), which are associated with abnormalities affecting the giant nerve fiber. Vaj and Jayakar (1976) investigated the importance of autosomal genes in the determination of locomotor activity in *D. melanogaster* and found that chromosome 4 is most influential in controlling the locomotor activity. The pyokori behavior is genetically controlled by major genes(s) on the second chromosome. However, some minor genes affect the manifestation of the pyokori behavior (Nakashima-Tanaka and Matsukara, 1980).

In the present work, males and females of five different stocks have been utilized to study locomotory behavior and speed of locomotion. One wild type stock and four mutant stocks have been selected for this study. The purpose of this study is to see whether there is variation at the level of speed of locomotion in different mutant stocks of this species. We know that mutation at a single gene may lead to its effect on a number of other activities of the fly. Such pleiotropic effects of a gene can be envisaged in the present experiment as mutant types may show variations in their locomotor activity owing to differences in the loci carrying the mutations.

**Materials and Methods**

Locomotory behavior was observed in five different stocks of *Drosophila melanogaster*. These stocks have spent several generations in the lab. The details of these stocks are as follows.

Wild Type: Flies of this stock are normal for all the visible phenotypic traits, *i.e*., the flies have normal wings, normal eye color, normal body color, and they have spent several generations in the laboratory condition.

White Eye: These flies possess white eye color and the gene determining this trait is located on the X chromosome.

Sepia Eye: Flies of this trait possess brownish black eye color and the gene determining this trait is located on III chromosome.

Curled Wing: These flies are mutant for wing shape. Their wings are turned upward at the terminal region. They are unable to fly because of the abnormal shape of the wings. The gene determining this character is also located on III chromosome.

Black Body Color: These flies have darkly pigmented body color. The gene determining this character is located on II chromosome.

A square box with a lid was used for observing locomotion. For introducing and taking out flies, an aspirator was used. The box had 100 squares drawn on its roof, each with an area of 1 $cm^2$. A single fly was observed at a time. A fly was allowed to adapt in the new environment for 90 seconds. Locomotion was observed for the next 60 seconds. The number of squares (l $cm^2$ area each) treaded by a fly in that 60 seconds was counted and recorded. Speeds of locomotion of individual flies of both the sexes were also recorded from all the stocks.

Student t- Test was done to compare the speed of locomotion between the two sexes, in all the five stocks. One way ANOVA was applied to compare the mean speeds of locomotion among the flies (males and females taken together) of the five stocks.

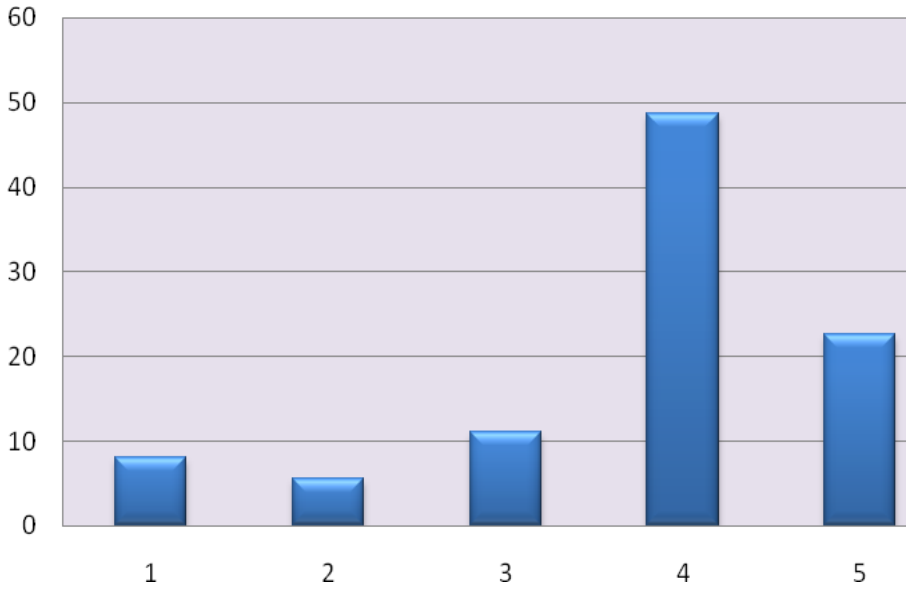


Figure 1. Mean rate of locomotion (in cm) in five different stocks of *D. melanogaster*.

1, wild type; 2, white eye; 3, black body; 4, curl wing; and 5, sepia eye.

Table 1. Student t- Test analysis to show differences in the mean speed of locomotion in the two sexes of five different stocks of *D. melanogaster*.

| | Wild type | White eye | Black body | Curl wing | Sepia eye |
|---|---|---|---|---|---|
| t values | 0.230 | 0.428 | 0.994 | 0.0586 | 0.440 |
| P | 0.820 | 0.671 | 0.327 | 0.954 | 0.662 |

*df* = 38 (for all five paired comparisons)

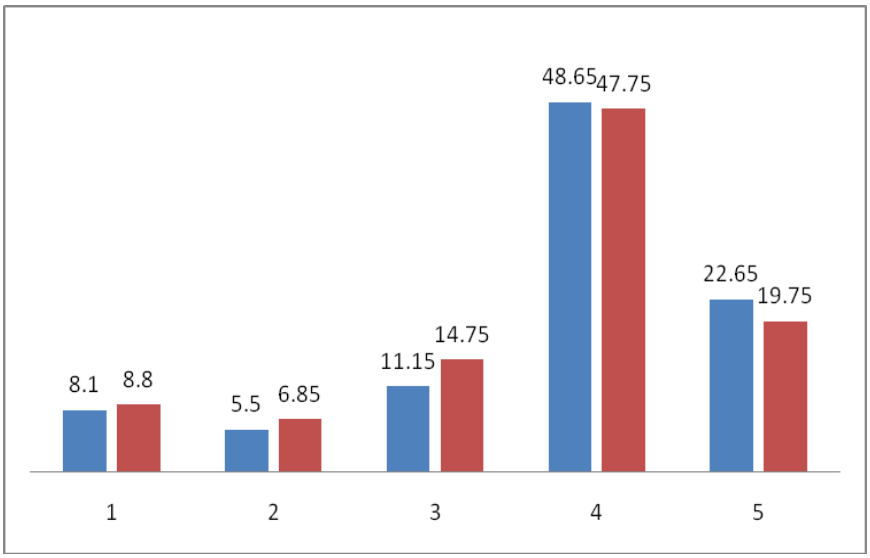


Figure 2. Mean rate of locomotion (in cm) in males and females of five different stocks of *D. melanogaster*.

1, wild type; 2, white eye; 3, black body; 4, curl wing; and 5, sepia eye.

Table 2. Result of one way ANOVA, to compare the mean speeds of locomotion across the five stocks.

| Source of variation | *df* | ss | ms | F | *p* |
|---|---|---|---|---|---|
| Between | 9 | 46285 | 5142.8 | 25.4 | |
| Within | 190 | 38459 | 202.4 | | < 0.001 |
| Total | 199 | 84744 | | | |

## Results and Discussion

Comparison of mean rates of locomotion shows that white eyed flies moved less than other types (Figure 1). This was observed in both the sexes of the white eyed flies. Flies with curl wing were seen moving with much speed and almost non-stop throughout the period they were allowed to remain in the box. Both the sexes of this stock showed this trend. The wild type flies did not show a greater speed of locomotion than others except the white eyed flies. The second runner in this study was sepia eyed flies whose males moved faster than their females. When sex-wise comparisons were made, it was observed that males moved faster than females in three stocks (wild type, white eyed, and black bodied). However the difference was not significant in any of the cases (Table 1). Table 2 shows the result of one way ANOVA, comparing the mean speeds of locomotion of flies in five different stocks. It is clear that curl winged flies moved maximum distance in the stipulated time period compared to other flies. White eyed flies moved minimum distance. Figure 2 shows mean rate of locomotion per minute in males and females of five different stocks of *D. melanogaster*.

Wild type males were seen to move that side of the chamber where light intensity was comparatively more. They were moving faster but they were suddenly seen to stop at a place and started rubbing their forelegs. Female flies did not show inclination towards light and they moved to all directions. Some flies were observed hopping frequently in the chamber. White eyed males and females were often seen beating their wings frequently and rubbing their forelimbs while sitting at one position. Both sexes were observed to be unaffected by the presence or absence of light conditions. Sepia eyed females preferred to move towards the source of light. Few flies were observed hopping in the chamber instead of moving in a direction. Some were seen moving in a circular fashion and frequently rubbing their forelimbs. Some were walking in a zig-zag manner and a few beating their wings. Curl winged males and females were showing neutral response to light conditions. Flies were mostly hopping to short distances. These flies were also often seen moving in a zig-zag manner. Flies with black body color preferred to move towards higher intensity of light. Most flies of both sexes preferred to move on the roof of the chamber. These flies either moved in a zig-zag manner or in straight line. Most of the male flies of this stock were seen beating their wings. Thus, distinct variation in the speed of locomotion, recorded among the different stocks of *Drosophila melanogaster*, indicates genic effect on this behavior.

Acknowledgment: We are thankful to Dr. Seema Sisodia for her suggestions and help during the present study.

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**Inversion polymorphism in a few south Indian populations of *Drosophila ananassae*.**

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## Introduction

The inversions were first detected in *Drosophila melanogaster* through the suppression of crossing-over by inversion heterozygotes (Sturtevant, 1926). Since flies with different karyotypes produced by inversions are externally indistinguishable, many investigators including Dobzhansky believed that the inversion karyotypes are adaptively neutral (White, 1977). This assumption is proved to be wrong, since many investigations demonstrated that inversion polymorphism in *Drosophila* are subject to natural selection and is an adaptive trait (Ayala *et al.,* 1974). The degree of inversion polymorphism varies in different species and also in different populations of the same species. Species like *D. pseudoobscura* (Dobzhansky and Sturtevant, 1938), *D. persimilis* (Mohn and Spiess, 1963), *D. subobscura* (Sperlich, 1961), *D. willistoni* (Prevosti, 1964), *D. ananassae* (Kikkawa,1938), *D. melanogaster* (Barnes, 1983), *D. robusta* (Carson and Stalker, 1947), *D. pavani* (Brncic, 1957), *D. paramelanica* (Stalker, 1960), *D. euronotus* (Stalker, 1963), *D. nasuta* (Ranganath and Krishnamurthy, 1978), *D. immigrans* (Toyofuku,1957), D. *nebulosa* (Pavan, 1946) possess large stores of inversions. These species have been termed as "champion species" by White (1977). On the other hand species such as *D. simulans, D. virilis,* and *D. novamexicana* do not seem to possess inversions in their natural populations (Aulard *et al*., 2002; Singh, 2008; White, 1977).

The most convincing evidence for the selective control of inversion frequencies comes from observations on inversion frequencies in geographic populations of different *Drosophila* species which showed seasonal, geographic, altitudinal, and latitudinal variations. In certain species, north-south clines in inversion frequencies (increase towards equator) have been reported (Krimbas and Powell, 1992). Dobzhansky *et al.* (1950), and Da Cunha and Dobzhansky (1954) have found a good correspondence between the mean number of heterozygous inversions and an index expressing environmental heterogeneity in natural populations of *D. willistoni*. Superiority of inversion heterokaryotypes over homokaryotypes has been demonstrated by Dobzhansky (1970). This led Dobzhansky and coworkers (1950) to suggest that chromosomal polymorphism is a device to cope with the diversities of environments.

*Drosophila ananassae* is one such species which exhibits high level of inversion polymorphism. It is a cosmopolitan domestic species having a unique status among *Drosophila.* Due to certain peculiarities such as male crossing over, high mutability, and high level of chromosomal polymorphism, it has been used for many genetic studies. This species harbors large numbers of inversions. Further it carries three well knit coextensive inversions, namely, 2LA on the left arm of the 2$^{nd}$ chromosome, 3LA on the left arm of the 3$^{rd}$ chromosome, and 3RA of the right arm of the 3$^{rd}$ chromosome. The extent of genetic polymorphism in various populations of *Drosophila ananassae* carrying these inversions has been studied; the adaptive significance of them has also been studied (Futch, 1966; Rajeswari and Krishnamurthy, 1969; Reddy and Krishnamurthy, 1974,

Singh and Chatterjee, 1988; Singh and Som, 2001). Therefore, in the present study inversion frequencies have been analysed in a few south Indian populations of *D. ananassae*.

## Materials and Methods

*D. ananassae* flies collected from Dharwad, Bellur, Manglore, and Mysore following the procedure described by Hegde *et al* (1999) were used for the present study. After the flies were brought to the laboratory, the females were individually placed in glass vials (2.5 cm × 8.5 cm) containing wheat cream agar medium, and males were used for identification. These flies were then maintained in the vivarium at constant temperature of 22 ± 1°C and relative humidity of 70%. When larvae appeared, eight third instar larvae from each isofemale line were used for analysis of inversion frequency following the procedure of Jayaramu (2009).

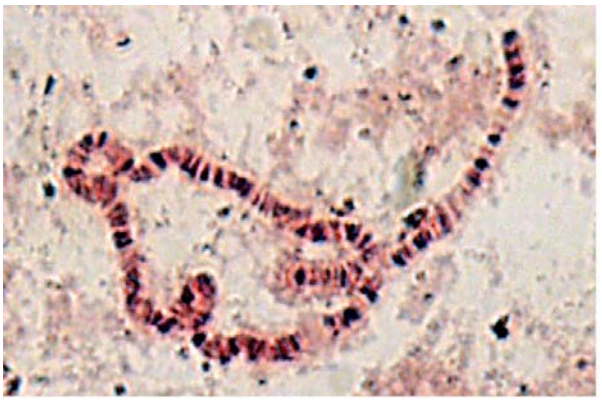
## Results and Discussion

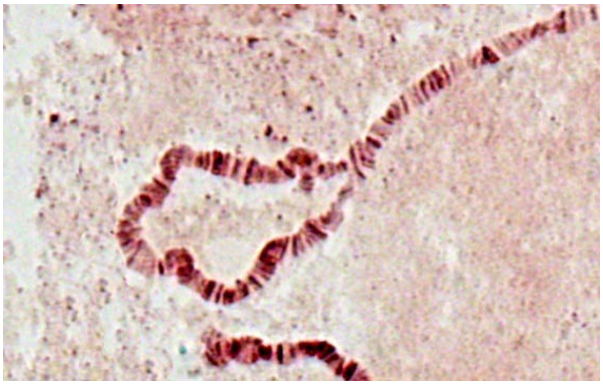
One of the most important aspects of evolutionary biology is the study of how natural selection modifies the genetic structure of populations. For this to happen, populations must encompass some degree of chromosomal or genetic variation or other kinds of modifications in the gene pool. Analysis of inversion polymorphism is one of the strategies to know the extent of genetic variation between populations. In the present study inversion polymorphism has been studied in four different geographical populations of *Drosophila ananassae*. A comparison of percentage of inversions (Table 1) of the four different populations (Mysore, Mangalore, Bellur, and Dharwad) showed that the inversion frequencies varied significantly between different populations. The same inversion was found to be present in different frequencies in different populations. For example, the frequency of 2LA inversion in Dharwad population was 20 percent, while in Mangalore population it was 36.7 percent. It was noticed that in all the population studied, 3LA inversion was most frequent, while 2LA+3LA was least. The difference in the percentage of inversions in different populations suggests that the frequency of each of the three paracentric inversions was not the same in all the populations studied. This agrees with earlier studies of inversion frequency in different species of *Drosophila* (Brncic and Koref-Santibanez, 1964; Singh, 1991; Sperlich, 1966; Spiess and Langer, 1961).

Table 1. Inversion frequency (%) in different geographic populations of *D. ananassae*

| | Inversion frequency (%) | | | | |
|---|---|---|---|---|---|
| Strains | N | 2LA | 3LA | 2LA+3LA | Inversion free (Monomorphic) |
| Dharwad | 38 | 20.0 | 50.0 | 20.0 | 10.0 |
| Mangalore | 42 | 36.7 | 53.3 | 6.7 | 3.3 |
| Mysore | 40 | 33.3 | 43.4 | 10.0 | 13.3 |
| Bellur | 32 | 16.7 | 40.0 | 26.7 | 16.6 |

The earlier studies on inversion polymorphism in *D. ananassae* (Da Cunha, 1960; Krimbas and Powell, 1992; Singh, 1998) have demonstrated that most of the inversions are distributed either on second or third chromosomes. Two hypotheses have been proposed to account for the concentration of inversions on single chromosomes, the co-adaptation hypothesis and mechanical hypothesis. Although the present study does not provide evidence in favor of any of these hypotheses, it can be said that inversion polymorphism is a means of reducing recombination in a chromosomal segment. Consequently, frequency dependent selection has been





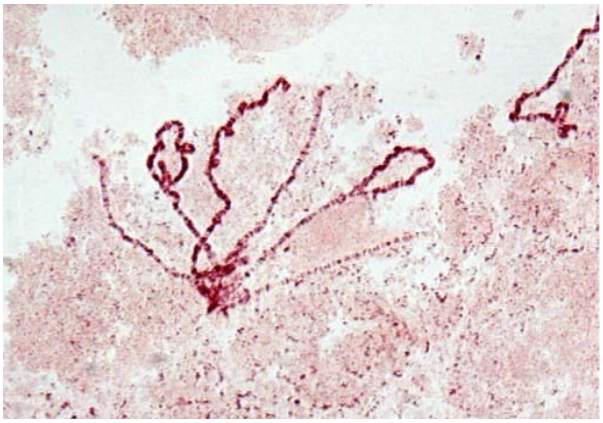


Figure 1 (Top). 2LA inversion of *D. ananassae.*

Figure 2 (Middle). 3LA inversion of *D. ananassae.*

Figure 3 (Bottom). 2LA+ 3LA inversion of *D. ananassae.*

reported to influence second and third chromosome inversions in *D. ananassae* (Kojima and Tobari, 1969). In the present study the persistence of inversion polymorphism in these populations could be explained by an advantage of inversion heterozygotes over corresponding homozygotes. The author in the present study has noticed more heterokaryotypes than homokaryotypes. This confirms the fact that the inversion polymorphism is adaptive and balanced due to higher Darwinian fitness on inversion heterozygotes (Dobzhansky, 1951).

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## Drosophilids of Male Mahadeshwar hills of Chamarajanagar District Karnataka State, India.

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### Introduction

The term "Biodiversity" encompasses different ecosystems, species, genes, and their relative abundance. The most succinct definition of biodiversity is the structural and functional variety of life forms such as genetic, population, species, community, and ecological levels. Biodiversity is a buzz word, which is attracting the researchers to understand the diversity of biological systems. Insects are supposed to be the major contributors and comprises 80% of faunal constellation. Genus *Drosophila* is one such group of insects, which has attracted the attention of biologists in terms of biodiversity. A better understanding of how different species are affected by current climates and why they sometimes respond differently to climate change is necessary for predicting future effects of climate change (Weather head, 2005). In view of this the chapter deals with the survey of Drosophilids fauna distributed in south Karnataka, India during 2010, 2011, and 2012 at variable altitudes and to record the impact of seasonal stress on Drosophilid composition.

### Materials and Methods

Chamarajanagar District, Male Mahadeshwar Hills is situated about 215 km from Bangalore and is a sacred place, with its famous Shiva temple. Amidst dense forest, the temple attracts not only the pilgrims but also nature lovers. The foot of the hill (658 m) was surrounded by mango orchards along with trees such as *Acacia concinna*, *Zizipus jujube*, *Vitex negundo*, and *Phyllanthus* species; the middle region (809 m) *Andrographis serpellifolia*, *Bridalia* species, *Tectona grandis*, *Tamarindus indica*; the top hill (912 m) *Vitex negundo* and other small shrubs.

*Collection procedure of* Drosophila *flies*

Both bottle trapping and net sweeping methods were used. For bottle trapping, milk bottles of 250 ml capacity containing smashed ripe bananas sprayed with yeast were tied to the twigs underneath to small bushes and trees. Five bottles were kept at each altitude. The following day the mouth of the bottle was plugged with cotton and brought to the laboratory. These flies that were collected in the bottles were transferred to the fresh bottles containing cream of wheat agar medium as food. Net sweeping was also done for collecting the flies using banana rotting fruits with equal

quantity of approximately 250 gms, which were placed beneath the shaded areas of trees and bushes one day before collection. Such bait as fermenting fruits retain its attractive odor for a long time. The collections were made early in the morning by sweeping in each trap at least three times and transferring samples to six quarter milk bottles filled with standard agar medium sprayed with yeast. Likewise from each collection spot, the flies were collected in ten bottles. Then the traps were brought to the laboratory, flies were isolated, counted, and categorized. The species identification was made according to taxonomic groups by employing several keys of Sturtevant (1927), Patterson and Stone (1952), Throckmorton (1927), and Bock and Wheeler (1972). The study was conducted during the months of May, August, and November.

## Results

*MM 2011*

- A total of 3163 flies were found with 10 species. More flies were observed during the summer season (1300), followed by the winter season (1032) and rainy season (831).
- *D. nasuta*, *phorticella striata*, and *D. neonasuta* were found increased in all the seasons and are variable in numbers with all the altitudes. More *D. nasuta* were observed during rainy season at 809 m and 912 m. (Figure 1b)
- *D. melanogaster* was observed more only during summer at the bottom region.
- *Phorticella striata*, *D. neonasuta*, and *D. nasuta* were found to be more dominating than other species at all the seasons.

In the year (2011) assessment, the total number of species 10 were found and the same species were recorded. In general more flies were observed in summer followed by winter and rainy season. *D. neonasuta*, *Phorticella striata*, and *D.* nasuta were found constantly throughout the year assessment in all the seasons.

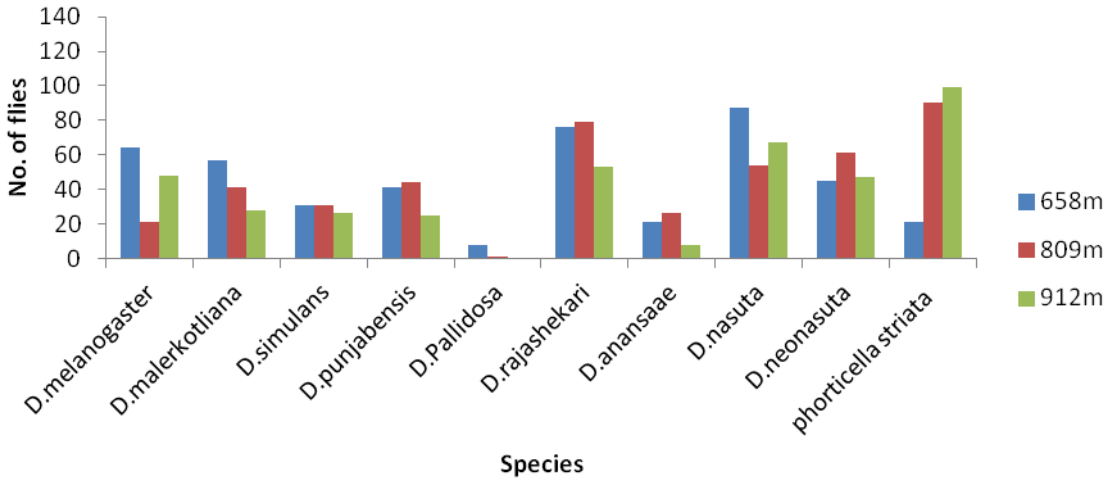


Figure 1 a.

Figure 1. Seasonal variation of *Drosophila* population at different altitudes of M M hill (2011): (a) in Summer, (b) in Rainy, (c) in Winter.

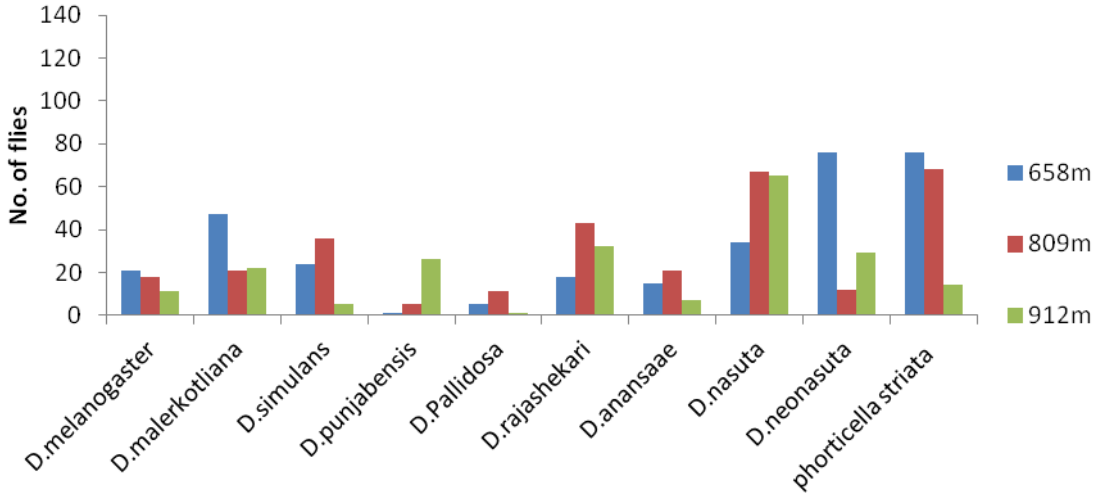


Figure 1 b.

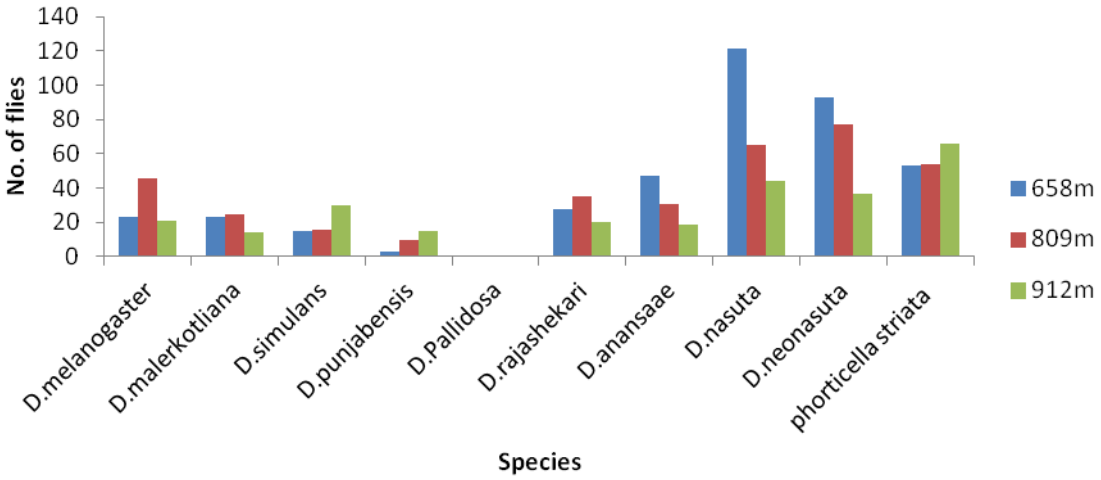


Figure 1 c.

Table 1. The *Drosophila* species and their numbers collected from the M M Hills 2011.

| Sl.No. | Species | Summer | Rainy | Winter | Total |
|---|---|---|---|---|---|
| 1 | *D. melanogaster* | 133 | 50 | 90 | 273 |
| 2 | *D. malerkotliana* | 126 | 90 | 62 | 278 |
| 3 | *D. simulans* | 88 | 65 | 61 | 214 |
| 4 | *D. punjabensis* | 110 | 32 | 28 | 170 |
| 5 | *D. Pallidosa* | 9 | 17 | 1 | 27 |
| 6 | *D. rajashekari* | 208 | 93 | 83 | 384 |
| 7 | *D. anansaae* | 55 | 43 | 97 | 195 |
| 8 | *D. nasuta* | 208 | 166 | 230 | 604 |
| 9 | *D. neonasuta* | 153 | 117 | 207 | 477 |
| 10 | *Phorticella striata* | 210 | 158 | 173 | 541 |
| | *Total* | 1300 | 831 | 1032 | 3163 |

## Discussion

M M Hills- A total of 3163 flies were recorded, 10 species were found (Table 1). The flies were found in summer season and flies tend to be increased in bottom region of the hill. Interestingly, flies were decreased with increasing altitude. *D. melanogaster, D. neonasuta, phorticella striata* and *D. nasuta* could be assigned as dominant species, as they are available at all the altitudes during 2011. The reason for this may be easily understood if we observe the quantity of the species if dominance at each altitude. When population sizes are relatively small and fluctuate, there is a possibility of populations going extinct due to demographic and environment stochasticity. Thus, from the present study the distribution of species is uneven in space and time.

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**Male age influence on pre-adult fitness in *Drosophila ananassae*.**

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## Introduction

In insects investigation on mate choice have shown effects of body size on mating events and reproduction (Hegde and Krishna, 1997). Apart from body size, the age of mating individuals also plays an important role in mate choice and reproduction (Pervez, *et al.*, 2004). It was widely held that females should prefer to mate with older males in species where males provide only sperm to females, because viability selection leads to older males of higher genotype quality than young males (Trivers, 1972; Manning, 1985; Andersson, 1994). In support of this hypothesis, a simulation model revealed a strong preference for older males under different eco conditions (Kokko and Lindstrom, 1996). Several empirical studies have suggested that females do indeed prefer to mate with older males for qualitative and quantitative progeny production (Zuk, 1988; Manning, 1989; Simmons and Zuk, 1992; Simmons, 1995). However, some have argued that males of young and intermediate ages are better mates, as they have the highest breeding values for fitness and are less prone to deleterious mutations (Hansen and Price, 1995; Beck and Powell, 2000). The difference in conclusions of these studies is a probable result of their contrary approaches towards the change in physiological state of males with age and the existence of life history trade-offs.

Therefore, in the present study, *D. ananassae* which belongs to the *melanogaster* sub group of *ananassae* complex has been used (Bock and Wheeler, 1972). In this species males do not provide parental care or nuptial gift to mated females. He can provide only sperm and accessory gland proteins to the mated female. In this species female prefers to mate with old age males more frequently than young or middle age males. Further in this species, strains both with inversion and

without inversion are available. Therefore, the present study has been undertaken to understand male age influence on preadult fitness to test the hypothesis good gene model (Kokko, 1997).

## Materials and Methods

Monomorphic (inversion free) and polymorphic (with inversion) strains of *D. ananassae* were established from already established (Prathibha and Krishna, 2010) out-bred populations collected at semi domestic localities of Mysore, Karnataka, India. To study polytene chromosomes and to screen for the presence of inversions, the larvae were dissected in 0.7% sodium chloride solution. These salivary glands were fixed in 1N HCL for five minutes. The stained glands were individually placed on slides with two drops of 45% acetic acid. Then salivary glands were squashed by placing a clean cover glass and by applying uniform pressure. This achieves uniform spreading of the chromosomes. After squashing, edges of the cover slip were sealed with paraffin lanolin mixture. The observation of slides were done under Leitz Ortholux II scientific and clinical microscope both at low (10×) and high (45×) magnification. If an inversion loop is present in the larvae then the mother of those larvae was treated as polymorphic. If there is no inversion loop in the larvae, then the mother of those larvae was treated as monomorphic. Presence or absence of inversions was checked for 5 generations. Like this polymorphic and monomorphic strains were established. These experimental stocks were maintained at 21±1°C at relative humidity of 70% using 12:12 L: D cycle. At the 5$^{th}$ generation after testing the inversion, virgin females and bachelor males were isolated within 3 hrs of their eclosion separately from monomorphic and polymorphic strains, were aged as required, and were cultured using same laboratory conditions until they were used in the experiments.

*Selection of male age classes*

Before assigning male age classes, we studied the longevity of males in monomorphic and polymorphic strains of *D. ananassae* by transferring individually unmated males into a vial containing wheat cream agar medium once in a week and maintaining them in the same condition. This process was continued until their death and longevity was recorded. A total of 50 replicates were made for each of monomorphic and polymorphic strains and mean longevity data show 60±2 for monomorphic and 63±2 for polymorphic strains. Since mean longevity of *D. ananassae* ranges from 60-65 days, we assigned days for young, middle, and old aged male as follows: young age male (2-3 days); middle age male (24-25 days), and old age male (46-47 days)].

In addition to this, we also collected 5-6 day old virgin females from the respective stocks to be used in the present experiment.

*Male age influence on pre adult fitness* (*egg - larval hatchability and larva - adult viability*)

Unmated young, middle, old aged males and 5-6 days old virgin female were used to study male age influence on male mating activities. A female along with a male of different age classes were aspirated individually into an Elens-Wattiaux mating chamber and observed for 1 hr. Pairs unmated within 1 hr were discarded. Soon after, the mated pairs were individually transferred to a new vial once in 24 hr until their death. Hundred eggs were taken randomly using a spatula to study percentage of egg-larval hatchability and were seeded separately for each of the three male age classes in a small petridish containing wheat cream agar medium. Number of 1$^{st}$ instar larvae emerged from above eggs was counted to account for percentage of egg-larval hatchability. For studying percentage of larva-adult viability, 100 1$^{st}$ instar larvae were collected and were placed in a petri dish containing wheat cream agar medium, and the number of adult flies emerged from these larvae were also counted, to account for percentage of larva-adult viability.

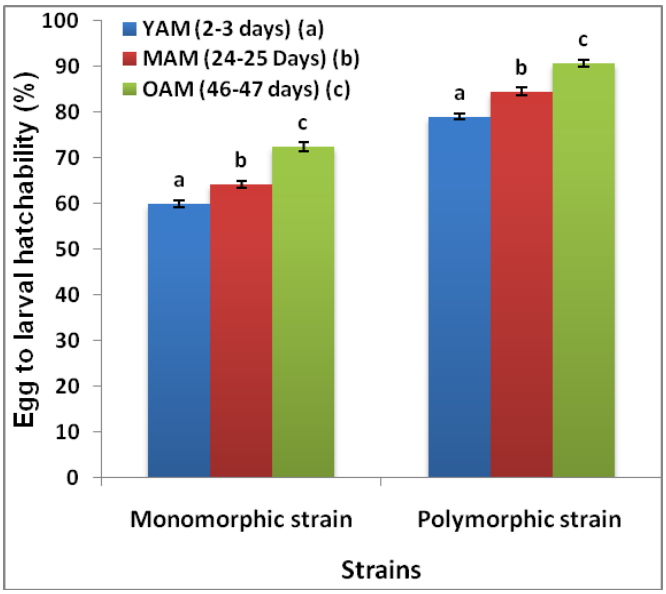


Figure 1. Male age influence on egg to larval hatchability in monomorphic and polymorphic strains of *D. ananassae.* (YAM – Young age male; MAM – Middle age male; OAM – Old age male).

Table 1. Two way Anova of male age influence on egg to larval hatchability in monomorphic and polymorphic strains of *D. ananassae.*

| % Hatchability | Source | Type III Sum of Squares | df | Mean Square | F-Values |
|---|---|---|---|---|---|
| Egg to larval Hatchability | Strains | 2815.203 | 1 | 2815.203 | 54.205** |
| | Male age | 37380.727 | 2 | 18690.363 | 359.873** |
| | Strains*Male age | 119.887 | 2 | 59.943 | 1.154$^{NS}$ |
| | Error | 15269.180 | 294 | 51.936 | |
| | Total | 1632315.997 | 300 | | |

$^{NS}$ Non-significant, **Significant at 0.0001 level.

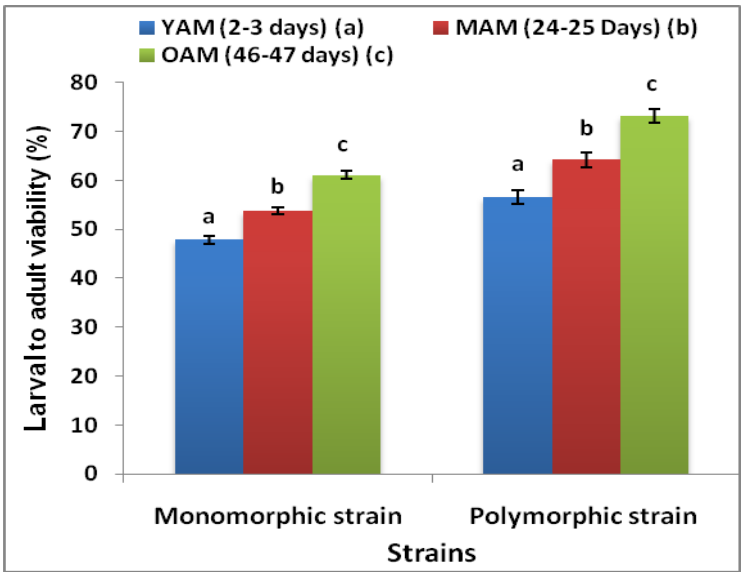


Figure 2. Male age influence on larval to adult viability in monomorphic and polymorphic strains of *D. ananassae.* (YAM – Young age male; MAM – Middle age male; OAM – Old age male).

Table 2. Two way Anova of male age influence on larval to adult viability in monomorphic and polymorphic strains of *D. ananassae.*

| % Viability | Source | Type III Sum of Squares | df | Mean Square | F-Values |
|---|---|---|---|---|---|
| Larval to adult viability | Strains | 4696.563 | 1 | 4696.563 | 84.822** |
| | Male age | 36584.607 | 2 | 18427.303 | 332.804** |
| | Strains*Male age | 720.927 | 2 | 360.463 | 6.510* |
| | Error | 16278.740 | 294 | 55.370 | |
| | Total | 1312179.000 | 300 | | |

* Significant at 0.005, **Significant at 0.0001 level.

**Results and Discussion**

Egg to larval hatchability and larva to adult viability are two important components of fitness in *Drosophila*, and they can be related to phenomena such as larval facilitation, as well as concepts of microniches and microenvironments (Castro and Mensua, 1985). Hence in the present study, these two parameters have been undertaken to study male age influence on preadult fitness in monomorphic and polymorphic strains of *D. ananassae*. Percentage of egg to larval hatchability and larva to adult viability data are provided in Figures 1 and 2, and in Tables 1 and 2. It was noticed from the table that in both monomorphic and polymorphic strains, mean preadult fitness of old aged male was found to be greater compared to preadult fitness of middle and young age males. Two way ANOVA followed by Tukey's post hock test showed significant variation in preadult fitness among males of different age classes, between monomorphic and polymorphic strains, and also in the interaction between male age classes and strains.

From the above data it was noticed that in *D. ananassae*, male age has significant influence on preadult fitness. Our study supports the theoretical models of good gene hypothesis (Kokko, 1997, 1998), suggesting that females of the species discriminate the male on the basis of male age and prefer to mate with old age males to obtain indirect genetic benefits. In the present study it was also noticed that polymorphic strains had significantly greater preadult fitness than monomorphc strain. This suggests the influence of inversion on fitness characters. This agrees with earlier studies of inversion role on fitness in different species of *Drosophila* (Spiess and Langer, 1961; Singh and Chatterjee, 1986; Singh, 1989; Sisodia. and Singh, 2001). Thus, in the present study it was clear that females of *Drosophila ananassae* preferred old age males to obtain better fitness.

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**Female age influence on mating activities in outbred populations of *Drosophila ananassae*.**

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Traditional models of sexual selection predict that in most animal species, males will be less discriminating in their choice of mating partners than females, because their investment in offspring is much lower (Bateman, 1948; Trivers, 1972). Costs of reproduction have been extensively studied in females, arising through offspring production but also male harassment, insemination, and maternal care (Chapman *et al.*, 1998; Roff, 2002; Harshman and Zera, 2007). However, it is becoming increasingly apparent that in many species males, nevertheless, have a high cost of reproduction (mating) due to costs arising from factors such as energetically expensive courtship displays (Judge and Brooks, 2001) and the production of ejaculates (Dewsbury, 1982; Galvani and Johnstone, 1998).

If all females in a male's pool of potential mates have equal reproductive potential, males should not preferentially mate with one over another, as maximizing only the number of female mates would give males the highest reproductive pay off (Bateman 1948). If females differ in their reproductive potential, males might exercise some degree of mate choice. Therefore, males must operate under time constraints, as well as possibly dwindling energy or sperm reserves, or both. Males that exercise mate selectivity might, therefore, have a reproductive advantage by wisely allocating their time, sperm, and energy. This is possible with females (Andersson, 1994) that provide them with the greatest gain in reproductive success.

In most of the studies on age effects of *Drosophila*, parental age on progeny fitness has not been directly looked into, but instead have considered physiological changes associated with changes in parental age, molecular aspects, selection experiments, and comparison of populations that have been generated from individuals of different ages (Parsons, 1964; Wattiaux, 1968; Ganetzky and Flanagan, 1978; Luckinbill *et al.*, 1984; Partridge and Fowler, 1992; Chippindale *et al.*, 1994; Hansen and Price, 1995). In most of these studies it was difficult to separate male age effect from female age effect. Therefore, the present study of female age effect on mating behavior has been undertaken in *D. ananassae*, a cosmopolitan domestic species of *Drosophila* belonging to *melanogaster* group of *ananassae* subgroup.

## Material and Method

In the present study an experimental stock of *D. ananassae* has been established by mixing together progenies of each of the 150 naturally inseminated isofemale lines collected at domestic

localities of Mysore, Karnataka, India and redistributed to twenty different culture bottles each with 40 flies (20 males and 20 females) containing wheat cream agar media. Here onwards this stock is designated as O population. These culture bottles were maintained at 21 ± 1°C at a relative humidity of 70% using 12:12 L:D cycle for three generations to allow them to acclimatize to the laboratory condition. These flies were used to collect eggs at the 4$^{th}$ generation using Delcour's procedure (1969). Hundred eggs were seeded in a vial containing wheat cream agar medium. When adults emerged, virgin female and unmated males were isolated within three hrs of their eclosion and were aged individually in a vial containing wheat cream agar medium until they were used in the experiment. These flies were used to study female age influence on mating activities.

Selection of female age classes

The mating activities of females of different age classes were studied and found that female above 35 days will not show any courtship activities and she did not mate within 1 hr of observation. Therefore, in our study we assigned days for young, middle, and old aged female as follows [young age female (2-3 days), middle age female (17-18 days), and old age female (32-33days)].

Influence of female age on mating activities

A male with a female (younger, middle aged, or older) were aspirated into an Elens- Wattiaux mating chamber (1964), and observed for 1 h. We studied mating latency, courtship acts such as tapping, scissoring, vibrating, licking, circling, ignoring, extruding, and decamping, and copulation duration following the procedures of Hegde and Krishna (1997). One-way analysis of variance (ANOVA) followed by Tukey's honest post-hoc test was carried out on courtship activity data using SPSS vers 10.1 software (SPSS, Chicago, IL, USA).

**Results and Discussion**

Earlier studies in mating behavior of *Drosophila* have suggested that male activity and female receptivity are important for successful mating in *Drosophila* (Manning, 1961; Spieth, 1968). In *Drosophila* mating latency or courtship to copulation is a good estimate of sexual receptivity of females and sexual activity of males (Spieth and Ringo, 1983). During this period the male fly performs various courtship acts such as tapping, scissoring, vibration, circling, and licking to increase the receptivity of females (Spieth, 1968; Hegde and Krishna, 1997). Table 1 revealed that middle-aged females had taken shorter time for initiation of copulation and copulated longer when compared to either young or old aged females suggesting influence of female age on mating latency and copulation duration. As the speed is reverse of the time, flies which take more time were slow maters while those which take less time were fast maters. This suggests that in *D. ananassae* middle-aged females were fast maters while old age females were slow maters. This supports age influence mating activities in *D. pseudoobscura* (Noor, 1997). Since female receptivity is also related to time of initiation of copulation, therefore middle-aged females were more receptive than young or old aged females in *D. ananassae*.

We also quantified the male and female courtship activities such as tapping, scissoring, vibration, circling, licking, ignoring, extruding, and decamping (Table-1). It was noticed that males of *D. ananassae* showed greater courtship activities to middle-aged females compared to young or old-aged females' in turn middle-aged females showed less rejection activities to suggesting influence of female age on mating success in *D. ananassae*. This supports the earlier studies of age and other environmental factors influence on courtship activities in different species of *Drosophila*,

too (Speith, 1952, 1968; Hegde and Krishna, 1997; Noor, 1997). Through these courtship activities, males of *Drosophila* convey chemical, auditory, and visual signals to middle-aged females better and try to convince the middle-aged female faster for mating than young or old-aged females. This agrees with earlier studies of *Drosophila* that males which perform greater courtship activities are better mates and obtained greater mating success than those males which do not show high level of courtship activities (Hegde and Krishna, 1997).

Table 1. Female age influence on mating activities in outbred population of *D. ananassae* (Values are Mean ± SE).

| Parameters | Young (2-3days) | Middle (17-18 days) | Old (32-33 days) | F-Value |
|---|---|---|---|---|
| Mating latency (in minutes) | $13.52 \pm 0.62^{b}$ | $9.94 \pm 0.38^{a}$ | $25.56 \pm 0.1.89^{c}$ | 48.53** |
| Tapping | $10.96 \pm 0.35^{b}$ | $12.78 \pm 0.33^{c}$ | $8.08 \pm 0.30^{a}$ | 50.82** |
| Scissoring | $10.82 \pm 0.36^{b}$ | $11.82 \pm 0.55^{b}$ | $8.36 \pm 0.36^{a}$ | 16.57** |
| Vibration | $10.12 \pm 0.41^{b}$ | $11.32 \pm 0.53^{c}$ | $8.24 \pm 0.27^{a}$ | 13.63** |
| Circling | $4.12 \pm 0.20^{b}$ | $6.40 \pm 0.28^{c}$ | $2.88 \pm 0.18^{a}$ | 61.57** |
| Licking | $3.58 \pm 0.18^{b}$ | $5.32 \pm 0.20^{c}$ | $2.54 \pm 0.19^{a}$ | 52.26** |
| Ignoring | $4.88 \pm 0.15^{b}$ | $3.26 \pm 0.19^{a}$ | $7.42 \pm 0.25^{c}$ | 100.65** |
| Extruding | $4.26 \pm 0.22^{b}$ | $2.76 \pm 0.14^{a}$ | $5.72 \pm 0.31^{c}$ | 38.81** |
| Decamping | $2.76 \pm 0.13^{a}$ | $2.12 \pm 0.14^{a}$ | $4.40 \pm 0.29^{b}$ | 32.96** |
| Copulation duration ( in minutes) | $4.12 \pm 0.04^{b}$ | $4.56 \pm 0.06^{c}$ | $3.17 \pm 0.04^{a}$ | 67.11** |

**P< 0.001; Note: 1) Different letter in superscript in each row indicates significant by Tukey's test

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**Biodiversity of *Drosophilidae* of Western Ghats (Coorg District) of Karnataka, India.**

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Since the times of Morgan until today, *Drosophila* has been one of the best model organisms for biological research. However, the taxonomic and population studies have progressed little due to the lack of interest of people in this area. Therefore, very few studies have been carried out to collect *Drosophila* from the field. Hence, the present study has been undertaken to study *Drosophila* fauna from three places of Western Ghats of Coorg district.

In the present study the *Drosophila* fauna was collected from three different places in the Western Ghats, namely, Irupu, Kutta, and Pookola in the month of October, 2012, using regular bottle trapping and banana bait methods. The study revealed a total of eight different species, namely *D. ananassae*, *D. bipectinata*, *D. kikkawai*, *D. malerkotliana*, and *D. takahashii* belonging to Subgenus sophophora. The species *D. nasuta* and *D. immigrans* belonging to Subgenus *Drosophila* and *D. nigra* belonging to subgenus *Scaptodrosophila* are being recorded. The frequency distribution of the above species in three places of Western Ghats of Coorg district is given in Table 1.

Table 1. Frequency distribution of the various species of *Drosophila* in the Western Ghats, Coorg District.

| S. No. | Name of the species | Frequency distribution at | | | | | | Total No. of flies | |
|---|---|---|---|---|---|---|---|---|---|
| | | Pookola | | Kutta | | Irupu | | | |
| | | (F) | (M) | (F) | (M) | (F) | (M) | (F) | (M) |
| 1. | *D. ananassae* | 60 | 67 | - | - | - | - | 60 | 67 |
| 2. | *D. bipectinata* | 15 | 23 | - | - | - | - | 15 | 23 |
| 3. | *D. kikkawai* | 75 | 79 | 10 | 16 | 130 | 152 | 215 | 247 |
| 4. | *D. malerkotliana* | 85 | 83 | - | - | - | - | 85 | 83 |
| 5. | *D. takahashii* | 26 | 35 | 32 | 43 | 86 | 102 | 144 | 180 |
| 6. | *D. immigrans* | 20 | 31 | 5 | 14 | 25 | 26 | 50 | 71 |
| 7. | *D. nasuta* | 260 | 278 | - | - | - | - | 260 | 278 |
| 8. | *D. nigra* | 100 | 116 | - | - | 40 | 37 | 140 | 153 |
| | Grand total | 641 | 712 | 47 | 73 | 281 | 317 | 969 | 1102 |

**Preliminary studies on genetic analysis of larval pupation site preference in *Drosophila*.**

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Larval pupation behavior in *Drosophila melanogaster*, *D. simulans*, *D. mauritiana*, and their hybrids was studied. The larvae of *D. melanogaster* prefer glass while its sibling species preferred

media for pupation. The inheritance of larval PSP has been studied by crossing the above species. The hybrids of *D. melanogaster* and *D. simulans* prefer media to pupate. The progenies of cross between *D. melanogaster* and *D. mauritiana* prefer glass for pupation, it is intermediate than the parents. Inheritance of *D. simulans* and *D. mauritiana* hybrids show media pupation.

## Introduction

Pupation site preference (PSP) is an important event in *Drosophila* preadult development, because the place selected by larva can have decisive influence on their subsequent survival as pupae (Sameoto and Miller, 1968). The PSP is one of the behaviors exhibited by late third instar larvae, and it has been studied in different species of *Drosophila*.

Genetically conditioned behavioral polymorphism of *D. willistoni* species used to study pupation behavior in cages revealed that the differences between larvae that prefer to pupate on food and larvae that prefer bottom of the cages is simple and due mainly to a single gene difference (de Souza *et al*., 1970). The inheritance of intra- and interspecies pupation behavior of *D. ananassae* is under the polygenic control (Singh and Pandey, 1993). Shivanna *et al*., (1996) studied the PSP in relation to quantity of glue protein and reported that *D. simulans* and *D. mauritiana* produced ample protein, thus preferred media, whereas *D. melanogaster* produced half of the quantity of its siblings preferred glass for pupation. Because of its difference in PSP and glue protein synthesis *Drosophila melanogaster* group has been the focus of the most recent advances in behavior genetics, inheritance, and so forth.

Hybrid sterility, lethality, gene expression, longevity, developmental stability, and so forth have been studied in hybrids of *D. melanogaster*, *D. simulans*, and *D. mauritiana* (Sawamura *et al*., 1993; Carracedo *et al*., 1998; Barbash and Ashburner, 2003; Ranz *et al*., 2004; Bhadra *et al*., 2006; Stamenkovic-Radaki *et al*., 2009). Whereas, the inheritance of PSP has not been studied in these species. Thus, the present work was taken to study the inheritance of PSP.

## Methods and Materials

*Crossing Experiment*

*Drosophila* species were obtained from *Drosophila* Stock Center, Dept. of Zoology, University of Mysore, Mysore, and cultured in wheat cream agar medium (Shivanna *et al*., 1996). The virgin male and female flies were collected within one hour of their eclosion from pupae, and they were kept in separate vials. After three days, they were used for crossing experiment as, *D. melanogaster* female × *D. simulans* male (A), *D. melanogaster* female × *D. mauritiana* male (B), and *D. simulans* female × *D. mauritiana* male (C). Whereas, their reciprocal crosses yield no progenies.

*Larval Pupation Site Preference*

The PSP has been studied using the procedure described by Shivanna *et al*., (1996). Synchronized first instar larvae were collected from the cultures of each species and hybrids. About 50 larvae were placed in different culture vials containing equal quantity of wheat cream agar medium. Ten replicates were maintained for each set at a constant temperature of 22 ± 1°C with a relative humidity (RH) of 70 - 80%. Yeast was added every day to feed the larvae. The number of larvae pupating at three different sites (cotton, glass wall, and media of culture vials) were counted and tabulated. The data were subjected to statistical analysis (using SPSS 13.0 software).

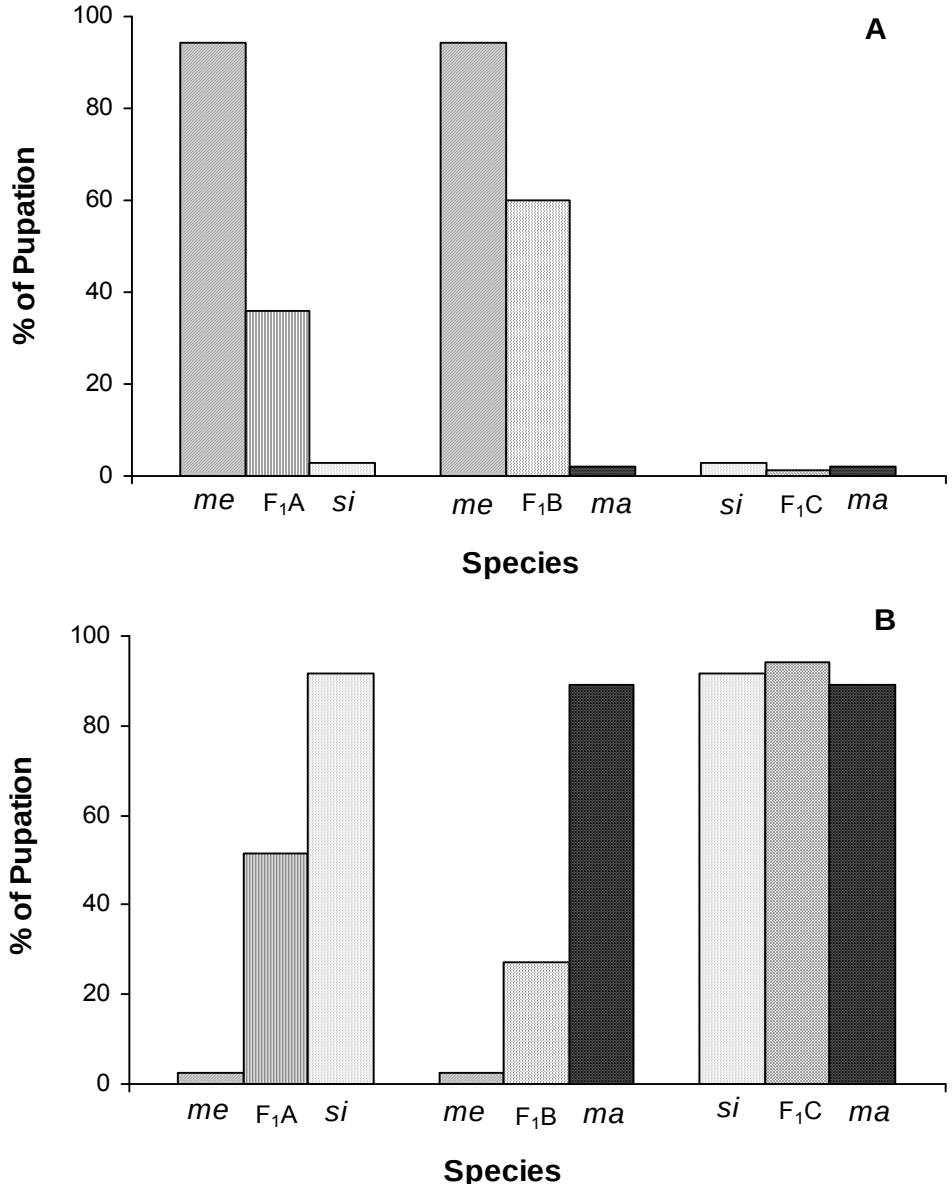


Figure 1. Percentage of pupation site preference in pure lines and hybrids of *Drosophila*. A, Glass pupation; B, Media pupation. (*me – D. melanogaster*; *si – D. simulans*; *ma – D. mauritiana*; $F_1A$ = *D. melanogaster* ♀ × *D. simulans* ♂; $F_1B$ = *D. melanogaster* ♀ × *D. mauritiana* ♂; $F_1C$ = *D. simulans* ♀ × *D. mauritiana* ♂).

**Results and Discussion**

The study of behavior is a relatively new field of investigation, and the behavior genetics largely began as a byproduct of their research investigations in a number of organisms including *Drosophila*. The genetic difference between larvae that prefer food and the bottom of the cages to pupate is simple and mainly due to a single major gene difference (de Souza *et al*., 1970). The type of sites selected by larvae affects pupal survival, and the choice of pupation sites by larvae has a genetic basis. Studies of Manning and Markow (1981) showed that the progeny from the cross of *D.*

*melanogaster* females and *D. simulans* males selected pupation sites exactly intermediate between parents in selection of light and dark, while the offsprings of reciprocal cross preferred light for their pupation.

Larvae of *D. melanogaster* preferred glass, whereas *D. simulans* and *D. mauritiana* preferred media to pupate. None of the species preferred cotton for pupation. The differences between three species at glass (F - 1005.346, df – 2, 27) and media (F - 1823.462, df – 2, 27) are significant. The inheritance of larval PSP of $F_1$A is media and $F_1$B is glass, and pupation is intermediate of their parents. The difference between glass and media pupation is less in hybrids than parents. $F_1$C preferred media to pupate and is more than their parents (Figure 1 A and B).

Sokolowski and Bauer (1989) investigated the inheritance of larval pupation behavior in sixteen reciprocal crosses between field collected lines of *D. melanogaster*. They reported that the second and third chromosomes act additively on pupation distance, and the third pair of chromosomes had a much larger effect than the second chromosome.

Present study reveals that the PSP between *D. melanogaster*, *D. simulans*, and *D. mauritiana* shows significant variations at both the sites (glass and media) except *D. simulans* and *D. mauritiana* at glass. The pupation of *D. melanogaster* shows significant difference with $F_1$A and $F_1$B at both glass and media. *D. simulans* pupation difference is found to be significant with $F_1$A at both the sites, whereas with $F_1$C at media only. The pupation variation of *D. mauritiana* is highly significant with $F_1$B at both the sites and $F_1$C at media only (Table 1).

Table 1. *t* - values of larval pupation site preference among pure lines and between hybrids and parents.

| Different combinations | Glass | Media | Cotton |
|---|---|---|---|
| *D. melanogaster* & *D. simulans* | 32.38*** | 47.01*** | 0.0 |
| *D. melanogaster* & *D. mauritiana* | 32.54*** | 44.17*** | 0.0 |
| *D. simulans* & *D. mauritiana* | 0.684 | 2.56* | 0.0 |
| $F_1$A & *D. melanogaster* | 7.49*** | 8.01*** | 0.0 |
| $F_1$A & *D. simulans* | 4.58*** | 6.77*** | 0.0 |
| $F_1$B & *D. melanogaster* | 3.44** | 2.45* | 0.0 |
| $F_1$B & *D. mauritiana* | 5.98*** | 6.33*** | 0.0 |
| $F_1$C & *D. simulans* | 1.74 | 2.09* | 0.0 |
| $F_1$C & *D. mauritiana* | 0.98 | 4.13*** | 0.0 |

df =18, *** $P < 0.001$ level, ** $P < 0.01$ level, * $P < 0.05$ level

Despite the intermediate nature of pupation in $F_1$A and $F_1$B, the larvae were influenced by the genes of one of the parents (*D. simulans* and *D. melanogaster*, respectively), whereas the larvae of $F_1$C are influenced by both parents irrespective of male or female.

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**Allele diversity of cross-species microsatellite amplification on populations of *Drosophila guarani* species group from Araucaria Forest in Brazil.**

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## Abstract

The allele amplification and diversity of 18 microsatellite loci described for *Drosophila mediopunctata* (*tripunctata* group) was studied in two species of the *Drosophila guarani* group, *D. ornatifrons* (*guarani* subgroup) and *D. maculifrons* (*guaramunu* subgroup), collected in fragments of Araucaria Forest from southern Brazil. The selected loci were already tested in lineages of both species from *D. guarani* group; however, they were not analyzed regarding allele diversity in natural populations. In spite of the 18 tested loci have been selected among more than one hundred loci, because they displayed good amplification pattern for both species in a previous work. Only 50% of them amplified in only one of the two *D. maculifrons* populations analyzed, and approximately 28% presented amplification on both *D. ornatifrons* populations. These results are in agreement with the phylogenetic relationships that allocate the *guaramunu* subgroup closer to *tripunctata* group instead to the *guarani* subgroup. The higher allele diversity of the positive amplified loci was detected in *D. maculifrons* (≈ 9 alleles per locus). The allele number detected in *D. ornatifrons* was also high (≈ 7 alleles per locus). These results suggest that the loci that displayed positive and quality amplification are useful markers to be applied in population genetic studies of these species. The low rate of amplification in the populations from Araucaria Forest sampled here suggests they have an accentuated genomic differentiation to the previous *D. maculifrons* and *D. ornatifrons* populations analyzed.

## Introduction

The polythene chromosome band pattern of the species that compose the *guarani* and *guaramunu* subgroups of the *guarani* group suggests that these subgroups could be changed to the group category as the chromosomes from the *guaramunu* subgroup species are more similar to the species from the *tripunctata* group than to the *guarani* subgroup (Kastritsis, 1969; Kastritsis *et al*., 1970). Hatadani *et al*. (2009), analysing the molecular phylogeny of the *D. tripunctata* and other groups, also agreed with the split of the *guarani* group in two. However, the phylogeny inferred

using molecular and morphological analyses performed by Robe *et al*. (2002) did not support such division.

More than a hundred microsatellite loci were described and had their allelic diversity checked for *Drosophila mediopunctata* (*tripunctata* group) (Laborda *et al*., 2009a). A cross-species amplification was tested in other species, including the *guarani* group species, *D. ornatifrons* and *D. maculifrons* (Laborda *et al*., 2009b). Some of these microsatellite loci were used to build a molecular linkage map that possibly revealed the region that controls the number of abdominal spots variation in *D. mediopunctata* (Laborda *et al*., 2012).

However, so far, the loci described by Laborda *et al.* (2009a) were not applied in natural population studies regarding allele diversity of species that belong to the *guarani* group, more specifically in *Drosophila ornatifrons* (*guarani* subgroup) and *D. maculifrons* (*guaramunu* subgroup). Thus, the goal of this work was to test the amplification rate and the allele diversity of some microsatellite loci described for *D. mediopunctata* in natural population samples of *D. ornatifrons* and *D. maculifrons* collected from fragments of Araucaria Forest located in southern Brazil.

## Material and Methods

The specimens analyzed were collected from three geographically isolated fragments of Araucaria Forest in southern Brazil: Parque Municipal São Francisco da Esperança – SSF (25° 03'S, 51° 16' W) and Parque Municipal das Araucárias – PMA (25° 23' S, 51° 27' W), both located in Guarapuava/PR; and Camping Recanto do Lazer, located in Canguçu/RS - CAN (31º 30' S, 52º 49' W). A total of 34 specimens of *Drosophila ornatifrons* were used in this study, 17 from SSF and 17 from CAN. For *D. maculifrons*, a total of 73 individuals were analyzed, 41 from SSF and 32 from PMA. The collections in SSF and PMA were performed according to Santos *et al*. (2010), with modifications, in September 2011 using open traps with banana, orange, and yeast baits (Sene *et al*., 1981). The individuals from CAN were provided by Dr. Lizandra J. Robe of Universidade Federal do Rio Grande (Rio Grande/RS).

From all microsatellite loci described by Laborda *et al*. (2009a) for *Drosophila mediopunctata* that also presented good quality amplification in the expected length for *D. ornatifrons* and *D. maculifrons* (Laborda *et al*., 2009b), 18 were utilized in this work (Table 1). The PCR were performed in a touchdown condition according to Laborda *et al*. (2009a), with the exception for $Dmed^{UNICAMP}$_ssr087 locus, which amplification was realized as follows: 1 cycle of 94ºC for 2 minutes, 30 cycles of 94ºC for 1 minute, 60ºC for 1 minute, and 72ºC for 2 minutes, ending at 72ºC for 5 minutes. The PCR products were separated in 6% PAGE, stained with silver nitrate (Sanguinetti *et al*., 1994; Machado *et al*., 2003).

## Results and Discussion

For 18 loci tested, selected among more than one hundred described because they showed good amplification conditions in the expected length for both *D. guarani* species (Laborda *et al*., 2009b), *D. maculifrons* showed only 50% of them amplified in SSF sample, approximately 44% amplified in PMA sample, and for *D. ornatifrons* approximately 28% presented amplification on both populations analyzed, with only three individuals from 17 tested showing amplification for the $Dmed^{UNICAMP}$_ssr034 locus (Table 1). The low rate of cross-amplification seems to be more remarkable if it is considered that among the 18 loci tested, 12 were on the list of the 15 loci

classified by Laborda *et al*. (2009b) as the microsatellites that displayed the best features for analysis disregarding the number of species that could be amplified.

Table 1. Allele diversity, amplification quality and rate of *Drosophila mediopunctata* microsatellite loci in two species of *D. guarani* group collected in fragments of Araucaria Forest from southern Brazil. SSF = Parque Municipal São Francisco da Esperança (Guarapuava/PR); PMA = Parque Municipal das Araucárias (Guarapuava/PR); CAN = Canguçu/RS; AQ = Amplification Quality; AN = Allele Number; n = number of analyzed individuals; AR = Amplification Rate. + = positive and good quality amplification; +/- = positive amplification in few samples; - = absence of amplification.

| | *Drosophila ornatifrons* | | | *Drosophila maculifrons* | | |
|---|---|---|---|---|---|---|
| | SSF n = 17 | CAN n = 17 | AN | SSF n = 41 | PMA n = 32 | AN |
| Loci | AQ | AQ | | AQ | AQ | |
| Dmed$^{UNICAMP}$_ssr034 | + | +/- | 06 | + | + | 12 |
| Dmed$^{UNICAMP}$_ssr039 | - | - | - | - | - | - |
| Dmed$^{UNICAMP}$_ssr041 | - | - | - | - | - | - |
| Dmed$^{UNICAMP}$_ssr053 | - | - | - | + | + | 09 |
| Dmed$^{UNICAMP}$_ssr054 | - | - | - | - | - | - |
| Dmed$^{UNICAMP}$_ssr056 | - | - | - | - | - | - |
| Dmed$^{UNICAMP}$_ssr057 | + | + | 10 | + | + | 10 |
| Dmed$^{UNICAMP}$_ssr065 | - | - | - | - | - | - |
| Dmed$^{UNICAMP}$_ssr079 | - | - | - | - | - | - |
| Dmed$^{UNICAMP}$_ssr087 | + | + | 07 | + | + | 07 |
| Dmed$^{UNICAMP}$_ssr095 | - | - | - | + | + | 11 |
| Dmed$^{UNICAMP}$_ssr096 | + | + | 07 | + | + | 07 |
| Dmed$^{UNICAMP}$_ssr099 | - | - | - | + | + | 08 |
| Dmed$^{UNICAMP}$_ssr102 | - | - | - | + | - | 05 |
| Dmed$^{UNICAMP}$_ssr107 | - | - | - | - | - | - |
| Dmed$^{UNICAMP}$_ssr118 | + | + | 07 | + | + | 14 |
| Dmed$^{UNICAMP}$_ssr126 | - | - | - | - | - | - |
| Dmed$^{UNICAMP}$_ssr133 | - | - | - | - | - | - |
| | AR ≈ 28% | AR ≈ 28% | Mean = 7.4 | AR = 50% | AR ≈ 44% | Mean = 9.2 |

The higher number of amplified loci in *Drosophila maculifrons* is in agreement with the phylogenetic relationships that allocate *guaramunu* subgroup closer to *tripunctata* group instead to *guarani* subgroup (Kastritsis, 1969; Kastritsis *et al*., 1970; Hatadani *et al*. 2009), as *D. maculifrons* (*guaramunu* subgroup) presented higher amplification rate than *D. ornatifrons* (*guarani* subgroup) for these microsatellite loci that were described for *D. mediopunctata*. These results suggest that the genome of *D. mediopunctata* has more similarity with the genome of *D. maculifrons* than with the genome of *D. ornatifrons*, although Laborda *et al*. (2009b) did not find any correlation between phylogenetic proximity and cross-species microsatellite amplification success.

Between the two analyzed species, *Drosophila maculifrons* showed higher allele diversity among the loci that presented positive amplification than *D. ornatifrons*, with mean of 9.2 alleles, against 7.4 for *D. ornatifrons* (Table 1). Nonetheless, both species showed higher allele diversity

when compared to the preliminary data of allele diversity of the same loci in 13 *D. mediopunctata* lineages (Laborda *et al*., 2009a). The mean allele number found by Laborda *et al*. (2009a) in *D. mediopunctata* for the loci amplified in *D. ornatifrons* was approximately 6.4, and for the loci that amplified in *D. maculifrons* was approximately 6.1. This fact, *i.e*., the higher allele diversity of these loci in heterologous amplifications than in the species from they were first described, could be due to the use of laboratory lineages of *D. mediopunctata* against the use of natural populations for the species of the *guarani* group analyzed in this work.

The results obtained suggest that the microsatellite loci that showed positive and good amplification quality are useful markers to be applied in population genetics studies using both species of the *D. guarani* group. The low amplification rate of the analyzed microsatellite loci in population samples from Araucaria Forest fragments suggest an accentuated genomic differentiation of these populations to the *D. maculifrons* and *D. ornatifrons* populations previously analyzed by Laborda *et al*. (2009b).

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**Rhodopsin traffic investigation with the heat shock promoter.**

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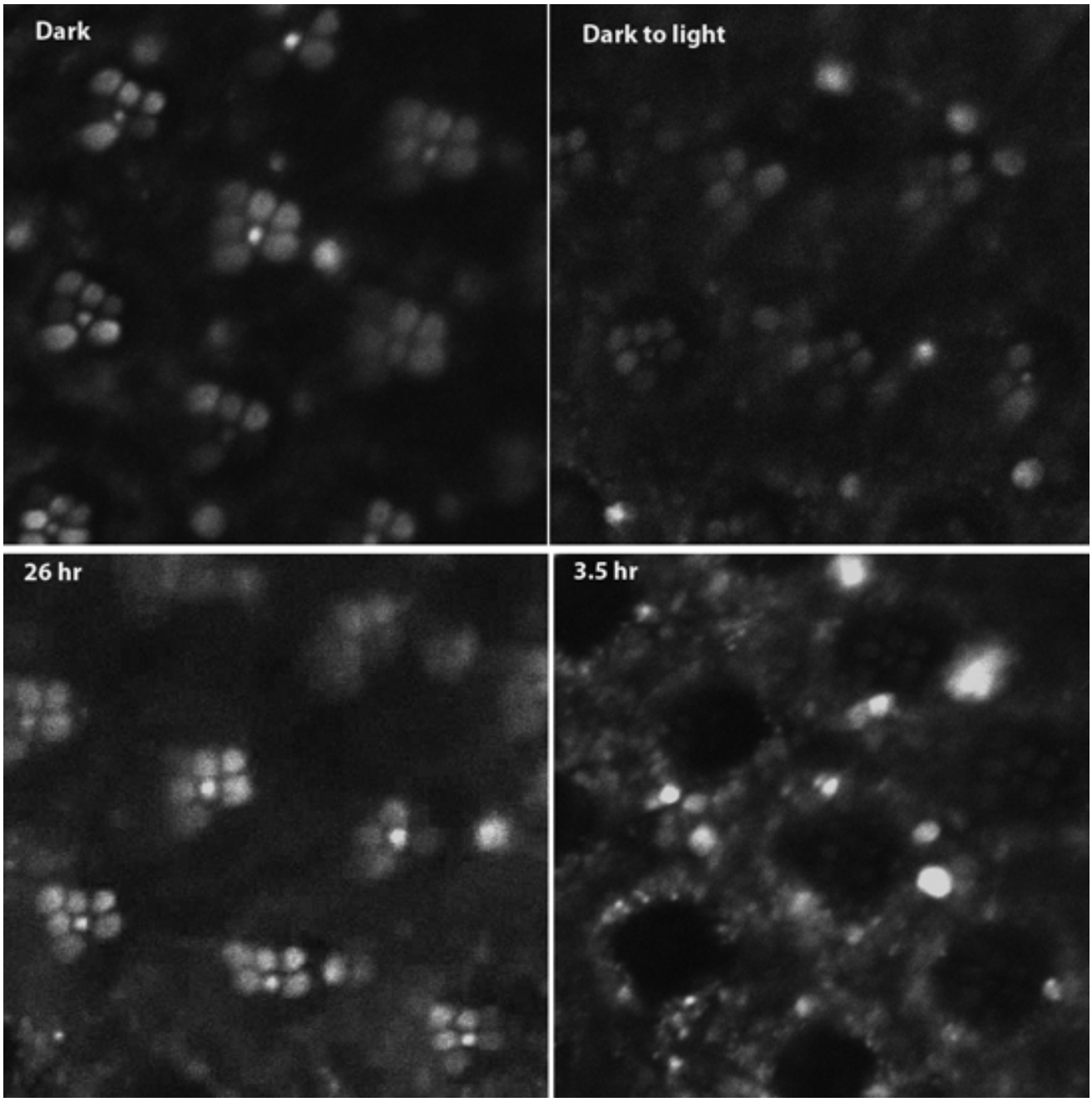
This laboratory has a long-standing interest in rhodopsin turnover in *Drosophila* (Stark, *et al*., 1988); rhodopsin is cleared from the photoreceptive organelle (rhabdomere) via coated pits then multivesicular bodies (MVBs) and lysosomes and imported into rhabdomeres via membranous vesicles. This ultrastructural description has had many molecular elaborations (*e.g*., Chinchore *et al.*, 2009) since our early work. Later, we showed that white-eyed flies maintained in the dark had considerably more rhodopsin than flies kept on a light-dark cycle (Zinkl, *et al.*, 1990; Selimovic, *et al.*, 2010). A white-eyed stock in which R1-6 rhodopsin (Rh1) attached to green fluorescent protein (GFP) was driven by a heat shock (hs) promoter (hs-Rh1-GFP, Belliveau, 2008) allowed us to visualize aspects of rhodopsin traffic using optical neutralization of the cornea in the confocal microscope (Stark and Thomas, 2004).

For heat shock, flies were lightly etherized and placed in a vial in a 37$^{o}$C water bath for 1 hr. Then, based on what we already knew and how our pilot observations guided us, we put them in a food vial in the dark. R1-6 and R7 rhabdomeres show fluorescence (Figure, top left). Since it was the heat shock promoter, not Rh1's promoter (ninaE), R1-6's rhodopsin (Rh1) should be expected to




be driven ectopically into R7 (and ocellar receptors) as well as into R1-6 (Belliveau, 2008). Some of the R1-6 rhabdomeres were dark, and this striking result was repeatable and unexplained. The fly (Figure, top left) had been maintained in the dark for 4 days, showing that, without light, the rhodopsin remains in the rhabdomere, and, in other work, we extended this obsevation out to 12 days. If, however, flies were kept in the dark for 3 days then in the light for 1 day (Figure, top right), the fluorescence was greatly diminished. This is in keeping with our earlier finding (Zinkl, *et al*., 1990) and Chinchore *et al*.'s (2009) work and reinforces the notion that light is necessary to trigger the clearance of rhodopsin from the rhabdomere.

We also sought to investigate import of rhodopsin into the rhabdomere. In pilot work, we showed that there was no fluorescence 2 hr after heat shock while the fluorescence was nearly fully established at 5 hr. Dissecting this time span, we saw dim rhabdomere fluorescence at 3.5 hr (Figure, bottom right). For a control, we show a fly 26 hr after heat shock (Figure, bottom left); as stated above, both were kept in the dark after heat shock. The striking aspect of the 3.5 hr vista is the haze of fluorescent bodies seen in the cytoplasm of the retinula cells. We presume that we are visualizing membranous vehicles (and, perhaps Golgi apparatus) involved in the import of rhodopsin into the rhabdomere.

All four of our figures, and hundreds of other images we have obtained, show large fluorescent bodies that appear to be in pigment cells between ommatidia. We have always assumed, though we have not proven, that these are the giant unpigmented pigment granules of white eyes (Stark and Sapp, 1988). We thought we should not gloss over this point because, again, with techniques more sophisticated than our 1980's ultrastructural work, there has been a vastly renewed interest in eye color pigment granules.

We hope that our observations are of use to the many research groups using modern techniques and the accessibility of rhodopsin and the compound eye in *Drosophila* to study the broader issue of protein traffic.

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## Polytene chromosome analysis in eye color mutants of *Drosophila willistoni* and their hybrids. The H inversion.

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### Introduction

During the course of the linkage analysis of (new) spontaneous mutations in *Drosophila willistoni* from populations of Uruguay, Brazil, and Argentina (Soler and Goñi, 2012, Dros. Inf. Serv., this issue), data on the genetic interaction of some eye color mutants were evaluated, among other genetic data to construct the linkage groups. Within the referent eye color mutations, *brown (bw)*, reported to be linked to chromosome 2 by Spassky and Dobzhansky (1950), produces white eye color in young flies to uniformly blotch brownish eye color in old flies with *cardinal (cd*) (Figure 1 in Soler and Goñi, 2012). The presence of this new eye color in $F_2$ progeny was interpreted as the occurrence of the eye color genetic interaction between the tested mutations and concluded that the

*cd* locus was linked to chromosome 3. When the *brown* mutation produces white eye color with the *cinnabar* (*cn*), as observed in *D. melanogaster* (Lindsley and Zimm, 1992), it was interpreted as resulting from the occurrence of crossing over within the *bw-cn* interval in the previous generation(s). Considering the usefulness of these mutations as genetic markers for genetic mapping studies in *D. willistoni*, we present the chromosome gene arrangement of several eye color mutants and their hybrids.

## Material and Methods

For the description of the eye color mutations and the experimental conditions of the mutant strains and genetic crosses, refer to Soler and Goñi (2012). Mutants were isolated from isofemale lines collected at the Faculty of Agronomy (34º 53´ S; 56º 16´ W), Montevideo City, Uruguay. The mutant strains used here are as follow:

- $bw^{SG23.00}$, $bw^{Sy11.03}$, $bw^{Q51F13}$, 2000, 2003, and 2009, respectively,
- $cd^{SG12.00}$, $cd^{SG4.01}$, 2000, and 2001, respectively, and
- $cn^{SM35.00}$, $cn^{Q51F13}$, 2000, and 2009, respectively.

The polytene chromosome preparation technique of Ashburner (1989, Protocol 18) was applied to obtain well-extended chromosomes of the eye color mutants and their hybrids. For chromosome identification and the description of inversions in *D. willistoni*, two important revisions were consulted. First, the research article of Schaeffer *et al.* (2008) presenting the genetic and physical maps of 11 *Drosophila* species, including the photomap of *D. willistoni.* As used in the previous article, we refer the chromosome (arms) to a single Müller chromosome element (A to F) as Müller (1940) and Sturtevant and Novitski (1941). Second, a recent revision by Rodhe and Valente (2012) presenting an exhaustive description of the arrangements in all five chromosome arms of 30 natural populations. Chromosomes were registered with Zeiss photomicroscope and phase contrast at 1000× magnification. Photomicrographs were edited using Adobe Photoshop 5.0.

## Results and Discussion

Table 1 summarizes data on the chromosome arrangements found in the eye color mutant strains and their hybrids. Only inversions in the left arm of chromosome II (IIL) and in the chromosome III (acrocentric) were detected. The XL, XR, and IIR chromosomal arms are free of inversions in all strains and slides analyzed, presenting the standard order shown in Schaeffer *et al.* (2008) and in Rohde and Valente (2012).

Four segregating inversions in the IIL chromosome (Müller C) and four in the chromosome III (Müller E/F) were detected in the mutant strains analyzed (Table 1; Figures 1 and 2). Unlike previous studies on chromosome polymorphism in Uruguayan populations of *D. Willistoni* that include data on heterozygous inversions (Valente *et al.*, 2001, 2003), here we identify the homozygous arrangement for the IIL H inversion in the $cd^{SG12.00}$ mutant strain. Most relevant is that until now IIL H inversion homozygote was not observed in natural populations (Valente, pers. comm.); its fixation in the $cd^{SG12.00}$ strain may be related by a chance event during the isolation of this mutation. Apparently, individuals that are IIL H inversion homozygotes show good viability under laboratory conditions.

Table 1. Chromosome gene arrangements observed in eye color mutant strains and hybrids of *D. willistoni*.

| Mutations, mutant strains and hybrids | | Chromosome arm | | Total larvae examined |
|---|---|---|---|---|
| | | IIL | III | |
| *brown* | $bw^{SG23.00}$ | F, D+E | J, B | 15 |
| | $bw^{Sy11.03}$ | --- | J | 7 |
| | $bw^{Q51.F13}$ | --- | J, B, C | 10 |
| *cardinal* | $cd^{SG12.00}$ | H* | J, B, A | 19 |
| | $cd^{SG4.01}$ | H, D+E | B | 6 |
| *cinnabar* | $cn^{SM35.00}$ | F, D+E | B | 6 |
| Double mutant | $bw^{Q51.F13}$ $cn^{Q51.F13}$ | --- | J, B | 5 |
| $F_1$ hybrids | $bw^{SG23.00}/+$; $cd^{SG12.00}/+$ | H, F, D+E | J, B, A | 6 |
| | $bw^{Q51.F13}/+$; $cd^{SG12.00}/+$ | H, D+E | J, B, C, A | 2 |
| | $bw^{Q51.F13}/+$; $cd^{SG4.01}/+$ | H, D+E | J, B,C | 16 |

*****Homozygous for IIL H inversion. In all mutant strains and hybrids, the IIR arm has the standard gene arrangement. The double mutant strain, $bw^{Q51.F13}$ $cn^{Q51.F13}$, was isolated from the recombinant progeny between the segregating mutations of the same isofemale line.

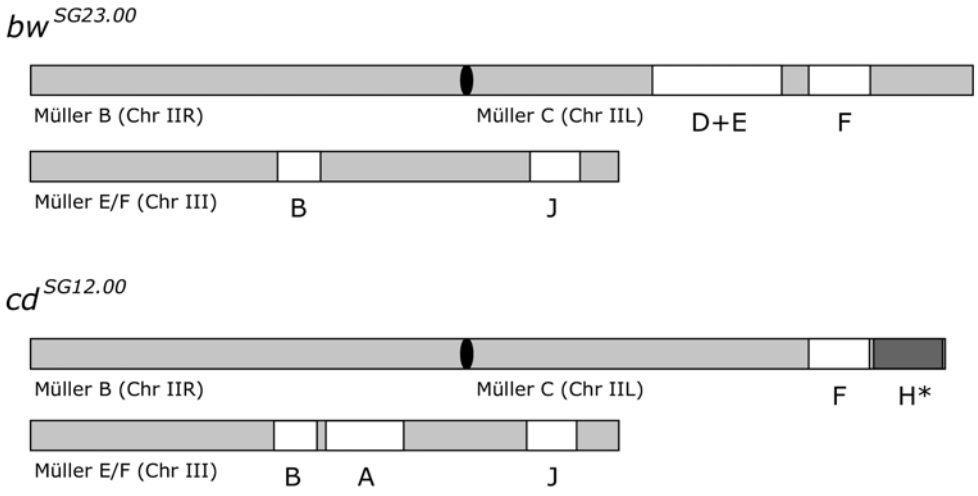


Figure 1. Genetic and chromosomal organization of the $bw^{SG23.00}$ and $cd^{SG12.00}$ mutant strains of *D. willistoni.*

The chromosome inversions observed in the mutants and their hybrids reveal that the *brown* and the *cinnabar* loci are unlinked to the IIL segregating inversions (F, D+E). Similarly, the *cardinal* locus is unlinked to any to the chromosome III segregating inversions (J, B, A). Spassky and Dobzhansky (1950) reported the eye color mutations *brown, orange, pink-wing,* and *purple* linked to chromosome 2, and the *claret* and *karmoisin* mutations linked to chromosome 3. These authors

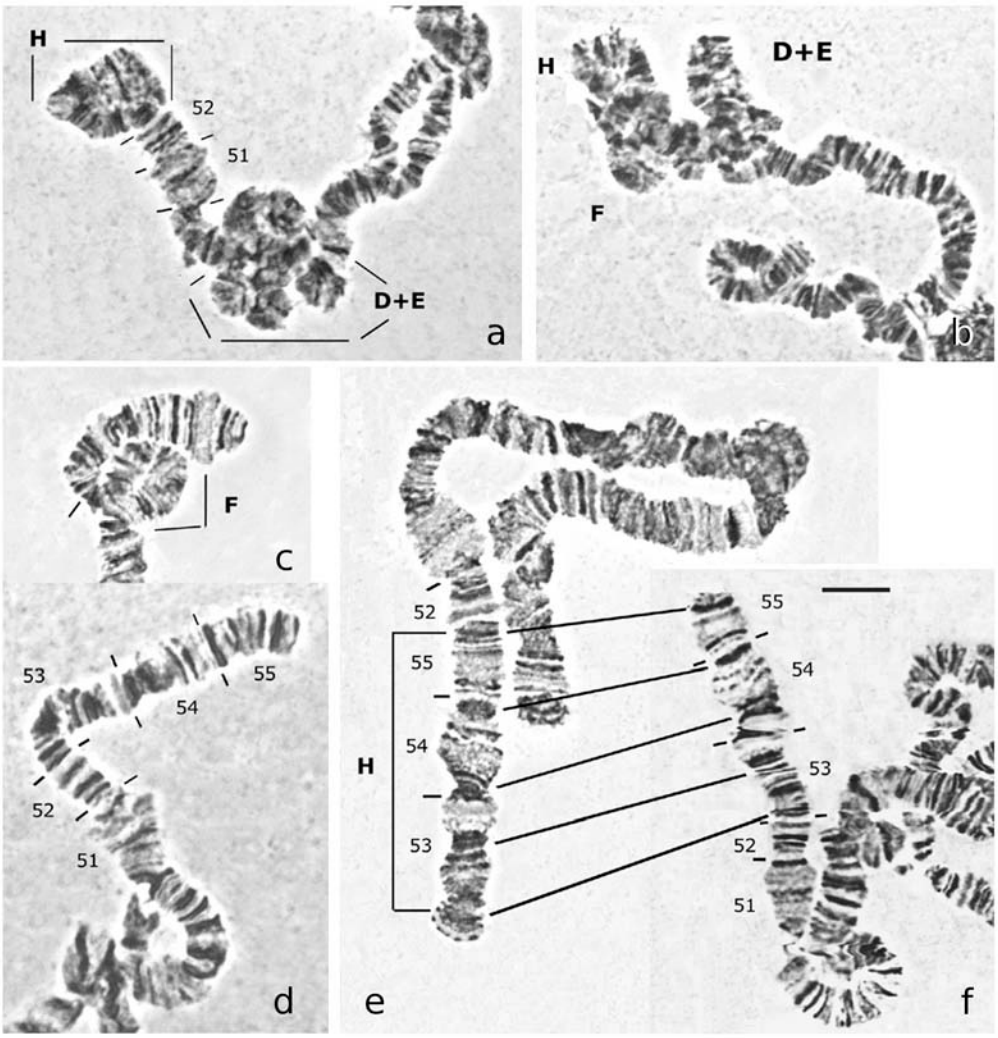


Figure 2. Chromosome gene arrangements at the IIL chromosome arm (Müller C) of *D. willistoni* described in this article. Complex gene arrangements in hybrids between the *bw* $^{SG23.00}$ and *cd* $^{SG12.00}$ eye color mutant strains (a, b). The tip of the chromosome IIL showing homozygous chromosome for the H inversion (e) in the *cd* $^{SG12.00}$ strain, and the standard chromosome gene arrangement (d, f) in the *bw* $^{SG23.00}$ strain. Characteristic IIL F heterozygous inversion in (c). IIL H: 52C – 55B; IIL F: 50A- 52C, and D+E: 42A-48B, as the cytogenetic map reference in Schaeffer *et al.* (2008) and in Rohde and Valente (2012).

mapped the *brown* locus on the distal region of the chromosome IIL, at 28 cM from *plexus*, and 70 cM from the *pink wing* locus. Mapping studies using new genetic and physical markers in *D. willistoni* will contribute to a better comprehension the genome of this species.

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## Effects of *α-synuclein* expression in the developing *Drosophila* eye.

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### Introduction

As the fly brain has over 300,000 neurons and is organized into specialized areas for learning, olfaction, vision and memory (Wolf and Herbelein, 2003; Cauchi and Heuvel, 2006; Hardaway, 2010), *Drosophila* has become an important organism in which to model human neurodegenerative disorders. Furthermore, the *Drosophila* eye is tolerant to genetic manipulations and is dispensable for the survival of the fly (Chan and Bonini, 2000; Celotto and Palladino, 2005; Jeibman and Paulus, 2009). The directed expression of *α-synuclein* results in flies that are viable, accumulate aggregated α-synuclein in perinuclear and neuritic filamentous inclusions similar to Lewy bodies and Lewy neurites, age–dependent loss of dorsomedial DA neurons, neuronal degeneration, age-dependent loss of climbing ability, retinal degeneration (Feany and Bender, 2000; Auluck *et al.*, 2002), and ommatidial degeneration (Todd and Staveley, 2008). Using the bipartite UAS/GAL4 system (Brand and Perrimon, 1993) to overexpress *α-synuclein* in eyes of *Drosophila melanogaster* and performed biometric analysis, we investigated the possibility that developmental phenotypes become more severe with increased expression of α-synuclein.

### Materials and Methods

Drosophila *stock and culture*

Dr. M. Feany of Harvard Medical School generously provided *UAS-α-synuclein* flies (Feany and Bender, 2000). The *GMR-GAL4*$^{12}$ (Freeman, 1996) and *UAS-lacZ* were obtained from the Bloomington Drosophila Stock Center at Indiana University. The *GMR-GAL4 UAS-α-synuclein/CyO* line was generated using standard recombination, tested via PCR, and used to overexpress *α-synuclein* in the developing eye in the Glass Multiple Reporter (*GMR*) pattern. Stocks and crosses were maintained on standard medium containing cornmeal, molasses, yeast, and agar. Stocks were

kept at room temperature (22 ± 2°C) while crosses and experiments were carried out at 29°C.

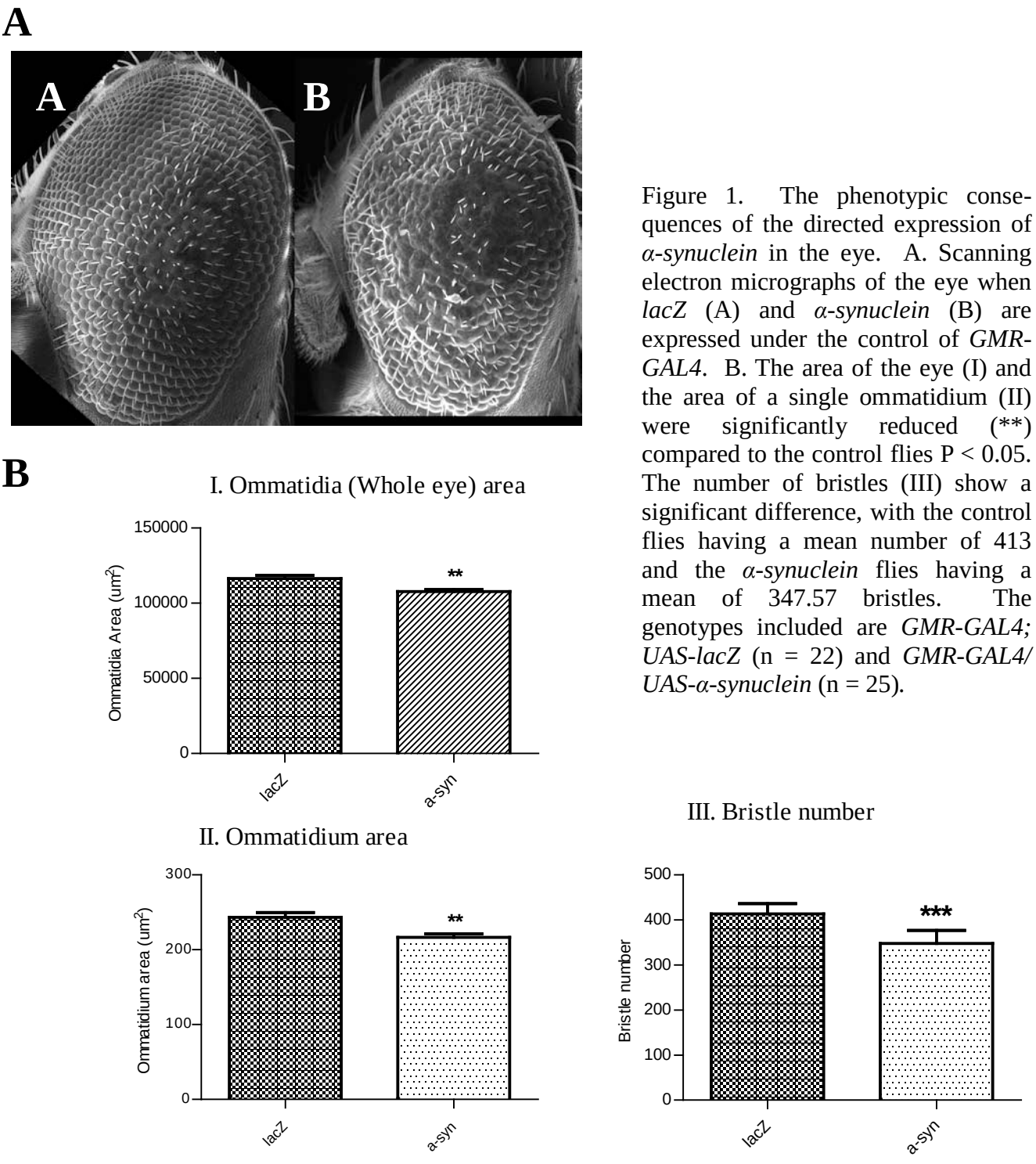


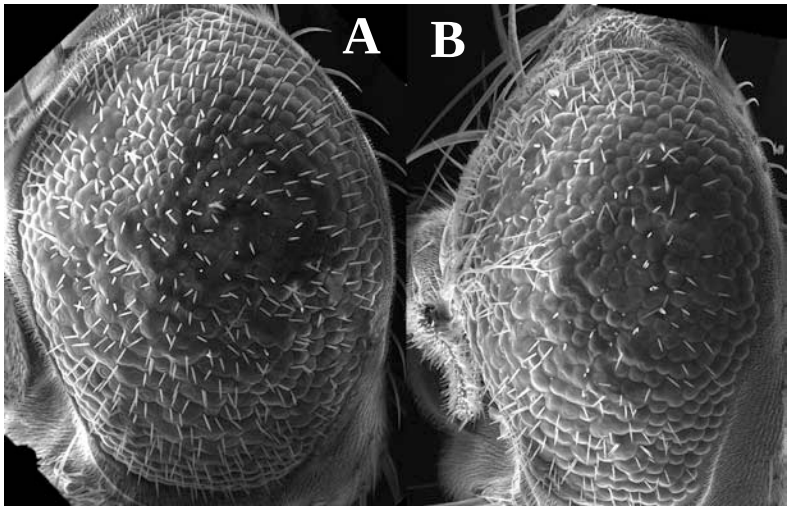

Figure 1. The phenotypic consequences of the directed expression of *α-synuclein* in the eye. A. Scanning electron micrographs of the eye when *lacZ* (A) and *α-synuclein* (B) are expressed under the control of *GMR-GAL4*. B. The area of the eye (I) and the area of a single ommatidium (II) were significantly reduced (**) compared to the control flies $P < 0.05$. The number of bristles (III) show a significant difference, with the control flies having a mean number of 413 and the *α-synuclein* flies having a mean of 347.57 bristles. The genotypes included are *GMR-GAL4; UAS-lacZ* (n = 22) and *GMR-GAL4/ UAS-α-synuclein* (n = 25).

*Biometric analysis of the* Drosophila *eye*

Several single vial matings of three to five females plus three to five males were made of each genotype at 29° C and a cohort of adult heterozygous male flies collected upon eclosion and aged for three days on standard cornmeal-yeast-molasses-agar before being frozen at -80° C. Whole flies were

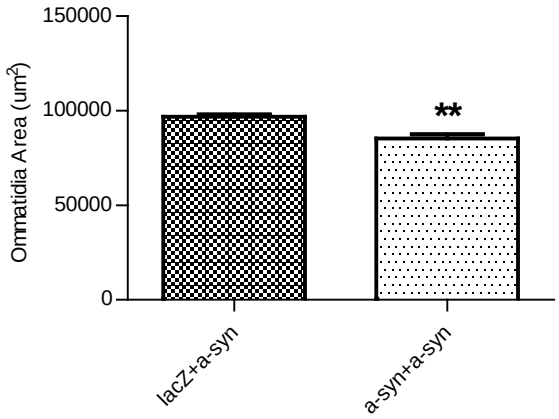
mounted on SEM studs, desiccated overnight and coated in gold prior to photography at 170× magnification with a Hitachi S-570 scanning electron microscope was done. For each cross at least 20 eye images were analysed using the NIH ImageJ software (Abramoff *et al.*, 2004) and biometric analysis performed. The ratio of the area of disruption was calculated from the total area of the eye divided by the total disrupted area. Disrupted area was considered as an area occupying two to three fused ommatidia.

**A**

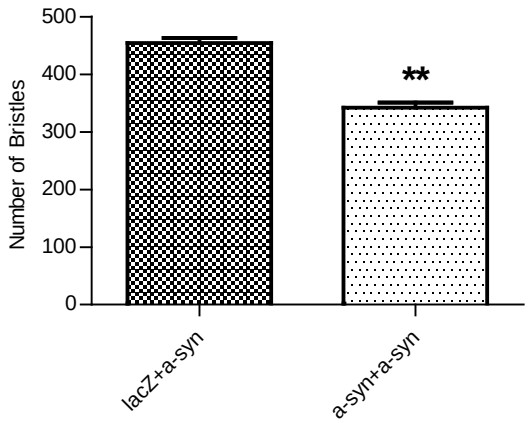



**B**

I. Ommatidia (Whole eye) area




II. Bristle number




III. Ratio of disrupted area of eye

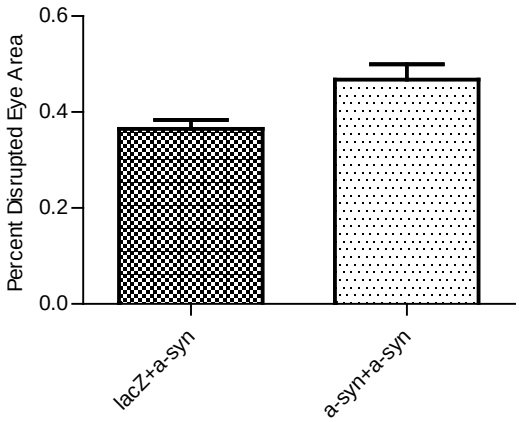



Figure 2. The consequences of the directed expression of an additional copy of *α-synuclein* in the eye. A. Scanning electron micrographs of both the control flies *lacZ+a-syn* (A), overexpressing a single copy of *α-synuclein* plus a copy of *lacZ* (as a control) and *α-synuclein* flies (B), overexpressing two copies of *α-synuclein (a-syn+a-syn).* Panel B; Biometric analysis of the eyes showing significance (*) for the whole area of the eye (I), the bristle number (II), and the ratio of disrupted eye area (III) when compared to the control flies ($P < 0.05$). The genotypes were *GMR-GAL4 UAS-α-synuclein/UAS-lacZ* (n = 24) and *GMR-GAL4 UAS-α-synuclein/UAS-α-synuclein* (n = 23).

**Results and Discussion**

Eye development in *Drosophila* is very precise with the maturation of each ommatidium and the organization of the ommatidial array being tightly controlled (Thomas and Wassarman, 1999). We expressed *α-synuclein* in the eyes using the eye specific driver *GMR-GAL4* crossed to the responding transgene, and secondly we expressed higher levels of *α-synuclein* in the eye using the *GMR-GAL4 UAS-α-synuclein* recombinant line. Analysis of SEMs of flies overexpressing a single copy of *α-synuclein* (n = 25) compared to the control flies (n = 22) overexpressing *lacZ* revealed differences in eye development, but notable was a slight decrease in the overall area of the eye of *α-synuclein* flies. The area of the eye (107802 ± 1311; 116459 ± 2153) (Figure 1-BI) and the area of a single ommatidium (216.6 ± 4.826; 243.2 ± 6.332) (Figure 1-BII) were slightly reduced for *α-synuclein* flies when compared to the control flies $P < 0.05$. The number of interommatidial bristles were significantly reduced (Figure 1-BIII), with the control flies having a mean number of 413 ± 22.92 and the *α-synuclein* flies having a mean of 347.57 ± 28.99 bristles.

We compared the SEMs of flies that developed while expressing one copy of *α-synuclein* plus one copy of *lacZ* (n = 24) to those of flies that were expressing two copies of *α-synuclein* (n = 23). We found that elevated levels of *α-synuclein* slightly altered overall eye development. The whole eye area (85346.4 ± 2250) (Figure 2-BI), bristle number (341.7 ± 9.276) (Figure 2-BII), and the ratio of disrupted area (0.4673 ± 0.0322) (Figure 2-BIII) for *α-synuclein* were significantly different from that of the control flies with whole eye area (96791 ± 1288), bristle number (454.4 ± 8.871), and ratio of disrupted area (0.3152 ± 0.0187). This suggests that elevated expression of *α-synuclein* alters the development of the eye.

In the pathology of Parkinson disease, the accumulation of *α-synuclein* is implicated with the progression of PD, and the intra-cytoplasmic inclusions or Lewy bodies have been shown to contain aggregates of *α-synuclein*, ubiquitin, and other proteins (Forno, 1996; Polymeropoulos *et al*, 1997; Leroy *et al*, 1998). The accumulation of these proteins is believed to result in cellular toxicity and pathogenesis. The *Drosophila α-synuclein*-induced models display retinal degeneration and other disease-like symptoms (Feany and Bender, 2000). We further investigated the overexpression of *α-synuclein* in eye development. The directed expression of *α-synuclein* in the eye of flies with *GMR-GAL4* revealed significant differences in the morphology of the eye when compared to the *lacZ*-expressing flies. The area of the whole eye and ommatidium was slightly decreased in *α-synuclein* flies, and the interommatidial bristle number was reduced. This may suggest that expressing *α-synuclein* in the eye of flies alters neurogenesis and might be attributed to the loss or death of the neurons due to *α-synuclein*-induced toxicity. Expression of *α-synuclein* in flies that were overexpressing a second copy of *α-synuclein* in the *GMR-GAL4* pattern slightly affected the development of the eye and in particular, 1) the overall area of the eye was reduced, 2) the interommatidial bristles were reduced in number, and 3) the ratio of disrupted area of the eye was slightly greater when compared to the control flies overexpressing *lacZ* and a single copy of *α-synuclein.* It is possible that the elevated levels of *α-synuclein* result in greater biological protein toxicity that causes the system for clearing malformed proteins to be stressed and lead to more neuronal cell death.

Recent studies have suggested that *α-synuclein* toxicity results in chaperone-mediated autophagy and lysosomal dysfunction by interfering with its ability to degrade *α-synuclein* and other products and seems to lead to the up-regulation of autophagy (Auluck *et al.,* 2002; Martinez-Vicente *et al.,* 2008; Winslow *et al.,* 2010; Xilouri and Stefanis, 2010). Indeed, neuronal death has been attributed to mitochondrial damage resulting from stress-induced by *α-synuclein* and causing an age-dependent decrease in substrate specific respiration along with an increase in mitophagy (Chinta *et al.,* 2010). It, therefore, seems that accumulation of *α-synuclein* promotes mitochondrial depletion

and neuronal death.

Acknowledgments: This research was funded by Memorial University of Newfoundland School of Graduate Studies Fellowship to PGM and by a National Sciences and Engineering Council of Canada Discovery Grant to BES. Thanks to Dr. Liqiu Men for her technical assistance.

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**Exposure to heat stress modulates DNA methyltransferase activity in the embryonic S2 cell line of *Drosophila melanogaster*.**

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## Summary

*D. melanogaster* is a dipteran model system for many diverse phenomena including animal development. The first report on the presence of 5-methylcytosine in the genomic DNA was by Deobagkar nee' Achwal (Achwal *et al*, 1984), where by use of sensitive and specific immunochemical staining and photoacoustic spectroscopy, the amount of 5mC was shown to be of

the order of 0.008mol%. Along with 5mC other methylated nucleotides like 6-methylAdenine and 7-methylGuanine were also shown to be present in genomic DNA of *D. melanogaster* using immunochemical methods (Achwal *et al*, 1983). Epigenetic changes in eukaryotic biology regulate diverse processes predominantly due to the cross-talk between DNA methylation and histone modifications. An appropriate environmental stimulus can reprogram the development of an organism (Felsenfeld, 2007; Allis *et al.*, 2007). The amount of methylation varies with different stages of life cycle in all organisms (Hendrich *et al*., 2003). It is known that there is differential expression of genes in response to external stimulus, and the stimuli for this change could be temperature, medium condition, or presence of oxidative stress (Gonsalves *et al*., 2011). *Drosophila* has been shown to possess a single known methyltransferase, dDNMT2 (Schaefer *et al*., 2008), which belongs to the enigmatic DNMT2 family. The DNMT2 family of proteins is found to be conserved, but their functions are still elusive. Molecular and biochemical experiments show that Dnmt2 can localize in the cytoplasm as well as nucleus (Lyko *et al*., 2000) and is supposed to help the fly against transposable elements and retroviral infections (Schaefer *et al*., 2010).

Prior studies indicate change in the expression of the genes in response to change in temperature (Sorensen *et al*., 2005; Kristensen *et al*., 2003). The cellular heat stress response is well studied in *Drosophila* with respect to the role of heat shock proteins (HSP), molecular chaperones that are highly expressed during and after exposure to numerous stress types. All HSPs appear to be regulated by a common transcription factor, the heat shock factor (HSF), which may also regulate uncharacterized heat-responsive genes (Jensen *et al*., 2008) besides Hsp genes. It has been suggested that HSPs also play a direct role in the extended longevity and stress resistance of flies exposed to non-lethal stress at a young age (Tatar *et al.*, 1997).

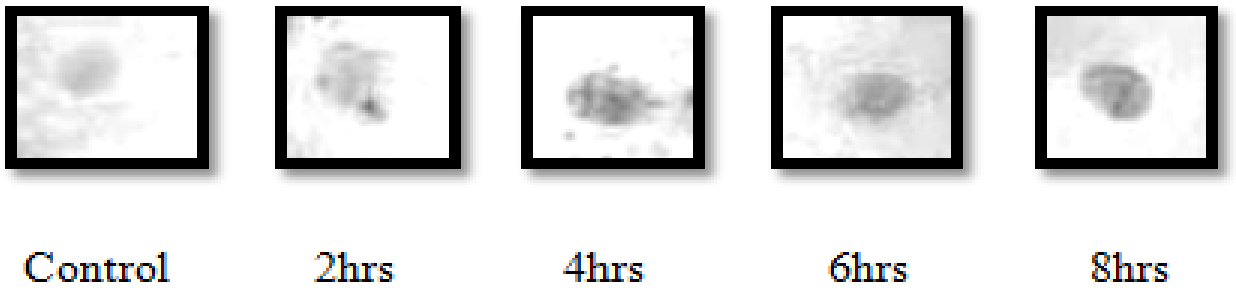


Figure 1. Immunochemical staining of unmethylated DNA substrate with 5 methylcytosine antibodies after incubation with protein extract from S2 cells under stress conditions.

**Results**

In this work we have studied the effect of heat stress on the 5 methyltransferase activity at the various time points in S2 embryonic cell line of *Drosophila melanogaster*. The S2 cell line was derived from a primary culture of late stage (20-24 hours old) *Drosophila melanogaster* embryos. This versatile cell line grows rapidly at temperature of 22ºC without $CO_2$ and is easily adapted to suspension culture. S2 cells can be grown in both serum-containing (Schneider's *Drosophila* Medium) or serum-free medium (*Drosophila* SFM). These are used represent a studies on early embryos. We have exposed the S2 cell line to a higher temperature for different time intervals and assayed for DNA methyltransferase activity. The S2 cell line grown in Schneider's medium was exposed to 37ºC for 0 hrs (control), 2 hrs, 4 hrs, 6 hrs, and 8 hrs, respectively. Cellular protein was

extracted and used for *in vitro* methyltransferase assay followed by immunochemical staining using 5-methyl cytosine antibodies (Paniker *et al.*, 2008). The concentration of the unmethylated substrate and cell protein used for the assay was 4 µg and 400 µg, respectively. The technique used to detect the activity of DNA methyltransferase and its effect on DNA methylation has been published by our lab (Deobagkar *et al*., 2012).

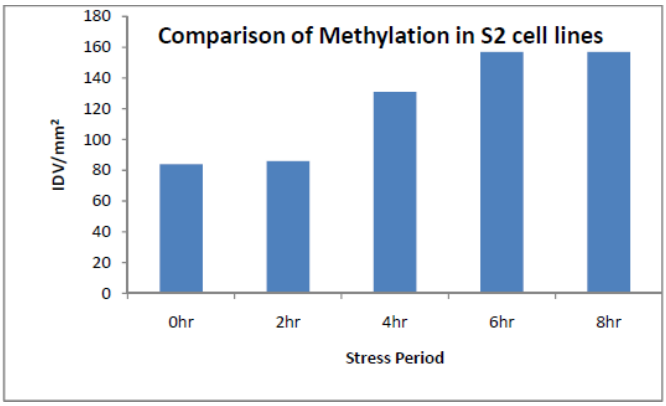


Figure 2. Graphical representation of DNA Methylation Assay for S2 Cell Lines of *Drosophila melanogaster* after exposure to higher temperatures (Deobagkar *et al*., 2012).

This result clearly documents an increase in cytosine methylation upon exposure to temperature stress. Although the exact role of DNA methylation in *Drosophila* is unclear, if involved in chromatin remodeling, physiological changes could result from epigenetic modifications due to change in the activity of methyltransferase. Different genotypes in the fly may respond differentially to the same environmental stress. The variation in these norms of reaction may be due to the genetic variation in their metabolic traits. The role of individual nutrients in stress resistance and longevity are largely unknown. Despite these variable parameters it can be affirmed that a progressive change in the activity of methyltransferase is observed under stress conditions in the embryonic stages of *Drosophila melanogaster*. The activity goes on increasing in case of S2 cell line as the time of exposure to stress is increased. The enigmatic role of DNA methylation in *Drosophila* physiology provides a model to integrate and analyze molecular and genetic mechanisms which are governed by methylation changes.

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**Confocal microscopy of light-induced holes deep in compound eyes of white-eyed *Drosophila*.**

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For two decades, this laboratory has utilized confocal microscopy and optical ("pseudopupil") techniques (Stark and Thomas, 2004) to investigate rhodopsin levels and retinal degeneration in the *Drosophila* compound eye (Zinkl, *et al*., 1990). All the time, strikingly clear images of tips of the rhodopsin-containing organelles (rhabdomeres) were seen in a narrow band delimited by the small "optical section", which is the hallmark of confocal microscopy, especially when green fluorescent protein (GFP) labeled the rhodopsin (Shah, *et al*., 2011).

With newer techniques (Selimovic *et al.,* 2010), we have appreciated our rediscovery of Zinkl *et al.*'s findings that (1) white-eyed flies reared on a day-night cycle of room lighting have less rhodopsin than those maintained in the dark, and (2) if kept in constant room light, they have receptor degeneration.

Never, until recently, did we think that there was useful information in confocal images at a deeper plane of section than that where the rhabdomere tips were seen. Here we show that "holes" or "islands" of ommatidial irregularity show up deeper in the retina as light-induced degeneration progresses. The accompanying Figure (top four images) presents confocal micrographs; most of the fluorescence is from the cornea, seen around the perimeter, and the unpigmented pigment granules of these white-eyed flies, revealing the ommatidial array. Notice the regularity in the ommatidial array in flies that had been maintained in the dark for two weeks; the dim autofluorescence of rhodopsin (the R1-6 rhabdomeres) can even be seen. Structure is still intact for flies that had been moved from dark to light for 1 day; as expected, rhabdomere autofluorescence is diminished since there is less rhodopsin. However, by 6 and 13 days in constant light, holes become apparent. These are, of course, selected images representative of a substantial body of data. We thought, while making these observations, that there might be more "misbehaviors" in the interfacetal bristles which should be at every other corner of each hexagon. However, scanning electron microscopy failed to reveal any difference between light- *vs* dark-reared flies.

The conclusions relevant to our research focus are that visual pigment decreases, then structural disintegration, can be seen non-invasively in the eyes of living white-eyed flies. In addition, other researchers may want to take advantage of the knowledge that they can see deeper than the surface into compound eyes using confocal microscopy.

Acknowledgments: Funding was from SLU's Beaumont and Presidential funds. This study was a follow-up from a serendipitous observation by George Denny (now in Washington University Medical School) when he used to work in this laboratory. We thank Prof. Jan Ryerse of Saint Louis University's Research Microscopy Core for help with the scanning electron microscopy.

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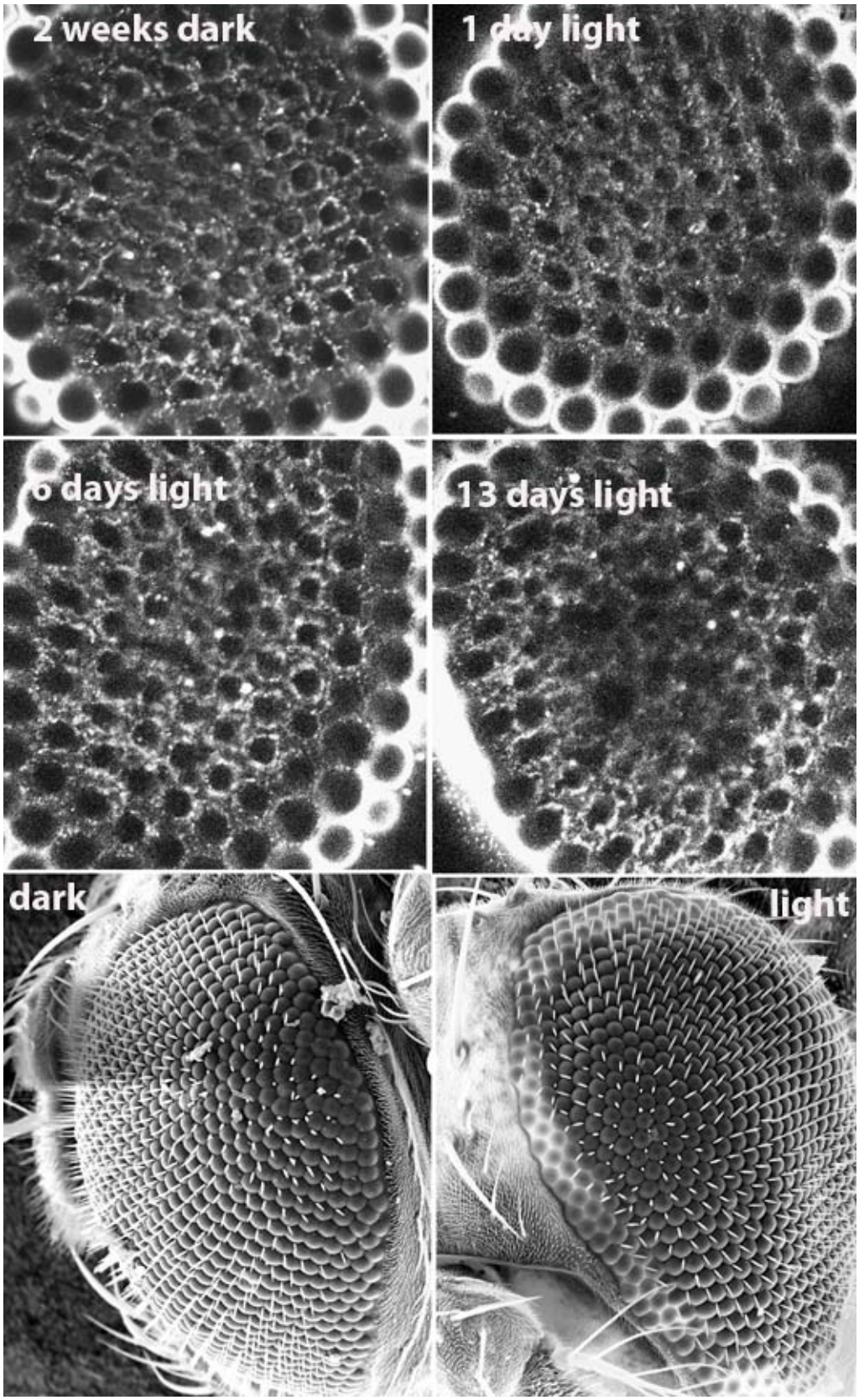
Figure 1. See next page.


2 weeks dark
1 day light
o days light
13 days light
dark
light

**First records of *Zaprionus tuberculatus* (Diptera: Drosophilidae) from the Mediterranean Region, Turkey.**

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## Introduction

The drosophilid genus *Zaprionus* Coquillett, 1902 is classified under two subgenera, and a total of 59 species are recognized with respect to recent phylogenetic findings using molecular and morphological characters (Yassin and David, 2010). The genus *Zaprionus* is widespread through the African continent (Tsacas *et al*., 1981), and it exhibits that the most common species of the genus are *Zaprionus indianus* Gupta, 1970 and *Zaprionus tuberculatus* Malloch, 1932 with their expanded distribution to the Afrotropical region and Palearctic (Chassagnard and Tsacas, 1993).

*Zaprionus tuberculatus* is assigned to the subgenus *Zaprionus*, species group *inermis* and species subgroup *tuberculatus* (Yassin, 2008). The species *Z. tuberculatus* commonly known as "Vinegar fly or Pomace fly," is an Afrotropical drosophilid native to the Afrotropical region and the islands of the Indian Ocean (Chassagnard and Tsacas, 1993). It has acquired invasive capacities after the geographical expansion to the southern boundaries of Europe. Even though it is the second most widespread species especially compared with the agricultural pest *Z. indianus*, very little is known about its biology and ecology. The present study intends to report the first record of *Zaprionus tuberculatus* from the city of Adana (37.0000° N, 35.3167° E) located on the southern coast of Turkey. Based on our observations and due to the some possible similarities between the range expansions of *Z. tuberculatus* and *Z. indianus*, it is supposed that *Z. tuberculatus* could be a potential agricultural pest for the fig cultures around the area in recent times.

## Materials and Methods

The sampling was done in August 2011, from more coastal parts of Adana to inner parts of the adjacent region. The locations chosen to be sampled were different in terms of altitude, and some of them are agricultural lands that surround the urban area (Geographical locations are listed in Table 1). We set quite a number of traps which contained fermented banana and peach baits with a distance of at least a few meters between each of them, to collect wild-living adults. The flies were picked from the traps in each region at approximately the same time of the day. The collected individuals were transported to the laboratory with each of them in a separate vial to allow females to lay eggs to produce progenies. Species identification was performed using the first generation progeny of wild-caught females with respect to the identification key by Yassin and David (2010).

**a.** **b.**

Figure 1. Forefemur with (a) a tubercule bearing a long bristle and (b) a tuft of heavy hairs on basitarsus segment of fore-legs of males in *Zaprionus tuberculatus.*

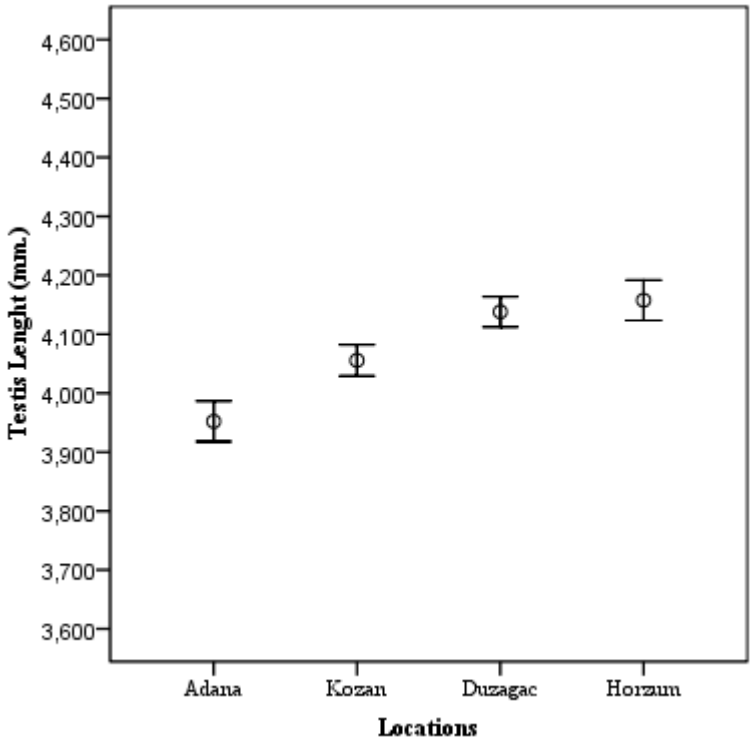


Figure 2. Testis length (mm) variation of F1 progenies in *Zaprionus tuberculatus* across the altitudinal gradient. Data are presented as mean and standart error.

**Results and Discussion**

The first generation progenies of wild-caught females collected from each location were identified step by step. Accordingly, it is shown that all individuals have the fore-femur with a protruding tubercule bearing a bristle (Figure 1a), frons with a median white stripe, thorax is dark brown, mesonotum and metanotum with aligned, white, longitudinal stripes. In males there exists a structure, a tuft of heavy hairs similar to *Drosophila*-sex comb, on basitarsus segment of fore-legs (Figure 1b) and aedeagus subterminally convex in dorsoventral view. Preapical egg filaments either spatulate or simple, testis repeatedly coiled and long, included in the range of 3.2 mm and 4.9 mm based on the results measured from approximately 100 males from the first generation of each location. According to Yassin and David (2010), *Z. tuberculatus* can only be distinguished from its siblings by testicular sizes. We found that

testicular size showed individual variation among brothers from the same isolines, and significant differences were found between population pairs [$F(3,514) = 12{,}182$, $p = 0.000$ ]. Interestingly, it seems that the testis length increases with increasing elevation (Figure 3). But that point needs a detailed study of a much more extensive sampling.

In conclusion, we are confident that these are very recent introductions for the region, as we had been sampling *Drosophila* specimens in the area previously without detecting *Z. tuberculatus* until 2010. The locations chosen were different in terms of altitude, and we could not collect *Z. tuberculatus* above 1000 meters above sea level despite more traps being laid in higher altitudes (Table 1).

Table 1. Number of adult specimens of *Z. tuberculatus* collected from different regions of Adana, Turkey.

| Geographical Locations | Habitat | Altitude | No. of traps | No. of individuals |
|---|---|---|---|---|
| ADANA (37.03°N, 35.82°E) | Urban | 35 m. | 20 | 100 |
| KOZAN (37.45°N, 35.80°E) | Suburban | 150 m. | 10 | 100 |
| DUZAGAC (37.58°N, 35.82°E) | Forested-rural area | 500 m. | 30 | 195 |
| HORZUM (37.62°N, 35.84°E) | Forested-rural area | 700 m. | 20 | 127 |
| TUFANBEYLİ (38.26°N, 36.22°E) | Suburban | 1.430 m. | 50 | - |
| SARIZ (37.81°N, 35.70°E) | Rural area | 1.612 m. | 45 | 3 |
| SAİMBEYLİ (37.98°N, 36.09°E) | Forested-rural area | 1.000 m. | 30 | 3 |

Acknowledgments: We thank Yavuz Turan from Department of Biology,Univesity of Hacettepe, for his helping with the fieldwork and Amir Yassin for courteous support about the specimen identifications.

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**Drosophilid collections at Moleques do Sul archipelago, southern Brazil.**

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Moleques do Sul is an oceanic archipelago near Santa Catarina Island, Santa Catarina state, southern Brazil. It is located 14 km from the coast and made up of three small islands with a total area of 10.5 hectares. The main island, also called Moleques do Sul, has 9.86 ha, from which 6.34 is covered with grass and bush vegetations and the rest by rocky terrain. The vegetation cover of this area has had many taxonomic surveys carried out (Gomes *et al*., 2005; Rogalski and Araújo, 2005).

Table 1. Absolute (ni) and relative (pi) abundance of drosophilids in each collection and in the total sample at Moleques do Sul Archipelago. Sum - Summer; Aut - Autumn; Win - Winter; Spr - Spring.

| Genus | Subgenus | group | subgroup | species | Sum04 | | Aut05 | | Win05 | | Spr05 | | Sample | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *ni* | *pi* | *ni* | *pi* | *ni* | *pi* | *ni* | *pi* | *ni* | *pi* |
| *Drosophila* | *Dorsilopha* | *buscki* | | *D. buscki* Coquillett, 1901 | | | | | | | 2 | | 2 | |
| | *Drosophila* | *annulimana* | | unidentified | | | | | | | 3 | | 3 | |
| | | *cardini* | *cardini* | *D. cardini* Sturtevant, 1916 | 2 | 0.00 | 1 | | | | | | 3 | |
| | | | | *D. neocardini* Streisinger, 1946 | 6 | | | | | | | | 6 | |
| | | | | *D. polymorpha Dobzhansky & Pavan, 1943* | 166 | 0.31 | 2 | | 5 | 0.05 | 11 | 0.06 | 184 | 0.17 |
| | | *immigrans* | | *D. immigrans* Sturtevant, 1921 | 2 | | | | | | | | 2 | |
| | | *pallidipennis* | | *D. pallidipennis Dobzhansky & Pavan, 1943* | 47 | 0.09 | | | | | 2 | | 49 | 0.05 |
| | | *repleta* | *fasciola* | *D. mapirensis* Vilela & Bächli, 1990 | | | | | | | 2 | | 2 | |
| | | | *repleta* | *D. zottii* Vilela, 1983 | | | | | | | 1 | | 1 | |
| | | | *mercatorum* | *D. mercatorum* Patterson & Wheeler, 1942 | 31 | 0.06 | 8 | | 3 | | 20 | 0.10 | 62 | 0.06 |
| | | | *mulleri* | *D. meridionalis* Wasserman, 1962 | 38 | 0.07 | 18 | 0.08 | 10 | 0.10 | 6 | | 72 | 0.07 |
| | | | | *D. serido* Vilela & Sene, 1977 or *D. antonietae* Tidon-Sklorz & Sene, 2001 | | | 1 | | | | | | 1 | |
| | | | | unidentified | 34 | 0.06 | 8 | | 10 | 0.10 | 24 | 0.12 | 76 | 0.07 |
| | | *tripunctata* | *ii* | *D. cuaso Bächli, Vilela & Ratcov, 2000 and D. paraguayensis Duda, 1927* | | | | | | | 2 | | 2 | |
| | | | | *D. mediopunctata Dobzhansky & Pavan, 1943* | 9 | | | | 1 | | 3 | | 13 | |
| | | | *iii* | *D. mediopicta* Frota-Pessoa, 1954 | 3 | | | | | | | | 3 | |
| | | | | unidentified | 25 | | | | 3 | | 8 | | 36 | |
| | | not agrouped | | *D. caponei Pavan & Cunha, 1947* | 1 | | | | | | | | 1 | |
| | *Sophophora* | *melanogaster* | *ananassae* | *D. ananassae* Doleschall, 1858 | | | 2 | | | | | | 2 | |
| | | | *melanogaster* | *D. simulans* Sturtevant, 1919 | 88 | 0.16 | 163 | 0.74 | 71 | 0.69 | 66 | 0.34 | 388 | 0.37 |
| | | | *montium* | *D. kikkawai* Burla, 1954 | 1 | | 3 | | | | 1 | | 5 | |
| | | *willistoni* | *bocainensis* | *D. capricorni Dobzhansky & Pavan, 1943* | 17 | | 1 | | | | | | 18 | |
| | | | | *D. fumipennis* Duda, 1925 | | | | | | | | | 0 | |
| | | | | *D. nebulosa* Sturtevant, 1916 | | | | | | | | | 0 | |
| | | | | *willistoni* Pavan, 1952 | 68 | 0.13 | 11 | 0.05 | | | 46 | 0.23 | 125 | 0.12 |
| | | *dreyfusi* | unidentified | unidentified | | | 1 | | | | | | 1 | |
| | *Zaprionus* | *armatus* | *vittiger* | *Z. indianus* Gupta, 1970 | 3 | | | | | | | | 3 | |
| **TOTAL** | | | | | 541 | 1.00 | 219 | 1.00 | 103 | 1.00 | 197 | 1.00 | 1060 | 1.00 |

The island of Moleques do Sul mantains the unique population of the small rodent *Cavia intermedia* Cherem (Olímpio and Ximenez 1999), the most endemic mammal known to date. This population has been isolated since the raising of the sea, approximately 7-10.000 years ago (Hesp *et al*., 2007), and it is classified by the IUCN red list as a critically threatened species (Salvador and Fernandez, 2008).

Following a series of collections in small islands around the main island of Santa Catarina, Florianópolis (De Toni *et al*., 2007), in the present study we report the first collections of drosophilids in this archipelago.

Collections were performed using five banana baited traps, made according to Tidon and Sene (1988), in four seasonal collections, from December 2004 to October 2005. The traps were placed in vegetated areas of Moleques do Sul Island (27º52'82"S; 48º25'89"W), in the shade, and left in the field for three days. Flies were brought to the laboratory and identified by external morphology or morphology of male genitalia (following Wheeler and Kambysselis, 1966, modified by Kaneshiro, 1969). Most females were identified just to species group level. Additionally, we swept over and collected flowers from *Verbesina glabrata* Hook. and Arn. (Asteraceae), the most abundant bush of the island (Gomes *et al*., 2005).

The total number of drosophilids collected in traps came to 1060, distributed in 20 species, (Table 1). Overall, *Drosophila simulans*, *D. polymorpha* and *D.*sgr. *willistoni* were the dominant taxa and made up more than 60% of the sample. These are ecologically generalist species and common on open areas (Sene *et al*., 1980; Gottschalk *et al*., 2007; Bizzo *et al*., 2010) as well as in other islands (De Toni *et al*., 2007).

The occurence of cactophilic species is intriguing. *Drosophila meridionalis*, *D. serido* (or *D. antonietae*), *D. mapiriensis*, and *D. zottii* are known to breed only on cacti, and we found a single large specimen of *Opuntia* which could not be inspected for larvae due to its coastal location. Gomes *et al*. (2005) and Rogaski and Araújo (2005) list two cacti species on this island, *Opuntia arechavaletai* Speg. and *Pereskia aculeata* Mill.

The absence of species groups characteristic of forested areas and humid places, such as the *saltans* group and most of the *tripunctata* group (Pavan, 1959; Sene *et al*., 1980), as well as the more ecologically restricted species of the other groups, is evidence that this harsh environment with scarce breeding and feeding resources cannot support complex communities. Also, the geographic isolation of the island is certainly limiting the incoming of flies, but still is not completely sufficient to avoid the introduction of exotic species such as *D. simulans*, *D. ananassae*, *D. kikkawai*, *D. immigrans*, and *Z. indianus*.

Our collection of inflorescences also gathered results. Eight adult individuals of the genus *Cladochaeta* Coquillet 1900 were captured on the inflorescences, and another 18 emerged from the capitules brought to the laboratory. This genus is known to have parasitic larvae, feeding on haemolymph and the spit of the spittlebugs, Cercopidae (Hemiptera: Auchenorrhyncha). There are currently two reports of *Cladochaeta* using flowers as breeding sites. *Cladochaeta floridana* emerged from *Bidenspilosa* (Compositae) in the United States, and larvae of *C. psychotria* were observed feeding on pollen of *Psychotria chiriquiensis* (Rubiaceae), in Costa Rica. Grimaldi and Nguyen (1999) point out that the former result is questionable; larvae might have developed on spits and later found an inflorescence to pupate. We cannot rule out this possibility from our data, but we saw no evidence of spittlebugs.

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## *Drosophila* in honeydew: an opportunistic resource.

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Drosophilids are small flies that usually breed on decaying vegetal material. They commonly feed on yeast and bacteria present during the fermentation process. The most studied genus, *Drosophila*, comprehends species that use mainly fruits, flowers, fungi, or cacti as feeding and breeding resources, although some other extreme niches are found (Carson, 1971).

In the last decade, there has been a sharp increase on the study of Neotropical *Drosophila* ecology. Temporal and spatial variation of groups and species has been a major theme (Tidon *et al*., 2006; Mateus *et al*., 2006; Bizzo *et al*., 2010, Schmitz *et al*., 2010; Poppe *et al*., 2012), and researchers are now moving towards the study of the mechanisms that determine the temporal and spatial patterns. One main topic in this area is resource use; decomposing material used by flies as feeding and breeding sites (Valadão *et al*., 2010). These observations are scattered among *Drosophila* literature, but were recently increasing on fruits (De Toni *et al*., 2007; Roque *et al*., 2009), fungi (Gottschalk *et al*., 2009), and flowers (Schmitz, personal communication). Here we report a new feeding resource for drosophilids: the honeydew of scale insects (Hemiptera: Coccoideae).

In the begining of July 2012, one of us (M.R.) observed a great mass of flies in a trunk of *Inga* sp. (Fabaceae) in an orchard at the campus of Universidade Federal de Santa Catarina (27°35'54"S; 48°30'54" W). The trunk was infested with scale insects that secreted a sugar-rich and sticky liquid that eventually dropped and soaked the ground. After being secreted, the honeydew started hardening, got waxy, and was covered by dark and green mould. We monitored the tree on the following week until the temperature dropped (it was mid winter) and no more flies were collected.

The mass of flies was most evident during the hottest hours of the day and consisted mainly of Milichiidae (Carnoideae) and were tentatively assigned to *Milichiella* Gigio-Tos and *Pholeomyia* Bilimek. Some Drosophilidae, Dolichopodidae, Mycetophilidae, and Syrphidae, as well as *Apismellifera* L. (Hymenoptera, Apidae), were also collected. Interestingly, flies were aggregated on places with direct sunshine and followed the sun movement. Initially we started to sweep for 10 minutes at morning, midday, and at noon, but it was clear that drosophilids occurred only in the morning. We swept the trunk and the soaked soil near the tree, as well as between its roots.

Table 1. Absolute (ni) and relative abundance (pi) of drosophilid species collected on honeydew.

| | | | | | 2-Jul | 3-Jul | 5-Jul | 9-Jul | 13-Jul | Sample | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Genus** | **Subgenus** | **group** | **subgroup** | **species** | ***ni*** | ***ni*** | ***ni*** | ***ni*** | ***ni*** | ***ni*** | ***pi*** |
| *Drosophila* | *Dorsilopha* | *busckii* | | *D. busckii* Coquillett, 1901 | | | 1 | | | 1 | |
| | *Drosophila* | *cardini* | *cardini* | *D. cardinoides* Dobzhansky & Pavan, 1943 | | 14 | | 5 | 4 | 23 | 0.14 |
| | | | | *D. neocardini* Streisinger, 1946 | | | 1 | | 1 | 2 | |
| | | | | *D. polymorpha* Dobzhansky & Pavan, 1943 | | | | | 2 | 2 | |
| | | | | unidentified | 1 | | 3 | | | 4 | |
| | | *guarani* | *guaramunu* | *D. griseolineata* Duda, 1927 | 4 | 5 | | | 3 | 12 | 0.07 |
| | | | | unidentified | | | 3 | 5 | 7 | 15 | 0.09 |
| | | *immigrans* | | *D. immigrans* Sturtevant, 1921 | | | 1 | | | 1 | |
| | | *repleta* | *mercatorum* | *D. mercatorum* Patterson & Wheeler, 1942 | | 2 | 6 | | | 8 | 0.05 |
| | | | *repleta* | *D. repleta* Wollaston, 1858 | | | 1 | | | 1 | |
| | | | | unidentified | | | 3 | | | 3 | |
| | | *tripunctata* | | *D. medioimpressa* Frota-Pessoa, 1954 | | 1 | 1 | | | 2 | |
| | | | | *D. paramediostriata* Townsend & Wheeler, 1955 | | 11 | 7 | | | 18 | 0.11 |
| | | | | unidentified | | | 3 | 2 | 1 | 6 | |
| | *Sophophora* | *bromeliae* | | unidentified | | 1 | | | | 1 | |
| | | *melanogaster* | *ananassae* | *D. malerkotliana* Parshad & Paika, 1964 | 1 | 1 | 4 | 6 | | 12 | 0.07 |
| | | | *melanogaster* | *D. melanogaster* Meigen, 1830 | 1 | | | | | 1 | |
| | | | | *D. simulans* Sturtevant, 1919 | 2 | 2 | 6 | 1 | | 11 | 0.07 |
| | | | *montium* | *D. kikkawai* Burla, 1954 | | | 7 | | | 7 | |
| | | *saltans* | *sturtevanti* | *D. sturtevanti* Duda, 1927 | | | | 3 | | 3 | |
| | | *willistoni* | *willistoni* Pavan, 1952 | | | | 3 | | | 3 | |
| | | unidentified | | unidentified | | 1 | | | | 1 | |
| *Scaptodrosophila* | | *latifasciaeformis* | | *S. latifasciaeformis* Duda, 1940 | | | 1 | | | 1 | |
| *Zaprionus* | *Zaprionus* | *armatus* | *vittiger* | *Z. indianus* Gupta, 1970 | 1 | 14 | 10 | 2 | | 27 | 0.16 |
| **TOTAL** | | | | | 10 | 52 | 61 | 24 | 18 | 165 | 1.00 |

With a modest number of drosophilids collected (165), up to 17 species were determined (Table 1). The flies collected consisted mostly of common species found in this urban environment when collecting with banana baited traps, which has a clear dominance of exotic flies such as *Zaprionus indianus* and *D. simulans* (Gottschalk *et al*., 2007). Within each species group, again we found mainly the most common and widely distributed species (*D. mercatorum* of the *repleta* group; *D. cardinoides* of the *cardini* group; *D. griseolineata* of the *guarani* group; and eventually *D. sturtevanti* of the *saltans* group). One of these, *D. cardinoides*, seems to have the widest niche within the *cardini* group, as it was found in many species of fruits (Blauth and Gottschalk, 2007; De Toni *et al*., 2007; Roque *et al*., 2009), flowers (Frota-Pessoa, 1952; Schmitz, personal communication), fungi (Gottschalk *et al*., 2009), and cacti (Bizzo, unpublished data).

Based on subtle differences of the morphology of the aedeagus, parameres, and decasternum, we believe *D.*aff. *paramediostriata* is potentially an undescribed species.

The reason why flies and bees where aggregating on this particular tree is not entirely clear, but with the exception of Syrphidae (known to be a predator of other flies), we believe they were feeding on this sweet secretion, the moulds, or even yeasts that are known to inhabit this kind of resource (Serjeant *et al*., 2008). Indeed, Brake (2012) reports that adult Milichiidae were observed feeding on honeydew. Flies clearly did not use the honeydew as a breeding site, since it hardens in few days and no larvae were seen. The honeydew of scale seem to be an opportunistic feeding resource for drosophilids and other close-related families of flies, especially in the winter, when there are fewer flowers, fungi, and fruits fermenting. Although we had a very limited collection time and captured mainly generalist common species, further collections in well preserved forests might show that honeydew is used by a greater diversity of drosophilids.

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**Isolation of a long lifespan strain of *Drosophila melanogaster*.**

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There is a substantial interest in the relationship between longevity and oxidative stress. It is well-known that longevity of an organism is a quantitative trait and is determined by the action of both genetic and environmental components. The longevity of an organism is thought to be evolved in relation to demands by the environment for fitness. The fruitfly *Drosophila melanogaster* has been used as a model in biological research on aging for exploring the longevity phenotypes, artificial and natural selection responses (Paaby and Schmidt, 2009). In the past decade or so, various groups have tried employing artificial selection to generate extended longevity strains and to use them as a tool to examine the mechanisms underlying aging. In *D. melanogaster*, much of the longevity research has been based on the artificial selection in the laboratory. The factors shown to be involved in longevity include coordinated and specific upregulation of different antioxidant genes, expression of heat shock proteins, mitochondrial differences, decreased ROS production or increased ROS scavenging activity, and calorie restriction (Arking, 2005). In *Drosophila*, energy metabolism and SOD account for less than 40% of the average difference in lifespan between long-lived and normal flies (Rose *et al*., 1992; Tyler *et al*., 1993). We have isolated a long lifespan (LLS) strain using laboratory population of *D. melanogaster*, which shows extended longevity when compared with their progenitor normal lifespan (NLS) flies. The LLS flies isolated will be a useful model to study the factors involved in the longevity of organisms.

*D. melanogaster* (Oregon K) was obtained from the Drosophila Stock Center, University of Mysore, Mysore, India. This stock was maintained in a vivarium at 22±1°C on standard wheat cream agar medium with 12:12 light and dark cycles. The virgin females and unmated males were collected within 6 h of eclosion. Isolation of LLS was carried out using this laboratory population of *D. melanogaster*. Freshly eclosed adults from vials set up with a density of about 25 eggs per vial were collected; the virgin females and unmated males were segregated within 6 h of their eclosion. Pair mating was conducted to obtain the progeny. Flies that lived longer than the NLS flies were selected, and the progenies of the same were monitored for further generations. The LLS lines were amplified further for future studies. The adult lifespan of reproducing flies was assessed by setting up 20 vials for each strain, with each vial containing 20 males or 20 females. Freshly eclosed NLS and LLS adults from vials set up with a density of about 25 eggs per vial were collected, and unmated flies of both the sexes were segregated within 6 h of eclosion. Flies were transferred to fresh food vials every

alternate day and mortality was recorded daily; any flies dying were not replaced during the course of the assay, and that was continued until all flies died (Bharathi *et al.*, 2003).

Marked differences in the rate of survivability of *D. melanogaster* flies were found. The LLS flies lived longer when compared with NLS flies. We also observed gender differences in survivability in NLS flies (male, 12 wk; female, 14 wk); whereas there was no such difference in LLS flies, wherein both males and females lived for 18 weeks (Figures 1 and 2).

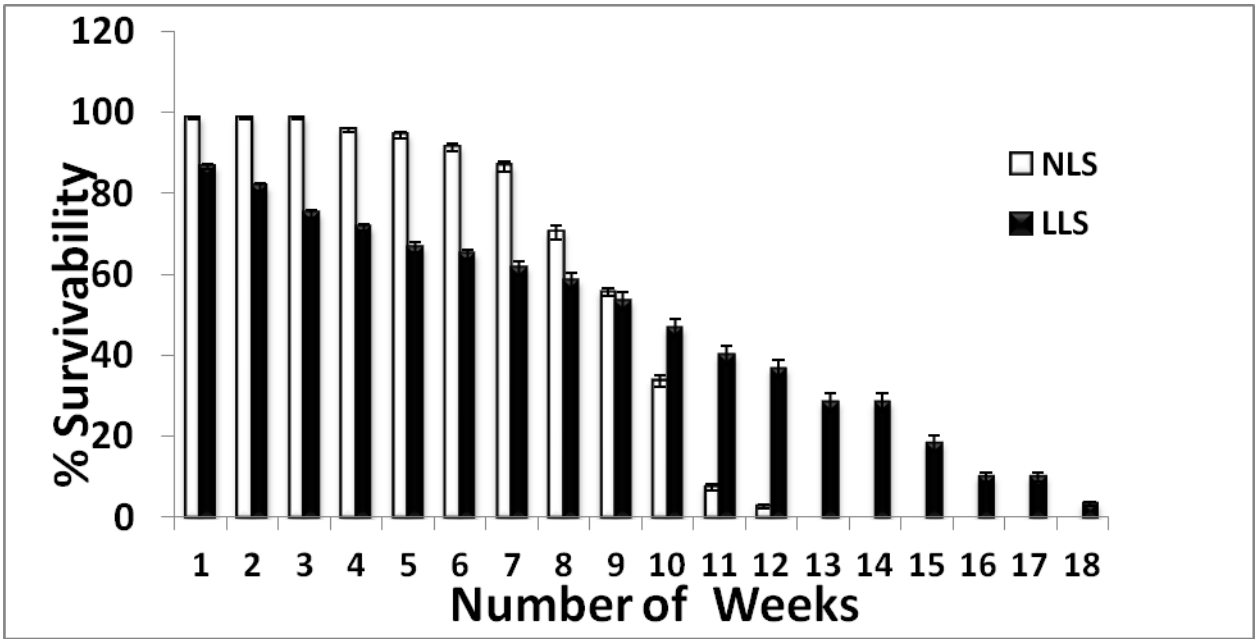


Figure 1. Mean lifespan of normal (NLS) and long lifespan (LLS) male *D. melanogaster* flies.

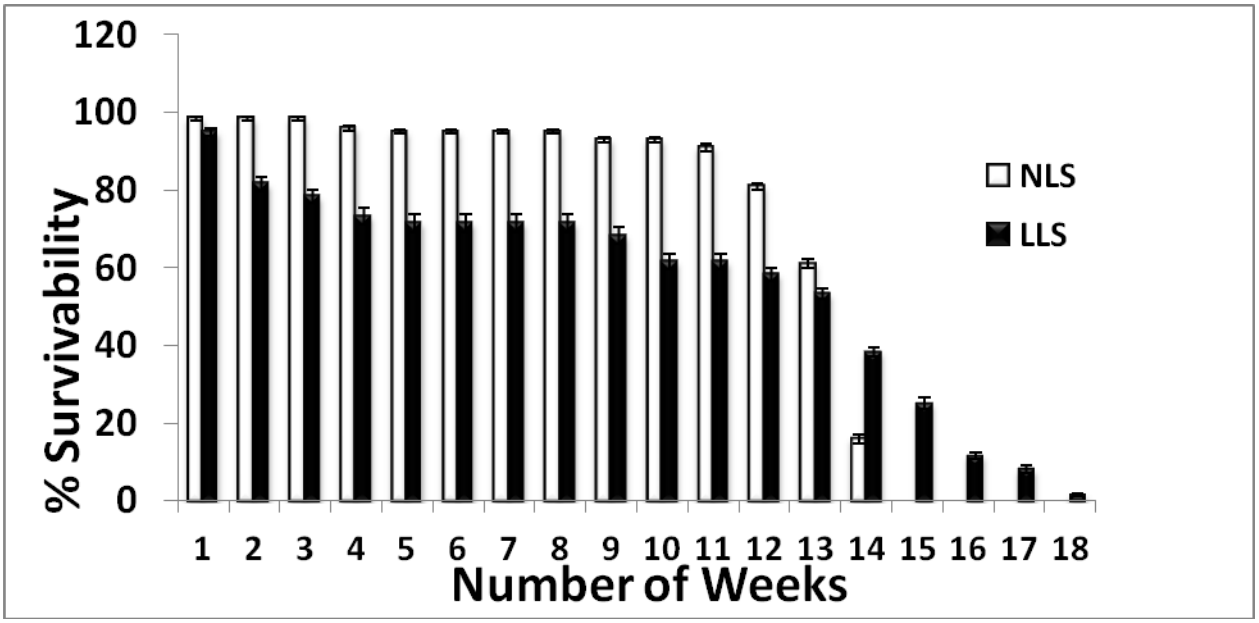


Figure 2. Mean lifespan of normal (NLS) and long lifespan (LLS) female *D. melanogaster* flies.

Various laboratories have reported long-lived strains of *Drosophila* using natural and artificial selection experiments. The long lifespan strains isolated in different laboratories are useful in unraveling the basic mechanisms of aging. According to free radical theory of aging (Harman, 1956), the "aging results from the deleterious effects of free radicals produced in the course of cellular metabolism." Luckinbill *et al.* (1984) and Arking (1987) made artificial selection to generate long-lived strains of *Drosophila* and compared them with the normal-lived progenitor strains. Selection for altered longevity was carried out by permitting reproductive success to occur at different times during the lifespan. Caloric restriction has been known for the past 75 years to be involved in extension of lifespan in rodents and *Drosophila*, and this attribute is conserved from yeast to rats (McCay *et al.,* 1989). Longevity strains have also been isolated through direct selection for delayed female fecundity (Luckinbill *et al.,* 1984; Arking, 1987; Partridge and Fowler, 1992). There are also reports of direct selection for desiccation resistance (Hoffmann and Parsons, 1989) or starvation resistance (Rose *et al*., 1992) and thereby indirectly selected for extended longevity. Many transgenic experiments have been carried out to test antioxidant theory of aging, which exhibited resistance to stress-inducing compounds and showed increased lifespan. Several laboratories have shown various characteristics of the long-lived strains, which include increased lipid content, increased starvation and desiccation resistance, longer flight duration, high glycogen content, higher stress resistance, and so forth (*c.f*., Arking, 2005). Therefore, longevity is associated with many factors. The LLS strain we have isolated will be useful to investigate the factors that influence extended lifespan when compared with the NLS strain, particularly with reference to oxidative stress and antioxidant enzymes.

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**Importance of non genetic factor (male age) in mating success of *Drosophila bipectinata.***

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Mating success is one of the fitness components in *Drosophila.* Numerous studies have shown the genotype dependent mating success (Spiess, 1970). However, non-genetic factors may also influence the mating success too such as temperature, larval density, and others.

For example, when *D. pseudobscura* is raised at low temperatures, males are larger and are more successful in mating than males of the same strain raised at high temperature (Ehrman, 1972). Similarly to larval density, age is known to influence mating success in *Drosophila* (Krishna *et al*., 2012). Recently in *D. bipectinata* it was noticed when females of *D. bipecinata* were given a choice to select young and old males, she preferred old males more frequently than young ones (Somashekar and Krishna, 2011). Therefore, in the present study we used brown eye mutant of *Drosophila* to test the female preference for male age classes to understand the relative importance of male age in mating success.

Flies used in the present study included the brown eye mutant originated by spontaneous mutation (Hegde and Krishna, 1995) in the natural population collected at Mysore in Chamundi hills. Eggs were collected from this stock using Delcour procedure (1969). Samples of 100 eggs were seeded in a culture bottle containing wheat cream agar medium and were maintained at 22±1°C and a relative humidity of 70%. When flies emerged, virgin females and males were isolated. Males were aged for 2-3 days (young) and 32-33 days (old); and 5-6 days virgin females were used for the female choice experiment. To study female choice experiment, a 5-6 day old mutant / wild female with two males (young and old) were introduced into an Elens Wattiaux mating chamber (1964) and observed for 1 hr. A total of 50 trials were made for each combination, and mating success of young and old age males were recorded.

Table 1. Male age influence on mating success of brown eye mutant and wild type flies of *D. bipectinata.*

| Female | Males | Female mated with young males | Females mated with old age males | $\chi^2$ |
|---|---|---|---|---|
| 5-6 days (W) | Young (M) Old (M) | 15 | 35 | 8.00* |
| | Young (W) Old (W) | 17 | 33 | 5.12* |
| | Young (W) Old (M) | 9 | 41 | 20.48* |
| | Young (M) Old (W) | 8 | 42 | 23.12* |
| 5-6 days (M) | Young (M) Old (M) | 17 | 33 | 5.12* |
| | Young (W) Old (W) | 20 | 30 | $2.00^{NS}$ |
| | Young (W) Old (M) | 10 | 40 | 18.00* |
| | Young (M) Old (W) | 7 | 43 | 25.92* |

* Significant at 0.001 level; NS- Non significant

Table 1 reveals that when wild females of *D. bipectinata* were given an opportunity to mate with young or old age males, irrespective of the genotype, females prefer to mate with old age males more frequently than with young age males. Even in trials of mutant females, females preferred to mate with old age males irrespective of mutant or wild males. This suggests that age of the male is important for mating success in *Drosophila bipectinata.* This study also supports influence of both genetic and non-genetic factors on mating success.

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**Female size does not determine ovariole number in *Drosophila*.**

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## Introduction

Body size in animals is an important trait associated with the reproductive success. It is generally believed that larger the individuals greater is the fitness, *i.e*., larger females can carry more ovarioles than small females. Thus fitness in general is believed to be an increasing function of body size in animals, particularly among insects (Krishna and Hegde, 2003). Although strong positive effects of body size on fitness are wide spread, there is a growing list of studies reporting no effect or even negative on body size effect on fitness components (Wayne *et al.*, 2006). However, studies within and among populations of the same species have produced conflicting results regarding the relationship between female size and fecundity through ovarioles number (Lefranc and Bundgaard, 2000; Wayne *et al.*, 2006). There is a growing list of evidence suggesting that the relation between female size and ovarioles number is not positive and it varies from time to time as it is positively associated with availability of nutrients. On the other hand, the relationship between ovariole number and fecundity was significantly stronger as compared to relationships between fecundity and female size (Branquart and Hemptinne, 2000). As more species are studied the number of examples where female size and fecundity Chenoweth *et al*, 2007) and female size and ovariole number are uncorrelated is increasing (Togashi and Life, 2007). In contrast to this, there is a strong positive relation between ovariole number and fecundity (Branquart and Hemptinne, 2000). Therefore, the present study has been undertaken in three species of *Drosophila*, namely *D. bipectinata*, *D. ananassae*, and *D. melanogaster*, to study the relation between female size and ovariole number.

## Methods

*Establishment and maintenance of experimental stocks*

Three *Drosophila* species, namely *D. bipectinata*, *D. ananassae*, and *D. melanogaster*, were used in the present study. All experiments were made separately for each of the three species. Experimental stocks of each of the above species were originated separately from 150 wild caught females collected at Mysore, Karnataka. When progeny appeared, flies were mixed together and redistributed to different culture bottles each with twenty pairs. These stocks were maintained using wheat cream agar medium in a constant temperature (21±1ºC) at a relative humidity of 70% under 12:12 light dark cycle. In every generation, flies multiplied in different culture bottles were mixed together and eggs were collected using Delcour procedure (1969). Eggs (100) were seeded in fresh quarter pint milk bottles with 25 ml of wheat cream agar medium to avoid larval competition during development (this procedure allowed us to reduce environmental variation in size). After, five

generations, when adults emerged, virgin females and males were isolated within 3 hr of their eclosion and maintained separately at 21±1ºC.

Five to six day old randomly collected hundred virgin females from each of the above *Drosophila* species (*i.e*., *D. bipectinata, D. melanogaster*, and *D. ananassae*) were used to study wing length and ovariole number following the procedure of Krishna Hegde (1997).

## Results and Discussion

Numerous morphometric traits in insects are often subjected to natural selection and related to fitness in different degrees. Variation of morphometric traits can be studied as intra population variability between individuals and traits (Imasheva *et al*., 1999) or as inter population variability (Coyne and Beecham, 1987), which reflects the source of variation. Table 1a and 1b revealed that female wing length varied significantly between three species of *Drosophila* studied. *D. melanogaster* had longer wing length (2.30 mm) compared to *D. ananassae* (2.15 mm) and *D. bipectinata* (1.88 mm). *D. bipectinata* was the shortest among the three species studied. This suggests the existence of species specific differences in female wing length in *Drosophila.* This conforms to earlier studies of wing size variation in different species of *Drosophila* (Santos *et al.*, 1992; Hegde and Krishna, 1997; Yadav and Singh, 2003). Krishna and Hegde (2003), while working on three sympatric species of *Drosophila* such as *D. malerkotliana, D. bipectinata*, and *D. ananassae*, have also found species specific difference in wing length. In *Drosophila* wing length is used as an index of body size (Hegde and Krishna, 1997). In addition to this, morphological traits such as wing width, thorax length, face width, and foreleg length have also been used as indices of body size (Ruiz *et al.*, 1991). Thus these studies suggest body size varies across the species in *Drosophila*.

Table 1a. Mean female wing length (in mm) and ovarioles number in three species of *Drosophila* (Values are mean ± standard error).

| Parameters | Species | | | F-value |
|---|---|---|---|---|
| | *D. bipectinata* | *D. ananassae* | *D. melanogaster* | |
| Wing length (in mm) | 1.88 ± 0.0068 [a] | 2.15 ± 0.006 [b] | 2.30 ± 0.0065 [c] | 1097.97*** |
| Ovarioles number | 28.02 ± 1.33 [a] | 33.08 ± 1.06 [b] | 31.14 ± 1.22 [a b] | 4.464* |

*P<0.05; ***P<0.001

- Same letter in the superscript indicate non-significant by DMRT
- Different letter in the superscript indicate significant by DMRT

Table 1b. Correlation matrices between female wing length (in mm) and ovarioles number in three different species of *Drosophila.*

| Species | | Ovarioles number | | |
|---|---|---|---|---|
| | | *D. bipectinata* | *D. ananassae* | *D. melanogaster* |
| *D. bipectinata* | Wing length | 0 .146 | | |
| *D. ananassae* | Wing length | | 0 .221* | |
| *D. melanogaster* | Wing length | | | 0.102 |

*correlation significant at 0.05 level

In our study female wing length also showed variation within the species. In *D. melanogaster* female wing length varies from 2.10 mm-2.42 mm with an average wing length of 2.30 mm, while variation in wing length of *D. bipectinata* ranged from 1.62 mm-2.00 mm with an average value of 1.88 mm. In *D. ananassae* female wing length also varies from 2.00 mm-2.29 mm with an average wing length of 2.15 mm. This suggests the occurrence of intraspecific variation in female wing length in *D. melanogaster, D. bipectinata,* and *D. ananassae*, respectively. This conforms to earlier studies of wing length and thorax length variation within species in different species of *Drosophila* (Wayne *et al.*, 2006; Yadav and Singh, 2007). Phenotypic variation or morphological difference among individuals is a regular feature in natural populations. These morphological features often vary among *Drosophila* populations, and this variation has some times been related to adaptations. Most of the morphometric characters are quantitative and, therefore, their variation is obvious. Therefore, in each species one can expect large variation in body size.

Like wing length, ovariole number is another morphological trait known to be involved in fitness in *Drosophila* (Wayne *et al.*, 1997). Table 1a and 1b show mean ovariole numbers of *D. melanogaster, D. ananassae* and *D. bipectinata*. It was noticed that mean ovariole number varied significantly between different species of *Drosophila* studied. *D. ananassae* had significantly greater ovariole number (33.08) compared to *D. melanogaster* (Gromko and Markow, 1993) and *D. bipectinata* (28.02). Among the three species studied, *D. bipectinata* had the least ovariole number. This suggests occurrence of species specific difference in ovariole number, too. This supports the earlier studies of variation in ovariole number in *D. melanogaster* (Wayne *et al.*, 2006), *D. malerkotliana* and *D. bipectinata* (Krishna and Hegde, 1997), *D. nasuta* (Harini and Ramachandra, 2003), *D. ananassae* (Yadav and Singh, 2007), and *D. buzzatii* (Santos *et al.*, 1992). They also found species specific variation with reference to ovariole number. Thus, these studies suggest that in *Drosophila* variation in ovariole number is seen across the species.

The adult female reproductive system is more or less typical for all *Drosophila* species. It consists of a pair of ovaries, the genital ducts with their accessory structures (Spermathecae, seminal receptacle and accessory glands), and the vagina (Santos *et al.*, 1992). Ovariole number variation is related to ecological conditions, among related species of fruit flies, a greater number of ovarioles is correlated with "niche breadth" measured as the number of different kinds of fruits used by ovipositing females (Fitt, 1990). Studies have shown a reduction in mean ovariole number has been linked to ecological specialization as the morinda fruit by *D. sechellia*, a close relative of *D. melanogaster* (R'Kha *et al.*, 1991), and a taxonomic study of 41 species of Hawaiian *Drosophila* showed that intraspecific variations in ovariole number is positively associated with the availability of nutrients in the larval feeding niches (Fitt, 1990; R'Kha *et al.*, 1991). A similar trend has been demonstrated for *Tephritid* flies in the genus *Dacus* with generalists again having more ovarioles than specialist (Fitt, 1990).

In the present study ovariole number also varied within species. In *D. ananassae* ovariole number varies from 14-57 with an average value of 33.08, while in flies of *D. melanogaster* it ranged from 11-81 with a mean value of 31.14. In *D. bipectinata* variation in ovariole number ranged from 10-55 with mean ovarioles number of 28.02. This suggests ovariole number varies widely within each species suggesting the occurrence of intraspecific variation in ovarioles number. This supports earlier studies of intraspecific variation in ovariole number in other species of insects (Yadav and Singh, 2007; Krishna and Hegde, 1997).

Like body size, ovariole number is a morphological trait with quantitative genetic control known in various species of *Drosophila* of the *melanogaster* complex (Stammer *et al.*, 2001) and is thought to be closely correlated with female reproductive success via a simple relationship between

the number of ovarioles and the rate at which eggs have been produced by the female (David *et al*., 1970; Chakir *et al*., 1995), hence their variation in natural population is also quite obvious. In *Drosophila* evidence on the adaptive nature of ovariole number comes from the observation of latitudinal clines in *D. melanogaster* (Azevedo *et al*., 1996). Such replication of clines strongly implies the action of selection in the creation and maintenance of the clines, as argued for an isozyme cline (Berry and Kreitman, 1993) or quantitative traits, such as body size (De Jong and Bochdanovits, 2003). These clines in ovariole number might be associated with differences in temperature, duration of reproductive period, the seasonal pattern of food availability, and geographical variation in over wintering conditions. The evidence between and within species suggests that there may be a relationship between environmental variation and ovariole number.

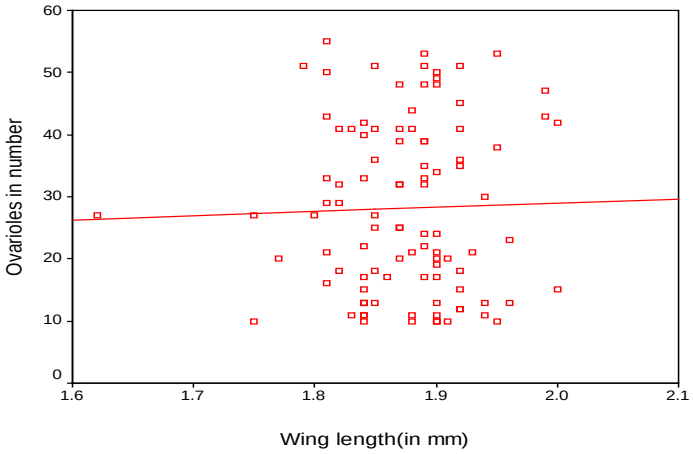


Figure 1a. Scattered plot showing relationship between female wing length and ovariole number of *D. bipectinata* (r = 0.146; n = 100; P > 0.05).

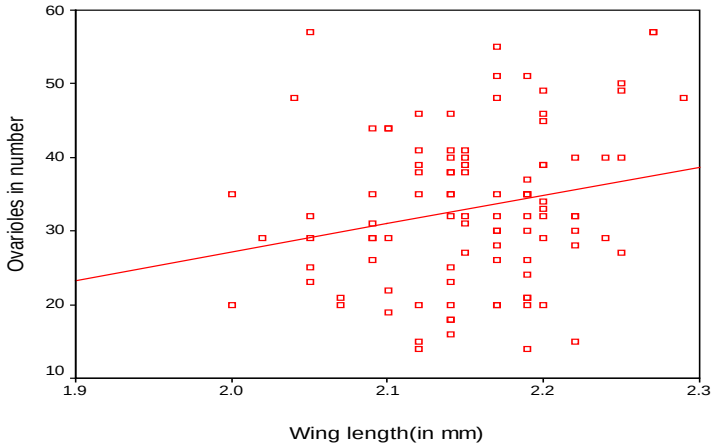


Figure 1b. Scattered plot showing relationship between female wing length and ovariole number of *D. ananassae* (r = 0.221*; n = 100; P < 0.05).

Correlation matrices calculated by applying Pearson correlation between female wing length and ovariole number in three different species of *Drosophila* are provided in Table 1b and Figures 1a-c. Table 1b revealed that there was no significant positive correlation between female wing length and ovariole number in *D. melanogaster* and *D. bipectinata*. However, in *D. ananassae* significant positive correlation was noticed between female wing length and ovariole number. This suggests the occurrence of species-specific differences in the relationship of female wing length and ovariole number. This supports the earlier studies of relations between female body size and ovariole number in *D. melanogaster* (Wayne *et al.*, 2006). They also found no relationship between ovariole number and body size either between selected or control lines or within each type of line. Studies of Yadav

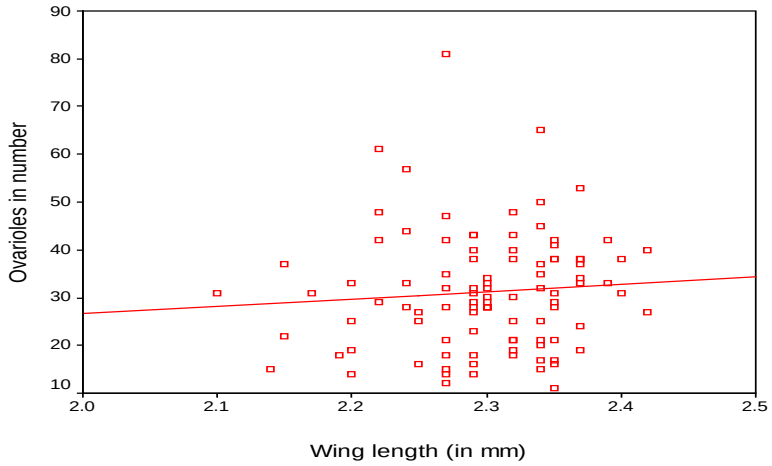


Figure 1c. Scattered plot showing relationship between female wing length and ovariole number of *D. melanogaster* (r = 0.102; n = 100; P > 0.05).

and Singh (2007), while working on evolutionary genetics of *D. ananassae*, have also found significant positive correlation between female wing length, thorax size, and ovariole numbers in G13 line, but in the same species in G10 line they did not find significant positive correlation between female body size and ovariole number. Santos *et al*. (1992), while working on *D. buzzatii*, have found positive phenotypic correlation between female size and ovariole number. Thus, these studies suggest that relationships between body size and ovariole number is not as strong as believed earlier. As more and more species are involved no correlation or negative correlation between female body size and ovarioles number is increasing (Wayne *et al.*, 2006). Thus, these studies also suggest that in most of the species of *Drosophila*, body size was not significantly positively correlated with ovarioles. Thus, our results do not support the theoretical prediction that large females have greater ovarioles than small females and are contrary to the studies of large female having greater ovariole number (Branquart and Hemptinne, 2000). This is because the strength of relationship between female size and ovariole number varies with a number of environmental factors (Santos *et al.*, 1992). Our study also suggests that ovariole number and female size are independent traits they are not interdependent.

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**On the hybrid tumor in the cross of *Drosophila melanogaster* females and *D. simulans* males.**

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*Drosophila melanogaster* and *D. simulans* are quite similar in morphology, but they are reproductively isolated. When *D. melanogaster* females are crossed with *D. simulans* males, hybrid females appear and hybrid males die at the larval stage. This type of cross is generally more successful than the reciprocal cross of *D. simulans* females and *D. melanogaster* males, in which hybrid males eclose and hybrid females die at the embryonic stage. Survivors are sterile in the both reciprocal crosses (Sturtevant, 1920). Uenoyama and Inoue (1995) found the S2 line of *D. simulans*, an isofemale line from a natural population collected in l988 at Mishima, Japan, and females of this strain showed remarkably high mating success with *D. melanogaster* males. This is the mutant for premating reproductive isolation. The high crossability was caused by at least two genes that act additively, one on the second and one on the third chromosome, but the X chromosome showed no effect.

In the present study, the cross of *D. melanogaster* females and males of the *D. simulans* S2 line were analyzed. The wild strains used for *D. melanogaster* were Oregon-R (OR). For *D. simulans* the S-2 line and the yellow mutant $y^{NS}$ line, being as the control, were used. The experiments were carried out as follows. Virgin females and males were collected within four hours after eclosion without anesthesia and aged separately for two days. Then each ten females and males were placed together in a 50 ml vial containing fresh medium. After two days the females were individually transferred to new vials and were allowed to lay eggs. The number of examined females [A] and number of females producing hybrid progenies [B] were examined, and the frequency [B/A] was calculated. A total of 253 females were analyzed in the present study. Table 1 shows the higher crossability of the *D. simulans* S-2 line to *D. melanogaster* by the frequency of females producing hybrid progenies at 24$^{o}$C. The S-2 line females showed the higher frequency of 0.696 to the *OR* males, whereas the $y^{NS}$ line showed zero value. The progenies, being all males, were morphologically normal. These results confirmed the previous experiments (Uenoyama and Inoue, l995) in which the insemination ability was analyzed. Although the frequencies were much lower than the reciprocal cross, the S-2 line males also showed significantly higher frequency of 0.200 to the *OR* females in comparison with the $y^{NS}$ line which showed zero value. Thus the S-2 lines showed the higher crossability in both reciprocal crosses. Moreover, the hybrid progenies were all females, which had frequently melanotic tumor on their legs when the S-2 line males were used for mating. The tumor was found around the connection part between femur and tibia (Figures 1 and 2).

In Table 2, pupa to adult lethality and frequency of hybrid pupae with melanotic tumor from crosses of the *OR* females and S-2 line males were examined by temperature shift experiment. The experiments were carried out as follows. To get the same developmental stage of the hybrids, each parental flies were placed in medium vials and were allowed to lay eggs in a short period of several

Figure 1. Hybrid progenies with a tumor from the cross between *OR* females and S-2 line males.

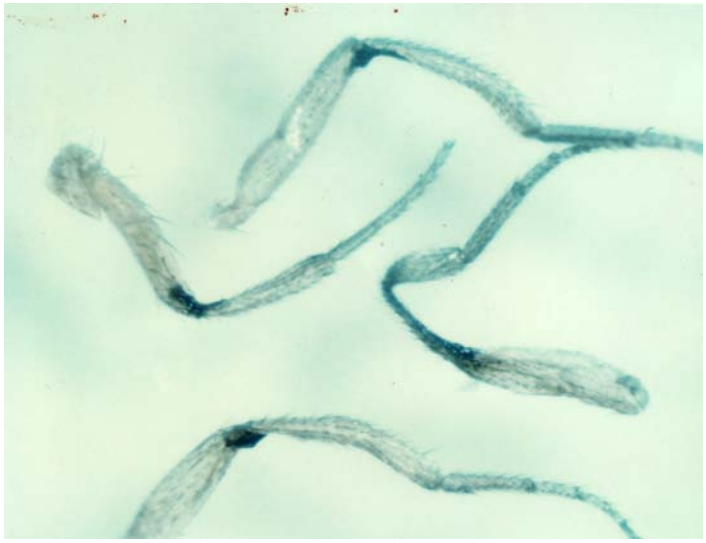

Figure 2. Tumors found around the connection part between femur and tibia of hybrid progenies between the *OR* females and S-2 line males.

hours. Temperatures were shifted at the following three stages during development; (A) early stage of the third instar larva, still being in medium surface, about 3 days of age; (B) late stage of the third instar larva, climbing glass wall just before pupation, about 5 days of age; and (C) two days after pupation, about 7 days of age. A total of 1606 pupae were analyzed. At 24$^{o}$C, the tumor frequency was high at 0.833 and lethality was 0.682, indicating that all dead progenies were not caused by melanotic tumor, and there were some survivors with melanotic tumor. Both lethality and tumor frequency were very low at 0.029 at 23$^{o}$C. The viable hybrid pupae were almost without tumor, and most dead pupae had tumor. Sturtevant (1929) reported that the hybrid females from the cross between *D. melanogaster* females and *D. simulans* males failed to emerge from puparium or died within a few hours after emergence at 25$^{o}$C. At 15$^{o}$C to 22$^{o}$C, they emerged normally and were fully viable. The borderline of the temperature sensitivity was found to be between 23$^{o}$C and 24$^{o}$C in the present study. Also the hybrid tumor seemed to have the same borderline of temperature sensitivity. Under shift down condition from 24$^{o}$C to 23$^{o}$C at early stage of the third instar larvae, all hybrid pupae were viable without tumor, indicating that the temperature sensitive period was after early

stage of the third instar larvae. Under shift up condition from 23°C to 24°C at the same developmental stage, tumor frequency was 0.586 and lethality was 0.224, both of which were unexpectedly lower in comparison with those of 24°C condition. The reason was unknown, probably being related to imperfection of the 24°C condition. More than half of tumor pupae seemed to be viable. On the other hand, the tumor frequency was 0.476 and lethality was 0.317 under shift down condition from 24°C to 23°C at late stage of the third instar larvae. These values were about half of the tumor frequency and lethality at 24°C condition. Under shift up from 23°C to 24°C at the same stage, tumor frequency was 0.390 and lethality was 0.309, both of which were slightly lower than the reverse shift condition. In addition, the tumor frequency and lethality were relatively high, at 0.645 and 0.667, respectively, under shift down from 24°C to 23°C at the stage of two days after pupation. Although the tumor frequency was somehow a little lower, lethality was almost the same as the case of the 24°C condition. In this case, all tumor pupae seemed to be dead. Under shift up from 23°C to 24°C at the same stage, the lethality was 0.130, being slightly higher, and tumor frequency was 0.037, being almost the same, in comparison with those of 23°C condition. In this case more than half of tumor pupae survived. These results indicated that the temperature sensitive period for lethality almost ceased at the stage of two days of pupation.

Table 1. The reciprocal crossability between *D. melanogaster* and *D. simulans* at 24° C condition.

| Cross | | No. of females examined [A] | No. of females producing progenies [B] | Frequency [A/B] |
|---|---|---|---|---|
| *D. simulans* female X *OR* male | | | | |
| S-2 | OR | 58 | 40 | 0.696 |
| y $^{NS}$ | OR | 53 | 0 | 0 |
| *OR* female X *D. simulans* male | | | | |
| OR | S-2 | 65 | 13* | 0.200 |
| OR | *y* $^{NS}$ | 77 | 0 | 0 |

* Most of hybrid progenies had melanotic tumor (see Table 2).

Table 2. Pupa to adult lethality and tumor frequency in hybrids between *D. melanogaster OR* females and *D. simulans* S-2 line males under temperature shift experiment between 23° C and 24° C.

| Temperature shift | No. of pupae examined [A] | No. of pupae with tumor [B] | Freq. of pupa with tumor [B/A] | No. of adults[C] | Pupa to adult lethality [1- C/A] |
|---|---|---|---|---|---|
| +++ (A)+++ (B) +++ (C) +++ | 466 | 388 | 0.833 | 148 | 0.682 |
| === (A)=== (B) === (C) === | 627 | 18 | 0.029 | 609 | 0.029 |
| +++ (A) === (B) === (C) === | 40 | 0 | 0 | 40 | 0 |
| === (A) +++ (B) +++ (C) +++ | 58 | 34 | 0.586 | 45 | 0.224 |
| +++ (A) +++ (B) === (C) === | 145 | 69 | 0.476 | 99 | 0.317 |
| === (A) === (B) +++ (C) +++ | 123 | 48 | 0.390 | 85 | 0.309 |
| +++ (A) +++ (B) +++ (C) === | 93 | 60 | 0.645 | 31 | 0.667 |
| === (A) === (B) === (C) +++ | 54 | 7 | 0.130 | 52 | 0.037 |

+++ : 24° C condition, and === : 23° C condition. (A) Early stage of the third instar larva, still being in medium surface, about 3 days of age. (B) Late stage of the third instar larva, climbing glass wall just before pupation, about 5 days of age. (C) Two days after pupation, about 7 days of age.

Thus the limited number of hybrid progenies emerged and were frequently accompanied with melanotic tumor at 24°C in the cross between the *OR* females and S-2 line males. The hybrid lethality and appearance of the hybrid tumor were strictly temperature sensitive; the 24°C condition was critical and 23°C was permissive. The sensitive developmental stages were found to be from the early third instar larvae to early pupa in both cases. Before and after the late third instar larva stage, both lethality and tumor seemed to appear in almost the same degree (Table 2). The present hybrid tumor seemed to be highly linked with reproductive isolation between these two sibling species, for the correlation coefficient between tumor frequency and hybrid lethality was calculated by the data of Table 2 to be 0.931, showing statistically significant positive correlation (d.f. = 6, $p < 0.01$).

References: Sturtevant, A.H., 1920, Genetics 5: 488-500; Sturtevant, A.H., 1929, Carnegie Inst. Wash. Publ. 399: 1-62; Uenoyama, T., and Y. Inoue 1995, Jpn. J. Genet. 70: 365-371.

## Availability of the University of Texas Publications Dealing with *Drosophila*

Marshall R. Wheeler

From 1940 to 1972 many research articles were published by the University Press in the series, "Studies in the Genetics of *Drosophila*" with J.T. Patterson as editor and later (from 1957-1972) with M.R. Wheeler as editor. In 1960 the series title was changed to "Studies in Genetics." There were also a few special issues. Many of these are now out of print (OOP); all known copies of the remaining issues have been made available by Dr. Wheeler. The copies are available from the office of the Editor, *Drosophila Information Service*; contact Dr. James N. Thompson, jr., (jthompson@ou.edu) for details.

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1940: UTP 4032 (OOP). 1942: UTP 4213 (OOP). 1942: UTP 4228 (OOP). 1943: UTP 4313, "Drosophilidae of the Southwest" (OOP). 1944: UTP 4445, with "Drosophilidae of Mexico" (OOP). 1947: UTP 4720, "Isolating Mechanisms" (OOP). 1949: UTP 4920 (OOP). 1952: UTP 5204 (25 copies). 1954: UTP 5422 (OOP). 1957: UTP 5721 (45 copies). 1959: UTP 5914, "Biological Contributions." Dr. Patterson's 80th birthday issue (59 copies). 1960: UTP 6014 (16 copies). 1962: UTP 6205 (63 copies). 1966: UTP 6615, Morgan Centennial Issue (28 copies). 1968: UTP 6818 (24 copies). 1969: UTP 6918, W.S. Stone Memorial Issue (12 copies). 1971: UTP 7103 (22 copies). Final volume, 1972: UTP 7213 (29 copies).

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# Technique Notes

**A system for measuring longevity and fecundity in flies.**

**Weber, K., E. Veilleux, and J. Harmon**. University of Southern Maine, Portland, Maine, USA; Email: keweber@usm.maine.edu

## Introduction

Longevity and fecundity are often studied using flies, and many experimental systems have been devised for this. We report here a simple apparatus we have used for measuring longevity plus fecundity in *D. melanogaster*. Our system was designed to track these parameters in individual flies, singly or in male-female pairs, housed in units of 100 cells. The basic materials are a few plastic "egg crate" lighting diffuser panels and a light box, plus some small metal pans, insulative foam-board, and fine nylon mesh.

## Design and Use

In the egg crate panels we used, the small square openings have inside dimensions of 16 mm on each side. We cut these panels into squares with 10 × 10 openings, with outside dimensions of 167 × 167 mm. We then glued these pieces together in stacks of six, with a sheet of nylon mesh between the top two pieces. This makes a box with 100 separate cells, each about 40 × 16 × 16 mm, bounded by mesh at the top, and open at the bottom (Figure 1). Each cell can hold a pair of flies. By gluing together two more squares of egg crate panel, we made a separate, matching, lower unit to combine with this, which can be pressed into a small metal pan of shallow agar medium as it solidifies, to provide the daily food and egg-laying surfaces below the flies (Figure 2). This lower unit is firmly stabilized in the agar pan by 1) a square sheet of one-eighth inch aluminum glued to the inside bottom of the pan and 2) four set screws through the sides of the pan that contact the plastic and can be adjusted by a hex key. The lower unit also has thin aluminum strips or flanges along its upper sides to provide guides so that the box containing the 100 cells, filled with flies, can be aligned exactly with the second grid of cells below, containing the 100 squares of agar. The two parts are then held together with rubber bands (Figure 3).

We replaced the agar pan each day. To accomplish this, the combined unit shown in Figure 3 containing the flies is inverted and lowered into the illuminator-anesthetizer box shown in Figure 4. This box is made of a light box (Porta-Trace, from Gagne, Inc., 41 Commercial Drive, Johnson City, NY, 13790), with four pieces of insulative foam attached to the top by an encircling band of duct tape, and four strips of aluminum on the inside of the foam to provide a supporting ledge, and finally a covering of white duct tape on the inside surfaces of foam and aluminum, to reflect the light.

The space inside the illuminator-anesthetizer can be filled with $CO_2$ from a port through the foam in the back. After a few minutes of $CO_2$ the flies fall onto the nylon mesh. (This is promoted by removing the inverted unit with the flies and dropping it onto the bench several times from a height of a few inches, during the anesthetization process.) Then the old agar pan and lower grid can be lifted off and replaced by fresh ones. If dead flies need to be removed (or replaced to maintain

sexual pairs) this can be done while the flies are anesthetized, before the new agar pan is attached. We also made a clear, sliding Plexiglas cover (not shown) to place over the box of cells while the flies are anesthetized, during the interval when the old agar pan is not attached. A small hole in this cover allows targeted access to individual cells without exposing all cells. Finally, the flies are given a few minutes to awaken fully, with the whole unit still in the inverted position, before turning it upright again. The eggs per cell on the agar surface of the old pan can be easily counted under a stereomicroscope. We numbered each cell in the agar grid with tiny digits so that the cell numbers are visible during counting. Pans can be stored in the refrigerator to stop egg development while they await counting.

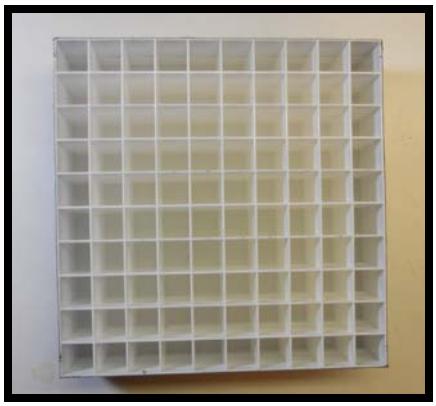

Figure 1. Fly box with 100 cells.

Figure 2. Base consisting of grid and pan.

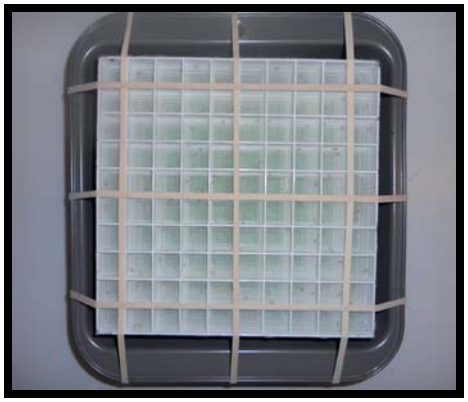

Figure 3. Combination of fly box and base.


Figure 4. Illuminator-anesthetizer.

At each agar transfer, care must be taken to minimize $CO_2$ exposure. When the experiment is initiated and the inverted box of cells is filled with flies for the first time, the box should not be kept on the anesthetizer at all, because it takes so long to fill all 100 cells the first time that the first flies added would receive much more $CO_2$ than the last. Instead the inverted box is placed on the benchtop, and as each row of 10 cells is filled with freshly and lightly anesthetized flies, the cells of that row are immediately covered with a strip of wide scotch tape. After all 100 cells are filled, the box is quickly gassed again on the anesthetizer, the whole covering of scotch tape is ripped off at once, and the agar pan is then attached. (The flies do not get stuck on the tape.)

Special care must be taken during construction of this apparatus to make sure all parts fit together tightly and exactly, taking into account any small irregularities in the egg crate material. In an experiment involving four simultaneous sets of (initially) 200 flies, no flies ever escaped or moved accidentally between cells in over 60 consecutive daily transfers.

**Fluorescein dye as a tagging agent for *Drosophila* dispersal studies.**

**Fingerut, Jonathan, Kristina Orbe, and Scott McRobert.** Biology Department, Saint Joseph's University, Philadelphia, PA 19083.

Dispersal, or the unidirectional movement of organisms in search of improved conditions, is a commonly investigated and ecologically important aspect of plants, invertebrates, and vertebrates (Howe and Smallwood, 1982; Johnson and Gaines, 1990; Levin, 2006). This action is particularly important for organisms that are born into habitats with high population densities such as *Drosophila*. For these animals, rotting vegetation provides a nutritional resource for adults and larvae, as well as a location to meet mates and lay eggs. And while these conditions may support large populations, *Drosophila* are known to disperse (see Dobzhansky, 1973; Johnston and Heed, 1975; Grossfield, 1978). Of the studies that have empirically investigated dispersal outside the laboratory, many have relied on dusting flies with fluorescent microparticles to delineate the released population from wild individuals (see Crumpacker, 1974). This dusting can be performed actively in a "shake and bake" methodology, or through passive contact as the larvae emerge from their pupal stage when their media is doped with the particles. While dusting is a useful technique for examining dispersal, its possible influence on such behavior has been questioned (Turelli *et al*., 1985).

As part of a larger project to determine the dispersal and feeding range of the invasive fruitivorous *D. suzukii*, we investigated an alternative to dusting, namely the use of the fluorescent marker fluorescein sodium ($C_{20}$-$H_{10}$-$O_5$-$Na_2$). This common, relatively non-toxic water-soluble laboratory chemical is used in a number of applications including ophthalmological examinations of the cornea and as a tracer in ecohydrodynamic experiments (Maurice, 1967; Zimmer-Faust *et al*., 1995; Pesticide Action Network). Its ability to fluoresce green under UV or blue light makes it easy to visualize, while its safety and inexpensive nature make it a useful product for *Drosophila* work.

## Methods and Results

*Fluorescent techniques*

Four application techniques were tested: dusting adults with fluorescein powder, spraying adults with a concentrated fluorescein solution (30 g/l), feeding adults media containing concentrated fluorescein solution (8 ml solution/vial), and feeding larvae the same media containing concentrated fluorescein solution. For the first two treatments (dusting and spraying), juvenile (<24 hours post-eclosion) flies from a laboratory population (Canton-S) of *Drosophila melanogaster* were collected and treated under $CO_2$ anesthesia and then placed (n = 15) in 5 replicate vials. Flourescein-fed-adults were collected after spending either 1 or 7 days on doped media, and flies from the flourescein-fed larval treatment were collected as emerging adults after spending their larval period in doped media. Pre-trial results determined that propionic acid, sometimes used when making the medium (Formula 4-24 Instant Media – Carolina Biological) to combat fungal infections, interacted with the dye decreasing or eliminating its fluorescence. Therefore, no propionic acid was added to the rearing media.

Effectiveness of all treatments was determined through visual comparison between treated flies and untreated control flies raised on the same media. Flies were observed at 30× using a dissecting scope while illuminated with a blue LED flashlight. Flies were qualitatively scored for their relative ease of discrimination when compared to untreated members of the same stock population. After initial observations were completed, the treated flies were all placed into vials containing standard media to determine how well the tagging held up over time, with observations taken again at 24, 48 and 72 hours.

Of the four techniques the best results were found in the groups in which flies or larvae had been fed media containing the fluorescein, both in terms of initial tagging and longevity of the tag. Dusting with fluorescein powder provided a discernable signal, but variation between individual flies, with respect to the amount of powder, was considerable and either through shedding of the particles or cleaning by the flies themselves the tag did not last long. Spraying adults with fluorescein made them visible, but this was attributed more to the dye mixing with the food and being ingested, rather than beading and sticking to the flies themselves. No discernable difference was seen between larvae raised on doped media and adults fed doped media for 24 hours. Fed flies possessed brightly fluorescing intestines (and feces) most easily seen in the abdominal region. Adults fed on doped media for a week, however, showed a noticeably greater level of fluorescence than all other treatments, primarily via increased fluorescence of the thorax. Observing the flies over the 72 hours following their initial inspection indicated that sprayed and dusted flies lost what fluorescence they had within 24 hours. In contrast, the fluorescein-fed flies still fluoresced at 72 hours, though it was diminished and primarily limited to the thoracic region. Fluorescent feces were noted in the vials containing fed flies, indicating that the gut residence time of the fluorescent food may be a determining factor on labeling longevity.

*Mark and recapture*

As proof of concept, a limited set of mark and recapture studies were undertaken on the lawn next to the laboratory. Bottles were placed every 2 m on either side of the release point and left in place for 12 hours after the release of ~800-1000 flies fed doped media for a week. Initial trials found extremely low recapture rates (< 0.1%) using yeasted fly medium. Subsequent trials delivered markedly higher recapture rates (up to 30%) using crushed fruit (bananas, peaches, raspberries, blueberries). While we were able to discriminate between fluorescing and non-fluorescing (wild) flies, the level of fluorescence was much lower than similarly treated flies that had not been released. This was likely due to the flies eating the fruit and therefore rapidly pushing out the labeled gut contents. As an alternative bait, a second set of bottles was deployed containing ~1 cm of cider vinegar. These were arranged in a line parallel and 4 m away from the fruit traps. While recapture rates were lower (6%) the fluorescence levels were much higher, likely due to the death of the flies due to drowning before they could feed.

## Conclusions

For short-term dispersal studies, raising flies on fluorescein-doped media appears to be a viable alternative to more invasive dusting techniques. While we did not study the effects of the dye on the behavior of the flies, fluorescein is considered to be non-toxic and considering its short (< 72 hrs) residence time in the gut is not expected to have any acute or chronic effects. That same short residence time is also its limiting factor in terms of its use as a label. However, for studies such as ours, which seek to determine dispersal rates and food preferences for flies at time periods up to 24

hours, its use provides a cheap, easy way to tag flies in order to study ecologically important aspects of their biology such as feeding choices and dispersal distances *in-situ*.

References: Crumpacker D.W., 1974, Amer. Midland Sci. 91: 118-129; Dobzhansky T., 1973, Am. Nat. 107: 312–314; Grossfield, J., 1978, Non-sexual behavior of *Drosophila*. In: *The Genetics and Biology of* Drosophila, vol. 2b (Ashburner, M, and T.R.F. Wright, eds.). New York: Academic Press**;** Howe, H.F., and J. Smallwood 1982, Ann. Rev. Ecol. Syst. 13: 201-228; Johnston, J.S., and W.B. Heed 1975, Am. Nat. 109: 207-216; Johnson, M.L., and M.S. Gaines 1990, Ann. Rev. Ecol. Syst. 21: 449-480; Levin, L., 2006, Int. Comp. Biol. 46: 282-297; Maurice, D.M., 1967, Invest. Ophthalmol. Vis. Sci. 6: 464-477; Pesticideinfo.org/Detail_Chemical.jsp?Rec_Id =PC41185; Turelli, M., *et al.* 1985, Dros. Inf. Serv. 63: 131-132; Zimmer-Faust, R.K., *et al*. 1995, Biol. Bull. 188: 111-116.

## Fresh yeast media for *Drosophila* egg-collecting.

**Martínez-Castillo, M.A., L.F. Santos-Cruz, E. Piedra-Ibarra, G. Vázquez-Gómez, I.E. Dueñas-García, L. Castañeda-Partida, and M.E. Heres-Pulido.**
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Fertility and fecundity studies in *Drosophila* have described several egg-collecting techniques with different media as cornmeal agar or banana agar added with animal charcoal for contrast, sprayed with a fine yeast suspension (Spencer, 1937), with colored Carpenter's medium and fresh or dead yeast (Gupta, 1980) or yeast-agar with grape juice (Nichols and Pak, 1985). Egg-collecting media has been added to watch glasses (Delcour, 1969), to spoons (Gupta, 1980), or to Petri dishes with small drops of fresh yeast (Nüsslein-Volhard, 1977; Sabio *et al.*, 2010). For more contrasting of eggs some techniques include small drops of food coloring (Gupta, 1980) or vital stain (Acosta *et al*., 2000). Any kind of egg-collecting media and vials must have these requests: i) to allow collection of large quantities of eggs; ii) to make accessible microscopic observation and incubation; iii) to assure enough amount of food for parents and larvae (Acosta *et al*., 2000). In order to describe a method that combines the features described above, we present a simple method that allows a short preparation time, low cost, besides eggs-collecting, observation facilities and incubation efficiency. Centrifuge plastic tubes of 90 × 30 mm with removable plastic cap, and without thread, to which the conical base was cut (Acosta *et al*., 2000) to replace with a foam plug (Figure 1) were used for egg collecting. The removable cap of the tube is completely filled with fresh yeast, previously sucrose activated and incubated in a water bath at 39°C for 5 min in three successive periods. After placing the fresh yeast in the cap with a sharp object slits are made on the surface (Figure 2). It takes 400 g of fresh yeast to prepare 80 caps with this media. Couples of males and female virgins are placed in vials for 5-6 h without food to prevent egg-laying (Delcour, 1969). About 2 h after placing the media in the removable caps, couples are placed in the vials for egg-collecting, and the tubes are closed with the foam caps. Then the tubes are placed in an incubator at 25°C and 60% relative humidity for 24 h. This method has the following advantages: i) fresh yeast promotes egg-laying and gives parents and larvae enough food; ii) yeast color contrasts with the color of the eggs, preventing exposure to dyes (Figure 3); iii) the media remains with the characteristics required for 72 h; iv) it is easy to remove the cap for complete stereo microscope observation of the media´s surface; iv) caps can be replaced for continuous egg-collecting every 24 h, and caps with eggs can be incubated in new tubes.

Figure 1. Centrifuge plastic tubes with removable plastic cap, and without thread, were used for egg collecting.

Figure 2. Fresh yeast, sucrose activated, is placed in the vials caps and with a sharp object slits are made on the surface.

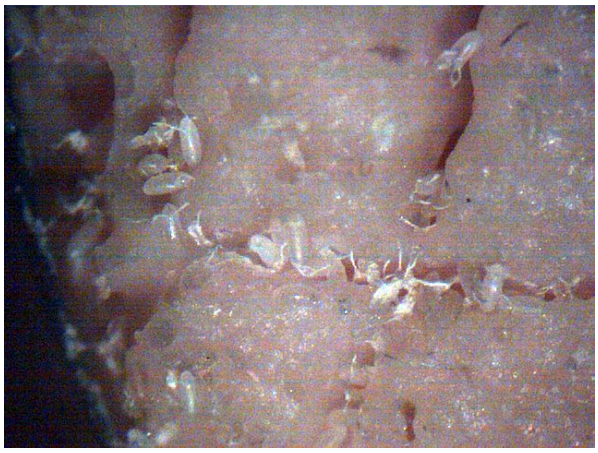

Figure 3. The oviposition is improved by slits in the yeast media and the natural color facilitates the counting of eggs.

References: Acosta, M., M. Costa, and B. Goñi 2000, Dros. Inf. Serv. 83: 174-175; Delcour, J., 1969, Dros. Inf. Serv. 44: 133-134; Gupta, A.P., 1980, Dros. Inf. Serv. 55: 152; Nüsslein-Volhard, C., 1977, Dros. Inf. Serv. 52: 166; Nichols, R., and W.L. Pak 1985, Dros. Inf. Serv. 61: 194-195; Sabio, G., L.A. Quesada-Allué, and M.M. Pérez 2010, Dros. Inf. Serv. 93: 175-178; Spencer, W.P., 1937, Dros. Inf. Serv. 7: 95-99.

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**Technical adaptations of instant medium for *Drosophila.***

**Bizzo, L.,[1,2] T. Vanderlinde,[1] B. Wildemann,[1,2] and D.C. De Toni[1]**. [1]Laboratório de drosofilídeos, sala 304C, Departamento de Biologia Celular, Embriologia e Genética, Universidade Federal de Santa Catarina. Campus Universitário Trindade, 88040-900, Florianópolis, Santa Catarina, Brasil; e-mail: detoni@ccb.ufsc.br; [2]Programa de Pós-Graduação em Biologia Celular e do Desenvolvimento - Centro de Ciências Biológicas, Universidade Federal de Santa Catarina.

Like all holometabolous insects, *Drosophila* occupy two very different habitats during their life cycle. Females lay their eggs on a soft substrate that is suitable for larval development. After the larval stage, the pupal stage sets in, inciting the imagos to emerge. The length of the life cycle varies from one species to another and is dependent on environmental factors such as temperature, type of substrate, and humidity (Powell, 1997).

The ease with which species can be cultured in the laboratory varies considerably. There is a clear correlation between the ease of culturing and the breadth of niche species, as well as with adaptation to be human commensals (Powell, 1997).

Domestic species, including the most studied model, *D. melanogaster*, have a broad range of habitat preferences and do not have particularly narrow nutritional requirements (Powell, 1997). However, there are species of this genus, that, due to the irrestricted ecology, have no reported studies concerning their biological and behavioral features, precisely because of the difficulty of keeping these species in a laboratory environment (Hofmann *et al*., 1984).

Drosophilids can live and forage on many types of substrates, such as flowers, fruit, leaves, sap, cactus, fungus, and also in decomposing organic matter. In a laboratory these substrates are replaced by culture medium in order to facilitate the maintenance and cut costs.

In species from the cardini group that occupy a narrow niche, the culture medium may be a reason for the limited population growth. The length of each genaration of the cardini group species is estimated to be 15 days (Markow, 2006). We were able to maintain our stock on standard agar-yeast-cornmeal medium; however, our strains of *D. polymorpha* did not develop properly, taking more than 25 days to complete their life cycle. Moreover, these conditions enabled some fungi to emerge, and the offspring also became considerably reduced, leading to the loss of many lines. Therefore, we decided to search for a culture medium more suitable for species from this group.

We reviewed the culture media used in stock centers and made some adjustments. We looked for an instant medium with a similar recipe to 4-24 from Carolina Biological. Therefore, we chose potato flakes, which are easily prepared because they do not require cooking, and only need the adding of the liquid portion. However, the liquid portion has to be enriched in order to ensure that the medium has all the essential components for the successful development of flies and also for yeast that eventually comes together with wild flies.

To this purpose, sugar, proteins, and minerals were added to the water, after which eventually an antifungal can beaded in. Because of the mixture's simplicity, independently we obtained a very similar recipe as proposed by Kliethermes *et al*. (2011).

We chose molasses as a source of sugar due to its greater diversity and quantity of sugar compared to regular sugar and honey. Yeasts were also added in, as a source of proteins and minerals. Propionic acid was chosen as an antifugal instead of nipagin (methylparaben), which is insoluble in water. The quantity of each ingredient is listed below:

400 ml water
20 g molasses
6 g yeast (*Saccharomyces cerevisiae*)
1 ml propionic acid

Firstly, we recommend dissolving the yeast and the molasses in a small quantity of water. This mixture needs to be boiled, thus ensuring the medium's sterilization. The remaining water and propionic acid can then be added and stirred. Finally, the liquid portion has to be added to the potato flakes, in a ratio of 2:1, or until it becomes a homogeneous mixture. Potato flakes will absorb the liquid portion in approximately 2 minutes after which the medium will be ready for use.

At first, we tried to use nipagin; however, only after we replaced it for propionic acid could we control the fungal contamination. The amount of propionic acid can be increased up to 4 ml if there are vestiges of fungi.

The medium was tested in *D. melanogaster*, and mainly in the species of the cardini group, such as *D. polymorpha*, *D. cardinoides*, and *D. cardini*, which reported a significantly shorter life cycle (approximately 2 weeks). Fungal contaminations were removed and the strains increased considerably. Species from other groups like annulimana, guarani, and tripunctata also developed successfully in this medium, and we are testing it with more species. We believe this can be an important step for the use of neotropical species as model organisms in genetic, ecological, and evolutionary studies.

References: Hoffmann, A.A., K. Nielsen, and P.A Parsons 1994, Devel. Genet. 4: 439; Kliethermes, C.L., 2011, Dros. Inf. Serv. 94: 132; Markow, T.A., and P.M. O'Grady 2006, Drosophila: *A Guide to Species Identification and Use*; Powell, J.R., 1997, *Progress and Prospects in Evolutionary Biology: The* Drosophila *Model*.

**New versions of trap and bait for the collection of the fig-fly *Zaprionus indianus* Gupta 1970 (Diptera: Drosophilidae).**

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## Abstract

*Zaprionus indianus* was accidentally introduced in Brazil in 1999 and is characterized as pest in purple-fig plantations. It has caused serious damage to the marketing of this fruit in the last decade, since these fruits are used as breeding and feeding sites, whereas this contamination has been favored by fruit morphology. Several measures have been tested against *Z. indianus*; however, none of them showed very satisfactory results. As an alternative, traps have been used, usually from plastic material, with or without light contrasts, possessing appropriate locations for the placement of attractive baits. In order to improve the confection of these traps and their performance, this paper presents a new version with adaptations made in the trap first proposed by Tidon and Sene (1988) and later modified by Medeiros and Klaczko (1999), considering the environmental conditions found in

purple-fig plantations of Valinhos region (São Paulo state), Brazil. These modifications were made through the substitution or addition of new materials, as well as changes in the method of bait preparation. These alterations were tested for a period of one year, through monthly collections in some fig fruit plantations in region of Valinhos. As a result of these modifications, it was observed a higher abundance for the trap model and bait presented here when compared with other traps developed earlier. Thus, it can be concluded that these changes are relevant and can increase the effectiveness in the capture of these drosophilids. Keywords: adaptation, *Ficus carica*, control measure, *Zaprionus indianus.*

## Introduction

The genus *Zaprionus* Coquillett, 1901 is composed of two sub-genera and 56 species (Kato *et al*., 2004) and is characterized by the ability to colonize new territories and for *Zaprionus indianus* Gupta 1970, a drosophilid originated from the Afrotropical region, this ability is favored by the global trade of fruits (Vilela *et al.*, 1999). The occurrence of this species has been recorded in Palaearctic, Eastern, and Australian regions, and recently in the Americas (Tidon *et al*., 2003). In Brazil, its introduction occurred accidentally, being collected for the first time in 1999, in persimmon fruits in the municipality of Santa Isabel, São Paulo state, Brazil (Vilela, 1999).

The fact that this fly is a polyphagous species, infesting still maturing fruits and showing adaptations that enable it to live in adverse weather conditions (Tidon *et al*., 2003; Ferreira and Tidon, 2005), contributed to its rapid distribution throughout Brazil, being currently present in all regions of the country (Kato *et al*., 2004). Since its first record, *Z. indianus* proved to be a potential pest for Brazilian fruit trees, and this estimate was confirmed after a considerable loss in the production of *Ficus carica* L. (Moraceae) "purple-of-Valinhos variety", that harmed both the domestic and exportation markets in 1999 (Stein *et al*., 2003).

The attack occurred by the use of figs, even in their early stage of maturation, as breeding and feeding sites (Stein *et al*., 2003; Belo *et al*., 2009). This infestation is favored by the morphology of these fruits, since this drosophilid uses of the opened ostiole to accomplish oviposition. Despite the fact that it is popularly known as the "fig-fly", the use of this culture by this drosophilid is facultative and its occurrence has been recorded in more than 74 species belonging to 31 different families (Vilela *et al*., 1999). However, this species was characterized as pest only in fig cultures.

Several measures have been tested against *Z. indianus*, like the application of insecticides (Belo *et al*., 2009), protection of the fig ostiole using "bordalesa syrup" and citronella gel, besides the bagging of fruits and obstruction of the ostiole by the use of adhesives (Raga, 2002). All of these have shown some effect on adults or on the immature development of *Z. indianus*, however, without to present satisfactory results.

As an alternative procedure, some drosophilid traps have been constructed, which can be used both for collections, in which the specimens are kept alive (Tidon and Sene, 1988; Klaczko and Medeiros, 1999; Roque *et al*., 2011) as for control, in which specimens are killed (Brotto, 2010). These traps are usually made from plastic materials and may present light contrasts or not, with appropriate locations for the placement of attractive baits.

In order to improve the confection and performance of these traps, this paper presents a new version with changes in relation to the trap first proposed by Tidon and Sene (1988) and then modified by Medeiros and Klaczko (1999). To carry out these adjustments, the environmental conditions found in the purple-fig monocultures in the region of Valinhos, São Paulo state, Brazil, were considered.

## Materials and Methods

*A*) *Making of traps*

The trap was developed using the following items: (1) three clean and transparent 2L PET bottles, being one with lid; (2) a pantyhose of the type "sneaker with elastic"; (3) a strip of filter paper with approximately 18 cm, applied with hot glue under the cover of the trap to retain moisture within the bottles; (4) mosaic glue applied at the junction of the transparent chambers, plus 35 cm of a string of foam mounted on the mosaic glue; (5) transparent adhesive tape to seal externally the junction of the trap chambers; (6) 50 cm of wire number 16, of which one extremity was attached to the mouth of the bottle, and from the other, a hook for hanging the trap in the branches was made; (7) 80 cm of textile elastic number 12, tied on the wire around the mouth of the bottle, to sustain the bait chamber with the rest of the trap (Figure 1a).

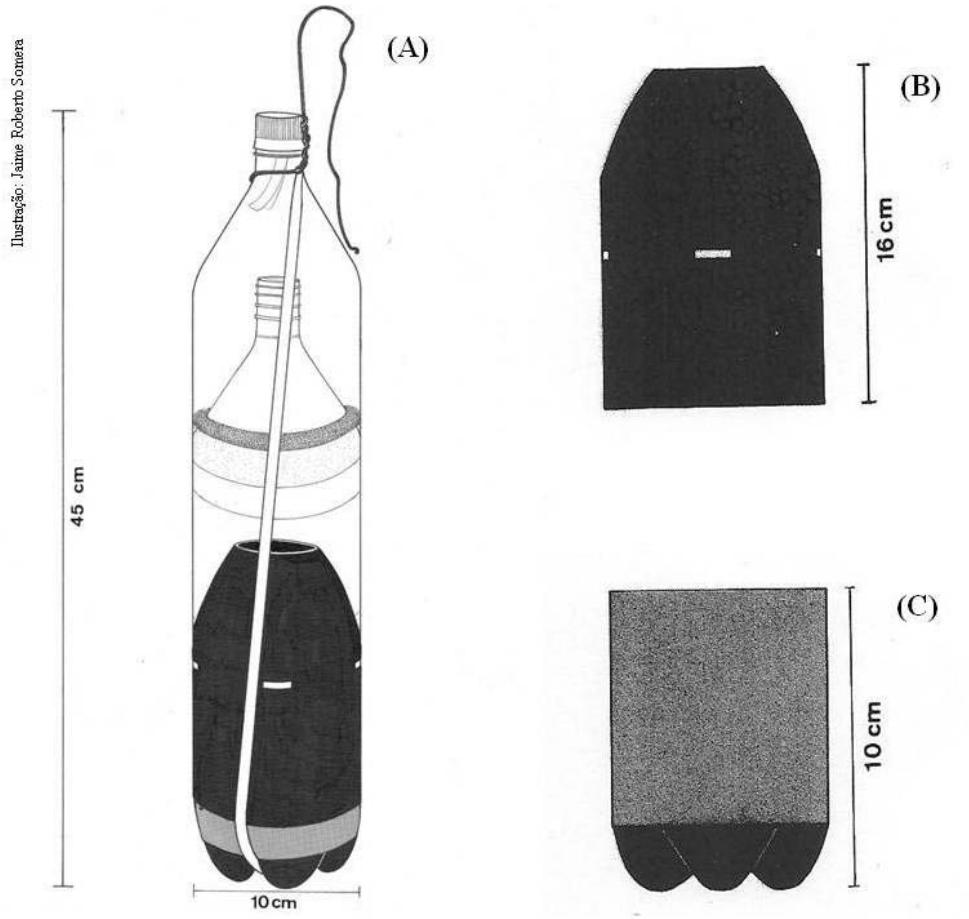


Figure 1. New version of the trap for the capture of the fig-fly *Zaprionus indianus* proposed by Matavelli and Von Zuben (2012): measurements and illustrations.

Since this model of trap presents contrast of light, some portions of the bottles were painted with two applications of water-based white primer for PET (and for other materials), with 48 hours

intervals, and some applications of water-based matte black acrylic at intervals of 24 hours, to obtain completely black parts.

Of the three transparent PET bottles, two were cut at 22 cm from their mouths (chambers 1 and 2). From the third bottle, mouth was cut, and from this cut, a new was made at 16 cm (chamber 3) (Figure 1b). In the last bottle, four windows of 0.5 cm × 2.0 cm were done, which allowed the entry of the insects. The base of one of the bottles was cut at 10 cm (Figure 1c). A piece of filter paper was pasted in a lid of chamber. Both the chamber 3 and the base of the bottle were painted black. At the junction of the chambers 1 × 2, mosaic glue was added (dotted area) and foam cord (shaded area) (Figure 1a). Between the chambers1 × 2 and 2 × 3, adhesive tape was passed externally to the bottle. A pantyhose was placed between the chamber 3 and the base of the bottle (Figure 1a), being the baits placed in this base (Figure 1c). The wire was tied at the mouth of the chamber 1 and an elastic band attached to this wire (Figure 1a).

*B*) *Preparation of the bait to be used in the trap*

The bait used in this trap was composed of 7 kg of mature banana (type "Nanica"), 3.5 L of water, 70 g of yeast (*Saccharomyces cerevisiae*). All ingredients were beaten in a blender and stored in PET bottles for at least 36 h. For this storage, the amount of bait placed in each plastic bottle not exceeded 400 ml, due to the large fermentation of this material. Because of this, the whole air contained inside the bottle was removed every 3 hours. With these proportions, the yield was 40 bait portions, and also a reserve quantity. The bottles containing the baits were transported to the field, and using a measuring cup, 150 ml of bait were placed in the base of each bottle. After the placement of the baits, the base was covered by pantyhose, involving the whole pot (extremely firm) in function of its elastic. Unlike Klaczko and Medeiros (1999), the pantyhose in question was not pressed against the bait, to prevent adherence between both. After fixing the pantyhose, the chambers 1, 2 and 3 were bound by textile elastic to the base of the bottle, and the traps were suspended in the branches by hooks formed by the wire, at approximately 35 cm height.

As also occurred in the traps proposed by Tidon and Sene (1988) and Medeiros and Klaczko (1999), the drosophilids are attracted by the smell of the bait placed in the dark portion of the trap (base of the bottle), and by the contrast of light, the flies fly toward the light in attempt to leave the trap and were trapped in the retention chambers 1 and 2. After the specified hours of exposure in the field of these traps, these were removed, the bait discarded on the ground and covered with soil and the pantyhose inverted (to the chamber 3), thus closing the openings that allowed the entry and exit of the insects. These openings remain closed until the withdrawal of the insects from the retention chambers.

## Results

These traps were utilized on purple-fig plantations, in the municipality of Valinhos, São Paulo state, for a period of one year (September 2010 to August 2011). The durability of the bottles proved to be more than a year, as well as the wires. However, the painting needed repairs after this period. The elastics were replaced every six months, and the pantyhose every four months, since exposure to sun and rain damaged its elasticity, which could adversely affect the effectiveness of capture and transport of the material collected.

Their efficiency can be verified by analyzing the number of individuals of *Z. indianus* that were collected, reaching to 1,509 in a single trap. This number is much higher than when drosophilids are analyzed in a general way. To achieve this value, several adaptations in relation to

the models proposed by Tidon and Sene (1988) and Medeiros and Klaczko (1999) had to be performed. For the formulation of these changes (Table 1), the environmental conditions found in the area of captures were especially considered.

Table 1. Characteristics of the traps proposed by Tidon & Sene (1988), Medeiros & Klaczko (1999), and of the present work.

| Utilized Material | TIDON & SENE (1988) | MEDEIROS & KLACZKO (1999) | MATAVELLI & VON ZUBEN (2012) |
|---|---|---|---|
| PET bottles 2L | 2 | 3 | 3 |
| Filter paper | Optional | Present | Present |
| Cotton sttoper | Optional | Absent | Absent |
| Nylon thread | Present | Present | Absent |
| Wire | Absent | Absent | Present |
| Adhesive tape | Absent | Absent | Present |
| Elastic | Absent | Absent | Present |
| Cord of foam | Absent | Absent | Present |

**Discussion**

In an attempt to develop a simple and effective method to capture drosophilids in tropical environments, Tidon and Sene (1988) developed a trap able to retain and keep alive all drosophilids collected. This trap of easy construction was composed of two plastic bottles attached, in which the placement of attractive baits for drosophilids was allowed. After this first type of trap, several other models for captures of drosophilids have been proposed, including open and closed traps (Penariol *et al*., 2008), McPhail type, based on PVC tubes (Martins *et al*., 2008) or based on PET bottles (Tidon and Sene, 1988; Medeiros and Klaczko, 1999). The latter, in turn, have shown a high durability in the field, and great resistance to attack by other animals and adverse environmental conditions (Tidon and Sene, 1988; Medeiros and Klaczko, 1999).

In 1999, Medeiros and Klaczko realized that some traps used for capture of *Drosophila* had problems in collecting the *tripunctata* group, and these authors began a battery of tests from the model proposed by Tidon and Sene (1988), in order to find a trap that minimized this problem. After eight laboratory and one field experiments and 32 proposed models, Medeiros and Klaczko (1999) came to a trap that had, among other criteria, the greater abundance of flies, a more homogeneous value for the frequency of the species collected, a good capacity of retention for these animals and some protection for flies from the risk of death.

The captures of drosophilids in the region of Valinhos, São Paulo state, began in purple-fig plantations based on the trap developed by Medeiros and Klaczko (1999). However, there was a need for new changes from the first captures, both in the preparation of the traps as in the bait.

*Bait preparation*

Tidon and Sene (1988) used only fermented mature banana as bait, while Medeiros and Klaczko (1999) used mature banana type "Nanica" with yeast (*Saccharomyces cerevisiae*), fermented for 36 hours. Both methods were tested here at the beginning of the captures in fig plantations. As the region of Valinhos is characterized, according to Koeppen's classification, as a region of humid temperate climate with dry winter and hot summer (CEPAGRI), there was the need of further dilution of the bait, since when used both the methodologies described earlier, the baits were drying, stiffening and not releasing more odors to attract insects. So, the dilution of the bait in water was tested, in order to find a consistency that does not dry with the exposure time in the field, and at the same time, keep the odor that can attract animals. This dilution occurred in a ratio of two measures of bananas to a measure of water (2:1).

*Bait utilization*

According to the methodology presented by Medeiros and Klaczko (1999), the bait being placed in the trap should be pressed against the pantyhose in order to join to it. In surveys carried out in Valinhos, São Paulo state, this procedure was tested. However, after an exposure time and because of the climate in the region, the bait attached to the pantyhose turned into a dry film that prevented the release of odor. Thus, this procedure was no longer running.

*The trap*

The adaptations presented here were necessary, mainly due to the ambient temperature, relative humidity of the air and high levels of wind in the capture region considered. Unlike the trap proposed by Medeiros and Klaczko (1999), which used nylon thread for support, we utilized adhesive tape between chambers 1 × 2 and 2 × 3 for this purpose. This fact prevented that these chambers got lost due to the wind. The nylon thread was also replaced by elastic, which aided in the junction of the bait pot to the other parts of the trap, fixing them more tightly. Moreover, the remaining threads that were used to hold the trap in the branches of fig plants were replaced by wire, which allowed a lower mobility of the trap, even in the presence of strong wind. This fact helped to prevent leakage of individuals according to the movement of the trap.

In addition to the replacement of the nylon threads by other materials, it was necessary to adjust the type and quantity of glue to be used between the chambers 1 × 2. Initially, several varieties of glue were tested in order to determine which is more resistant to environmental conditions to which traps would be exposed. The results show that the best was the glue used in handicraft (mosaic). A quantity of glue similar to that of Medeiros and Klaczko (1999) was applied; however, it was noted that this amount of glue had a very narrow supporting surface to hold collected individuals, and in more abundant months, some animals could be crushed. Moreover, in pilot collections, it was found that the presence of many individuals within this chamber increased its moisture content and caused the adherence of some individuals in the glue. Thus, a cord of foam was applied on this layer of glue. This cord has not only increased the support surface of the animals, it also prevented direct contact with the glue, thus facilitating the removal of the insects from these traps.

The utilization of the pantyhose was maintained to prevent contact of drosophilids with the bait. However, instead of using a piece of pantyhose as in the model of Medeiros and Klaczko (1999), we choose here the use of pantyhose of the type "sneaker with elastic", because of the elastic in the end. So, in addition to the better adherence to the bottles, when removed from the field and inverted to chamber 3, this kind was better fixed, preventing its sliding and a possible exposure of its openings. However, the use of pantyhose was contested in a new model of trap proposed by Roque *et al*. (2011), in which this use was abolished. According to these authors, the utilization of

pantyhose affects the sampling, since beetles are able to tear it up, leaving the animal collected in contact with the bait. However, the model proposed here was used in the field 480 times (40 traps/month) and this phenomenon has never been observed.

*About its effectiveness*

Penariol *et al*. (2008) showed in their work that the efficacy of closed traps is greater than the open ones, with respect to population abundance and species richness, and suggested a standardization of drosophilid captures, using this type of trap. The effectiveness observed in the model of trap presented here was larger than of the other already existing closed traps. Tidon and Sene (1988) collected about 850 drosophilids per trap using as bait only a fermented banana, and Medeiros and Klaczko (1999) collected more than 1,000 Drosophilidae per trap using banana as bait, whereas with the present version, 1,509 specimens (only of *Z. indianus*) were collected per trap, using diluted bait.

## Conclusions

The obtained results and the efficacy demonstrated by the new trap and bait here proposed, compared with previous versions, allow us to conclude that the enhancements discussed in this work were relevant and increased the performance of traps in the capture of *Z. indianus* and other drosophilids.

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# Mutation Notes

## Linkage relationships of spontaneous mutations in *Drosophila willistoni.*

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**Abstract**

New mutations of *Drosophila willistoni* are reported. Of 80 visible spontaneous mutants, mostly isolated from natural or laboratory populations of *D. willistoni*, 42 genes have been assigned to chromosomes. Twenty-one mutants map on chromosome 2, fifteen on chromosome 3, and six on the X chromosome. The isolation of mutants with a particular phenotype and data on the genetic interaction observed in eye color mutants were used to propose the linkage groups of *D. willistoni* in relation to *D. melanogaster*.

**Introduction**

The *willistoni* group comprises a group of cryptic species that inhabits the Neotropical region and has been the subject of evolutionary research studies for the past century (Ehrman and Powell, 1982; Cordeiro and Winge, 1995). *D. willistoni* is a member of the *willistoni* species subgroup, which inhabits domestic and natural environments of Uruguay (Goñi *et al.*, 1997, 1998).

The karyotype of *D. willistoni* is characterized by two pairs of autosomes (chromosomes 2 and 3) and a pair of heteromorphic sex chromosomes, the X chromosome and the Y chromosome. The earliest information on *D. willistoni* mutants and linkage maps was reported by several authors in the early 1920's. Lancefiled and Metz (1922) and Ferry *et al.* (1923) described 34 X-linked and 19 autosomal mutants (11 in chromosome 2, and 8 in chromosome 3) isolated from strains brought from Cuba in 1915. Later, Spassky and Dobzhansky (1950) described 21 X-linked mutants, 22 mutants on chromosome 2, and 11 on chromosome 3, some of them of spontaneous in origin from Belem, Pará, Brazil and others, which were X-ray-induced. The authors named these mutants by virtue of their phenotypic similarities with the classical mutants of *D. melanogaster*, as well as to those mutants described by Lancefield and Metz (1922). Subsequently, Poulson and Counce (1960) described additional mutants of *D. willistoni*. Unfortunately all of the mutant strains reported by these authors are no longer available.

Müller (1940) proposed that the ancestral genome of *Drosophila* species was composed of five pairs of acrocentric chromosomes and a pair of dot chromosomes. Using *D. melanogaster* as reference, he also proposed the letters A-F to designate each of the five chromosome arms and mini or "dot" chromosome, and used that nomenclature to identify homologous linkage groups within different species of of the genus *Drosophila*. Subsequently, a homology relationship between the chromosome arms of *D. willistoni* and *D. melanogaster* was proposed (Sturtevant and Novitsky, 1941). According to these authors, the X chromosome of *D. willistoni* is the result of the fusion of Müller elements A and D (chromosome X and 3L of *D. melanogaster*), chromosome 2 is the result of

the fusion of Müller elements B and C (arms 2L and 2R of *D. melanogaster*), and chromosome 3 is composed by elements E and F (chromosome 4 and 3R of *D. melanogaster*). This proposal was recently reevaluated by Schaeffer *et al*. (2008) using physical and genetic markers to anchor the genome assembly scaffolds to the polytene chromosome maps of 12 *Drosophila* species that have sequenced genomes. In that analysis, the scaffolds are oriented and joined on the basis of the conserved synteny at the ends of the scaffolds using the location of a single locus or the order of genetic marker(s) on the genetic map. In spite of using new markers on most of the chromosome arms of *D. willistoni*, Schaeffer *et al*. (2008) point out the paucity of genetic and physical markers available in this species resulting in a provisional orientation of the scaffolds. In addition to the aforementioned global analysis, Papaceit and John (1998), using molecular techniques and *in situ* hybridization, mapped several markers on chromosome 3 of *D. willistoni* to characterize the E-F Müller element fusion.

Since 1998, the laboratory of *Drosophila*, Evolutionary Genetics Section has been working in the isolation of spontaneous mutations in *D. willistoni*, which were reported in Goñi *et al.*, (2002) and Parada and Goñi (2003). More recently, Soler (Bachelor Thesis, 2012) presented new mutants along with relevant linkage data for autosomal mutations of *D. willistoni*. We report here a study of the formal genetics of *D. willistoni*: the collection of a broad spectrum of spontaneous mutations, mostly isolated from natural populations, and their linkage relationships.

## Material and Methods

*Flies*

Eighty spontaneous mutants of *D. willistoni* were analyzed. The majority of these were isolated from isofemale lines or mass mating cultures from natural or laboratory populations collected in several localities of Argentina, Brazil, and Uruguay, since 1998. Wild type strains used: WIP-4, from Ilha das Cobras, Bahia, Brazil, 1970, which has been kept in culture for many years, and GDH (Guadalupe), a monomorphic strain used in the recent citomap revision and genomic studies (Schaeffer *et al.*, 2008) (obtained by courtesy of Dr. V.L.S. Valente).

*Mutant descriptions*

The phenotypes of mutants of *D. willistoni* (Ferry *et al.*, 1923; Lancefield and Metz, 1922; Poulson and Counce, 1960; Spassky and Dobzhansky, 1950), *D. paulistorum* (Malogolowkin and Ehrman, 1960, see the revised genetic map of *D. willistoni* proposed by Dobzhansky and Powell, 1975), and the classical mutant descriptions of *D. melanogaster* (Lindsley and Zimm, 1992; The FlyBase Consortium, 2012) were used as a reference for a comparison and aid in the description of the new mutants of *D. willistoni* reported here.

*Allelism test*

Genetic crosses between individuals of mutant strains with similar phenotype were done to test for complementation in the $F_1$ progeny. In each experiment, two replicas of each reciprocal cross were performed. In some cases, individuals from wild strains were used in crosses with mutant strains to test for the recessive/dominance character of the mutation, or the segregation of more than one mutant phenotype from a mutant strain.

*Linkage test*

Mutant phenotype criteria affecting adult morphology (wing shape, eye color, eye shape, body color, or others) allowed us to select some strains as reference mutations for linkage analysis.

Crosses were performed between individuals from reference mutations to unmapped mutations. For each cross the $F_1$ progeny was examined. Male mutants appearing in the $F_1$ progeny of mutant females indicated an X-linked mutation. If the $F_1$ progeny were all wild type, an $F_1 \times F_1$ cross was performed, and the $F_2$ progeny analyzed. The presence of four different phenotypic classes indicated that the mutant genes are unlinked and thus located on different autosomal chromosomes. The data obtained allowed us to identify the linkage groups of mutants.

*Eye color mutant genetic interaction test*

Crosses between individuals with eye color mutations were performed. The $F_1$ progeny was analyzed. If the $F_1$ was wild type, $F_1 \times F_1$ cross was done. The presence of a new eye color in $F_2$ progeny was interpreted as the occurrence of eye color genetic interaction between the tested mutations. When testing for genetic interactions between eye color mutations that are linked on the same chromosome, the presence of a novel eye color in individuals in the $F_3$ progeny was interpreted as resulting from crossing over in the previous generation. In *D. melanogaster* crossing over is restricted to females; however, two observations suggest the existence of crossing over in males of *D. willistoni*. França *et al*. (1968) reported 0.4% recombination between heterozygous inversions in males and females of *D. willistoni*. More recently, Colares Dos Santos *et al*. (2004) reported a meiotic configuration in spermatocytes, that they interpreted as the result of exchange within the 2LH inversion loop in larvae from the wild type (G3) strain collected in the State of Santa Catarina, Brazil.

*Experimental conditions*

For mating experiments virgin flies were collected every three hours and mated to young males. In general, virgin females and young males used in the genetic crosses were selected with the help of an aspirator. The progeny were anethesized with triethylamine vapors (Fresia *et al.*, 2002). Progeny were scored only up to the 21$^{st}$ day post crossing to avoid overlapping generations. Wild and mutant strains and experimental crosses were maintained at 23-25$^{o}$C with standard cornmeal-yeast-agar media.

## Results and Discussion

The detailed list of (new) mutants and alleles is presented below. It includes relevant information concerning genetic interaction of eye color mutants, *e.g*., the observed interaction between *brown* and *cardinal* mutations, and others (Table 1, Figure 1) that were evaluated, among other genetic data to construct the linkage groups.

This list includes data on 42 mutations: twenty-one map on chromosome 2, fifteen on chromosome 3, and only six map on the X chromosome. The X linked loci include new *white* mutants and *yellow* alleles. Chromosome 2 linked mutants include some mutations that were previously mapped by Spassky and Dobzhansky (1950), *brown* and *plexus*, and new mutations with good homology with *D. melanogaster*: *blistered*, *cinnabar*, *eye absent*, *jaunty*, *orange*, *purple* and *speck*. Chromosome 3 linked mutants include several new mutations: *blistery*, *claret*, *ebony*, *glass*, *hedgehog*, *kayak*, *rough*, *rosy*, and *spineless*. Some of these mutants are shown in Figure 2. At present, the genome sequence of *D. willistoni* and the sequence annotation in *D. melanogaster* (see FlyBase, Dmel annotation version 4.3) provide information of orthologous genes for most of these autosomal mutations. In the case of *cinnabar*, the only information available is the annotated transcript of *D. melanogaster*. The linkage data of new mutants reported here along with the availability of new mutant strains provide an opportunity to review the genetic and physical map of *D. willistoni*.

## X Linked Mutants:

### *cm: carmine*

phenotype: Eye color: almost wild type in young flies to dull carmine in old flies. Pseudopupil less evident than wild type.

$cm^{FA30.05}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2005.

### *cv: crossveinless*

phenotype: Anterior and posterior crossvein absent or traces only present. Similar to *crossveinless* of *D. melanogaster.*

$cv^{EM1.00}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2000.
references: Parada and Goñi (2003).

### *g: garnet*

phenotype: Eye color: translucent brownish, darkening with age. No pseudopupil.

$g^{EM1.00}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2000.
references: Referred as *pink* mutation in Goñi *et al.* (2002); Parada and Goñi (2003).

### *l-1: lobe-1*

phenotype: Variable reduction in eye size. Eyes slightly smaller with a nick in the anterior edge. The lower part of eye is more reduced than upper part. Overlaps wild type. Similar to *Lobe* of *D. melanogaster.*

$l\text{-}1^{EM1.00}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2000.
references: Parada and Goñi (2003).

### *w: white*

phenotype: Eye color: white. Colorless ocelli. Eye color: dull light red with *cinnabar* at eclosion, colorless ocelli (Table 1). Similar to *white* of *D. willistoni* (Spassky and Dobzhansky, 1950).

$w^{EM1.00}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2000. Segregates a second linked mutant, referred as *dull red* mutation in Goñi *et al.* (2002).
references: Goñi *et al.* (2002); Parada and Goñi (2003); Ludwig *et al.* (2003).

$w^{TB44.02}$

origin: Segregates from the *eye absent* [$eya^{TB44.02}$] mutant strain. Low fertility.

$w^{a\,Q14F11}$

phenotype: Eye color: yellow-orange. Pale ocelli. Similar to *white apricot* of *D. melanogaster.*
origin: Buenos Aires City, Argentina, 2006.

$w^{cf\,Ey10.00}$

phenotype: Eye color: pinkish-red, darkening to sepia-like in old flies. Similar to *white coffee* of *D. melanogaster.*
origin: Faculty of Agronomy, Montevideo City, Uruguay, 2000.
references: Ludwig *et al.* (2003).

### *y: yellow*

phenotype: Wings and body cuticle yellow. Puparia much lighter than wild type. Similar to *yellow* of *D. melanogaster.*

$y^{EM1.00}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2000.
references: Goñi *et al.* (2002); Parada and Goñi (2003); Ludwig *et al.* (2003).

$y^{TB46.02}$

origin: Laguna Negra, Rocha, Uruguay, 2002.
references: Parada and Goñi (2003).

$y^{Q14F1}$

origin: Buenos Aires City, Argentina, 2006.

## Chromosome 2 Linked Mutants

### *amy: amethyst*

phenotype: Eye color: dull light purplish in young flies, darkening to purple with age. Pseudopupil less evident than wild type. Eye color: light reddish with *cardinal* in young flies (Table 1).

$amy^{RLN1.00}$

origin: Laguna Negra, Rocha, Uruguay, 2000.

$amy^{SG3.03}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2003.

### *bs: blistered*

phenotype: Wings inflated with hemolymph to produce blisters and vesicles. Venation weak. Texture discolored and muddy in appearance. Poor viability at low temperature. Similar to *balloon* of *D. willistoni* (Ferry *et al.*, 1923) and to *blistered* of *D. melanogaster.*

$bs^{D14.99}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 1999.

### *bur: burgundy*

phenotype: Eye color: dull brownish red in young and old flies. Pseudopupil less evident than wild type. Similar to *burgundy* of *D. melanogaster.*

$bur^{SMV28.00}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2000.

### *bw: brown*

phenotype: Eye color: light brownish in young flies to light reddish-brown color in old ones. No pseudopupil. Eye color: white with *cardinal* in young flies to blotchy brownish in old flies. Eye color: white with *cinnabar*, colorless ocelli (Table 1). Similar to *brown* of *D. willistoni* (Spassky and Dobzhansky 1950) and to *brown* of *D. melanogaster*.

$bw^{SG23.00}$, $bw^{Sy11.03}$, $bw^{Q51F13}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2000, 2003 and 2009, respectively.

### *Cl: Clipped*

phenotype: Wing margins notched, most often along marginal vein. Similar to *Clipped* of *D. willistoni* (Ferry *et al.,* 1923).

$Cl^{ES13.99}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 1999.
references: Parada and Goñi (2003); Ludwig *et al.* (2003).

### *cn: cinnabar*

phenotype: Eye color: bright red, approaching to wild type with age. Colorless ocelli. Eye color: white with *brown*, colorless ocelli. Eye color: apricot with *claret*. Eye color: light apricot with *maroon*. Eye color: dull apricot with *purple* at eclosion, colorless ocelli. Eye color: Light creamy-apricot with *rosy*. Eye color: dull light red with *white* at eclosion, colorless ocelli (Table 1). Similar to *orange* of *D. willistoni* (Spassky and Dobzhansky, 1950), *orange* of *D. paulistorum* (Malogolowkin and Ehrman, 1960) and *cinnabar* of *D. melanogaster*.

$cn^{REB1.00}$

origin: Laguna Negra, Rocha, Uruguay, 2000.
references: Parada and Goñi (2003).

$cn^{SM35.00}$, $cn^{SL7.01}$, $cn^{FA2.05}$, $cn^{FA22.05}$, $cn^{Q51F13}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2000, 2001, 2005 ($cn^{FA2}$, $cn^{FA22}$) and 2009, respectively.

$cn^{TB26.02}$

origin: Laguna Negra, Rocha, Uruguay, 2002.

$cn^{Q46F32}$

origin: San Pablo City, Brazil, 2007.

### *cp-2: clipped-2*

phenotype: Wing margins notched, most often along marginal vein. Similar to *clipped* of *D. melanogaster*.

$cp\text{-}2^{TB46.02}$

origin: Laguna Negra, Rocha, Uruguay, 2002.
references: Parada and Goñi (2003).

### *cui: curvi*

phenotype: Distal half of wings curved upward. Temperature sensitive expression. Curl is strong if wings unfolds at 24ºC but weakened or overlap wild type if wings unfold at 17ºC. At low temperature females do overlap wild type more than males. Very good viability. Similar to *curvi* of *D. melanogaster.*

$cui^{Q46M6}$

origin: San Pablo City, Brazil, 2007.

### *dke: dark eye*

phenotype: Eye color: dull dark red in young flies to brownish red in old flies. Less evident pseudopupil than wild type. Eye color: bright reddish with *cardinal* at eclosion (Table 1). Similar to *dark eye* of *D. melanogaster*.

*dke*$^{SL13.01}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2001.

*dke*$^{TB28.02}$

origin: Laguna Negra, Rocha, Uruguay, 2002.

### *dl-2: delta-2*

phenotype: Wing's second vein widened at their junctions with the margin to form delta-like structure. Mutant phenotype stronger in females than males. Similar to *Delta* of *D. willistoni* (Spassky and Dobzhansky, 1950) and *Delta* of *D. melanogaster*.

*dl-2*$^{Q46F9}$

origin: San Pablo City, Brazil, 2007.

### *dre: dark red*

phenotype: Eye color: dark red in young flies, darkening with age, but lighter than the *dark eye* mutation. Similar to *dark red* of *D. melanogaster*.

*dre*$^{SGV8.00}$

origin: Santa Lucia, Canelones, Uruguay, 2000.

*dre*$^{SL8.02}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2002.

### *eya: eye absent*

phenotype: Eye facets almost or completely absent, from flies with no eyes to those having few ommatidia. Head development normal; antennae and ocelli present. The aristae of the antennae is thinner and shorter than wild type. Similar to *eye absent* of *D. melanogaster*.

*eya*$^{TB44.02}$

origin: Laguna Negra, Rocha, Uruguay, 2002.
references: Mistakenly referred as the *eyeless* mutation in Ludwig *et al.* (2003).

### *j: jaunty*

phenotype: Wings curved upward throughout their length but less twisted than the classical *Curly* mutation of *D. melanogaster*. The wings are thick and with opaque texture. In general, the wings do not entirely unfold. Similar to *jaunty* of *D. willistoni* (Ferry *et al.*, 1923) and *jaunty* of *D. melanogaster*.

*j*$^{ST12.01}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2001.

*j*$^{TB38.02}$

origin: Laguna Negra, Rocha, Uruguay, 2002.

### *or: orange*

phenotype: Eye color: bright yellowish orange, browner with age. No pseudopupil. Colorless ocelli. Eye color: dull apricot with *claret*, colorless ocelli. Eye color: yellowish apricot with *pink* (Table 1). Similar to *orange* of *D. melanogaster*.

*or*$^{FA7.05}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2005.

### *pab: pale body*

phenotype: Whitish body color. Similar to *Pale body* of *D. melanogaster*.

*pab*$^{S46.99}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 1999.

### *po: pale ocelli*

phenotype: Ocelli colorless. Similar to *pale ocelli* of *D. melanogaster*.

*po*$^{Riv1.03}$

origin: Rivera City, Uruguay, 2003.

### *pr: purple*

phenotype: Eye color: purple at eclosion, darkening to purplish ruby with age. No pseudopupil. Eye color: dull apricot with *cinnabar* at eclosion, colorless ocelli (Table 1). Similar to *purple* of *D. willistoni* (Spassky and Dobzhansky, 1950) and to *purple* of *D. melanogaster*.

*pr*$^{Ey3.99}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 1999.

### *pu: punch*

phenotype: Eye color: translucent purplish at eclosion darkening with age. Similar to *Punch* of *D. melanogaster*.

$pu^{REM2.00}$

origin: Laguna Negra, Rocha, Uruguay, 2000.

### *px: plexus*

phenotype: Wings have network extra veins, specially towards tips and margins. First posterior cell between L3 and L4 widen toward tip. Similar to *plexus* of *D. melanogaster*.

$px^{SGV1.00}$

origin: Santa Lucia, Canelones, Uruguay, 2000.

$px^{SG1.01}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2001.

### *sp: speck*

phenotype: Axils of wings have black specks. Similar to *speck* of *D. melanogaster*.

$sp^{98e3.98}$

origin: Segregate from the *claret* $[ca^{98e3.98}]$ mutant strain. Females have poor fertility.

### *wr: wrinkle*

phenotype: Wings wrinkled, blistered and curved upward. Temperature sensitive expression. Overlaps wild type. At low temperature poor viability. Similar to *wrinkle* of *D. melanogaster*.

$wr^{TB46.02}$

origin: Laguna Negra, Rocha, Uruguay, 2002.
references: Parada and Goñi (2003).

## Chromosome 3 Linked Mutants

### *by: blistery*

phenotype: Wings with blisters in the proximal and medial regions. Wing surface dusky. Temperature sensitive expression. Females do overlap wild type more than males. Very good viability. Similar to *blistery* of *D. melanogaster*.

$by^{EB1.03}$, $by^{Q52F56}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2003 and 2009, respectively.

### *ca: claret*

phenotype: Eye color: bright brownish scarlet in young flies, approaching sepia-like with age. No pseudopupil. Eye color: apricot with *cinnabar*. Eye color: dull apricot with *orange*, colorless ocelli (Table 1). Similar to *claret* of *D. willistoni* (Spassky and Dobzhansky, 1950) and to *claret* of *D. melanogaster*.

$ca^{98e3.98}$, $ca^{ES13.99}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 1998 and 1999 (Parada and Goñi, 2003), respectively.

### *ca-l: claret-like*

phenotype: Eye color: bright brownish scarlet, but lighter and translucent than the *claret* mutation of *D. willistoni* reported here. No pseudopupil.

$ca\text{-}l^{Q15F10}$

origin: Buenos Aires City, Argentina, 2006.

$ca\text{-}l^{Q52F63}$, $ca\text{-}l^{Q52F51}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2009.

### *cd: cardinal*

phenotype: Eye color: bright yellowish vermilion at eclosion to dull red in old flies. No pseudopupil. Colorless ocelli. Eye color: light reddish with *amethyst* in young flies. Eye color: white with *brown* in young flies, to blotchy brownish in old flies. Eye color: bright reddish with *dark eye* at eclosion (Table 1). Similar to *cardinal* of *D. melanogaster*.

$cd^{SG12.00}$, $cd^{SG4.01}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2000 and 2001, respectively.

$cd^{TB40.02}$

origin: Laguna Negra, Rocha, Uruguay, 2002.

### *e: ebony*

phenotype: Body color grayish-black. Puparia much lighter than wild type. Larvae show wild type color of spiracle sheaths. Good viability at 25°C. Similar to *ebony* of *D. paulistorum*

(Malogolowkin and Ehrman, 1960) and to *ebony* of *D. melanogaster*.

$e^{REB1.00}$

origin: Laguna Negra, Rocha, Uruguay, 2000.
references: Parada and Goñi (2003).

$e^{SB26.02}$

origin: Solis, Maldonado, Uruguay, 2002.
references: Parada and Goñi (2003).

$e^{Q14F23}$

origin: Buenos Aires City, Argentina, 2006.

### *gl: glass*

phenotype: Eye color: blotchy with few patches of red, and creamy-white pigment. Eyes variable in size with a typically glassy surface. Few ommatidial bristles. Subtle anterior notch. Colorless ocelli. Reduced fertility. Similar to *glass* of *D. paulistorum* (Malogolowkin and Ehrman, 1960) and to *glass* of *D. melanogaster*.

$gl^{TB14.02}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2002.

### *hh: hedgehog*

phenotype: Eye size reduced and rough, with irregular facets. Ommatidia unordered. Ommatidial bristles absent except on the posterior border. Fragmented pseudopupil. Variable expression in both sexes. Similar to the *hh[bar3]* allele of *D. melanogaster*.

$hh^{Q02F7}$, $hh^{Q46M8}$

origin: San Pablo City, Brazil, 2006 and 2007, respectively.

### *kay: kayak*

phenotype: Mesothorax with a dorsal-antero-posterior cleft, which causes a gap in the bristle pattern. Temperature sensitive expression. Good viability and fertility. Similar to *kayak* of *D. melanogaster*.

$kay^{EB2.02}$, $kay^{Q51F14}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2002 (Parada and Goñi, 2003) and 2009, respectively.

### *ma: maroon*

phenotype: Eye color: dull ruby, approaching to wild type with age. Eye color: light apricot with *cinnabar* (Table 1). Similar to *maroon* of *D. melanogaster*.

$ma^{SB27.02}$

origin: Solis, Maldonado, Uruguay, 2002.

$ma^{TB40.02}$, $ma^{TB77.02}$

origin: Laguna Negra, Rocha, Uruguay, 2002.

$ma^{Riv9.03}$

origin: Rivera City, Uruguay, 2003.

$ma^{FA1.05}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2005.

### *p: pink*

phenotype: Eye color: similar to wild type in young flies, approaching to dull ruby with purplish tint in old ones. No pseudopupil. Eye color yellowish apricot with *orange* (Table 1). Similar to *pink* of *D. melanogaster*.

$p^{FA33.05}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2005.

### *ro: rough*

phenotype: Eyes rough with irregular facets and black specks. Similar to *rough* of *D. melanogaster*.

$ro^{FA11.05}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2005.

### *ry: rosy*

phenotype: Eye color: purplish reddish brown in young flies, darkening with age. Eye color: Light creamy-apricot with *cinnabar* (Table 1). Similar to *rosy* of *D. melanogaster*.

$ry^{ES7.99}$, $ry^{SG24.00}$, $ry^{SS48.00}$, $ry^{SG1.03}$, $ry^{FA8.05}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 1999, 2000 ($ry^{SG24}$, $ry^{SS48}$), 2003 and 2005, respectively.

### *sh-5:*

phenotype: Wings veins L5 and L2 short and do not reach wing margin. Variable expression. L2

may overlap wild type. Similar to *short-5* of *D. melanogaster*.

$sh\text{-}5^{SG11.00}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 2000.

### *ss: spineless*

phenotype: Flies show a loss or reduction in number of scutellar, dorsocentral, coxal, ocellar, orbital, anterior notopleural, postvertical, tergital and sternal bristles. Variable expression. Similar to *aristapedia* ($ss^a$) of *D. willistoni* (Spassky and Dobzhansky, 1950) and *spineless* of *D. melanogaster*.

$sc^{TB37.02}$

origin: Laguna Negra, Rocha, Uruguay, 2002.

### *tx: taxi*

phenotype: Wings held out at about 75° from body axis. Female sterile. Similar to *taxi* of *D. melanogaster*.

$tx^{ES13.99}$

origin: Faculty of Agronomy, Montevideo City, Uruguay, 1999.

Table 1. Genetic interaction of eye color mutations in *D. willistoni*.

| Mutants / chromosome linkage | Eye color | Mutant alleles tested |
|---|---|---|
| *Chromosome X ; 2* | | |
| *w ; cn* | Dull light red, colorless ocelli | *w EM1.00, cn SM35.00* |
| *Chromosome 2 ; 3* | | |
| *amy ; cd* | Light reddish in young flies | *amy RNL1.00, cd SG12.00* |
| *bw ; cd* | White in young flies to blotchy brownish in old flies | *bw SG23.00, cd SG12.00* |
| *cn ; ca* | Apricot | *cn SM35.00, FA2.02, ca 98e3.98* |
| *cn ; ma* | Light apricot | *cn SM35.00, FA2.05, ma FA1.05* |
| *cn ; ry* | Light creamy-apricot | *cn FA22.05, ry SS48.00* |
| *dke ; cd* | Bright reddish at eclosion | *dke TB28.02, cd SG12.00* |
| *or ; ca* | Dull apricot, colorless ocelli | *or FA7.05, ca ES13.99, 98e3.98* |
| *or ; p* | Yellowish apricot | *or FA07.05, p FA33.05* |
| *Chromosome 2* | | |
| *bw cn* | White, colorless ocelli | *bw SG23.00, Q51.F13, cn SM35.00, Q51.F13* |
| *cn or* | Dull yellowish orange at eclosion, light brownish in old flies, colorless ocelli | *cn FA2.05, or FA7.05* |
| *cn pr* | Dull apricot at eclosion, colorless ocelli. | *cn FA2.05, pr Ey3.99* |

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Figure 1. Photomicrographs of the eye color interaction seen in crosses between *brown* (*bw* $^{SG23.00}$) and *cardinal* (*cd* $^{SG12.00}$). From left to right are $F_2$ individuals as follows: *cardinal*, white eye color (young fly), creamy-white eye color (older fly), and *brown.*

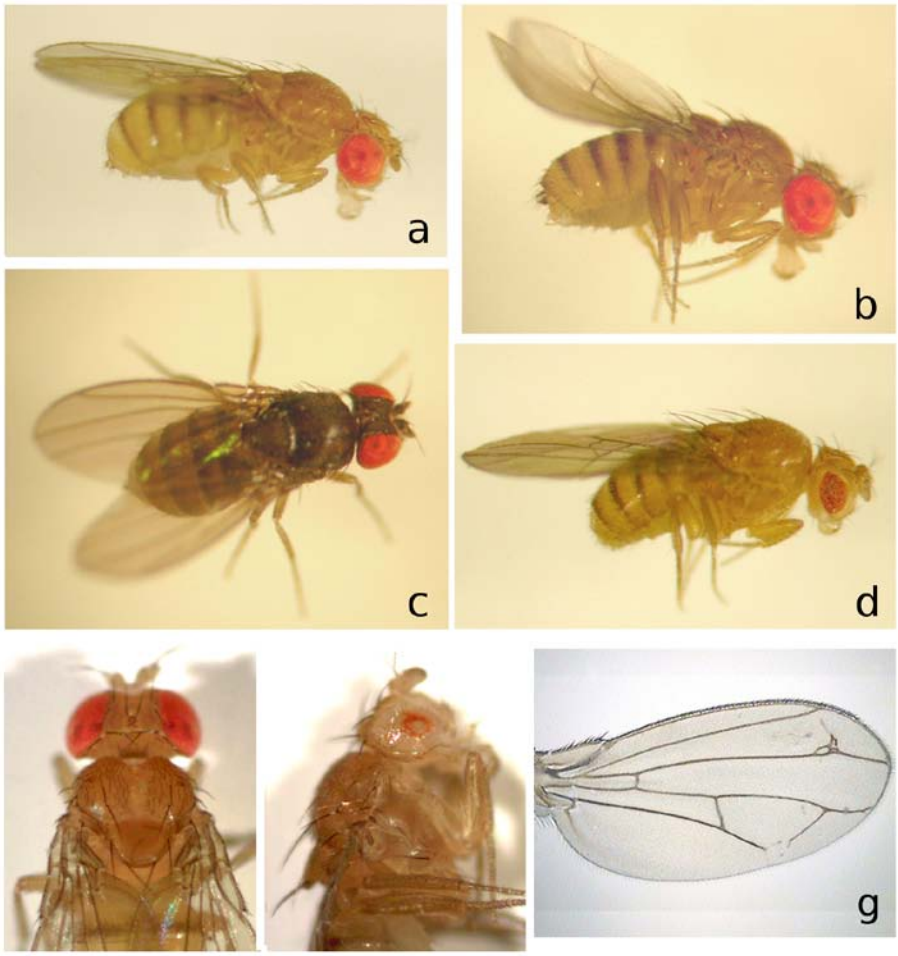


Figure 2. Photomicrographs of selected spontaneous mutations of *D. willistoni* described in this article: (a) wild type, (b) *jaunty*, (c) *ebony*, (d) *hedgehog*, (e) *kayak*, (f) *glass*, and (g) *plexus*.

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## New mutants in *Drosophila simulans.*

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Here we describe 20 novel spontaneous mutations in *Drosophila simulans* and provide updates of mutations previously reported.

### New Mutants

1- *jagged*[1] (*jgg*[1])
ORIGIN: Isolated in April 2012 from the stock Tabacón.
PHENOTYPE: recessive, wings divergent with severe notches along the wing margin or extremely reduced wings. Sometimes the longitudinal veins are interrupted.
LINKAGE: 1-95.5

NOTE: *jgg*$^{3}$ suppresses 85% of the crossovers between *v* and *f*.

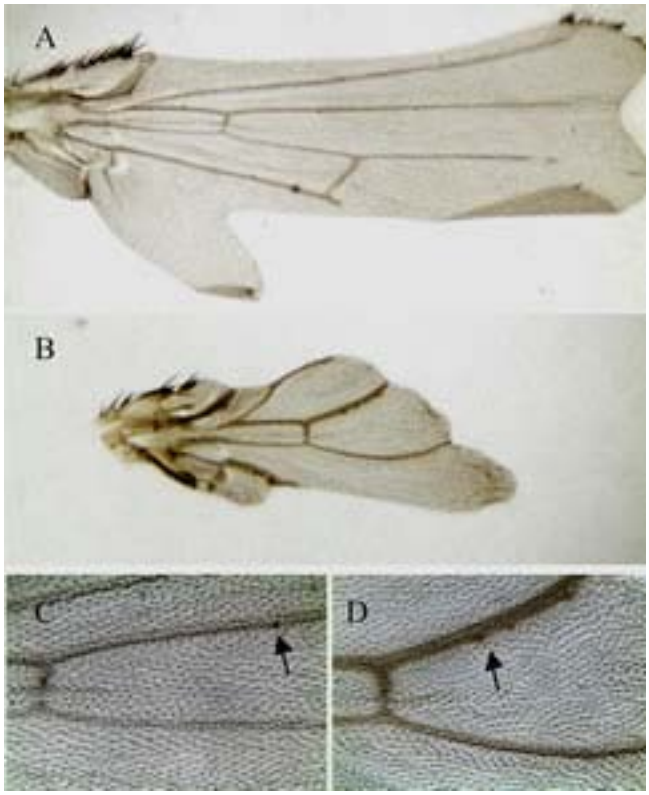


Figure 1. *jagged*$^{1}$ wings. A) Wing margin severely notched and L4 vein interrupted. B) Wing extremely reduced and the position of the three campaniform sensilla in L3 veins is readjusted to this reduction. C) Detail of the campaniform sensilla of the wing in A. D) campaniform sensilla of the wing in B). Note that the first sensilla is much closer to the crossvein (arrow).

2- *jagged*$^{3}$ (*jgg*$^{3}$)
ORIGIN: Isolated in April 2012 from the stock Tabacón.
PHENOTYPE: Identical to *jgg*$^{1}$.
LINKAGE: Not determined
NOTE: *jgg*$^{3}$ suppresses 85% of the crossovers between *v* and *f*.

3- *Delta*$^{R}$ (*Dl*$^{R}$)
ORIGIN: Isolated in April 2012 from the stock Rincón the la Vieja
PHENOTYPE: Dominant, strong deltas and vein thickening. *Dl*$^{R}$ exhibits very low transmission to the offspring. When males are mated to *roy* homozygous females more than 90% of the progeny die as embryos.
LINKAGE: Autosomal, linkage to the 3$^{rd}$ chromosome is tentative and based on phenotype.

4- *roughy*$^{1}$ (*roy*$^{1}$)
ORIGIN: Isolated in June 2012 from the stock Rincon de la Vieja.
PHENOTYPE: Recessive, rough eyes with irregular coloration in males and nearly wild type in females. Severity of the phenotype in females is influenced by the genetic background. Males emerge later than females and are short lived.
LINKAGE: 1-15.6
NOTES: Maps near the expected positions of the *D. melanogaster rap* and *rg* genes.

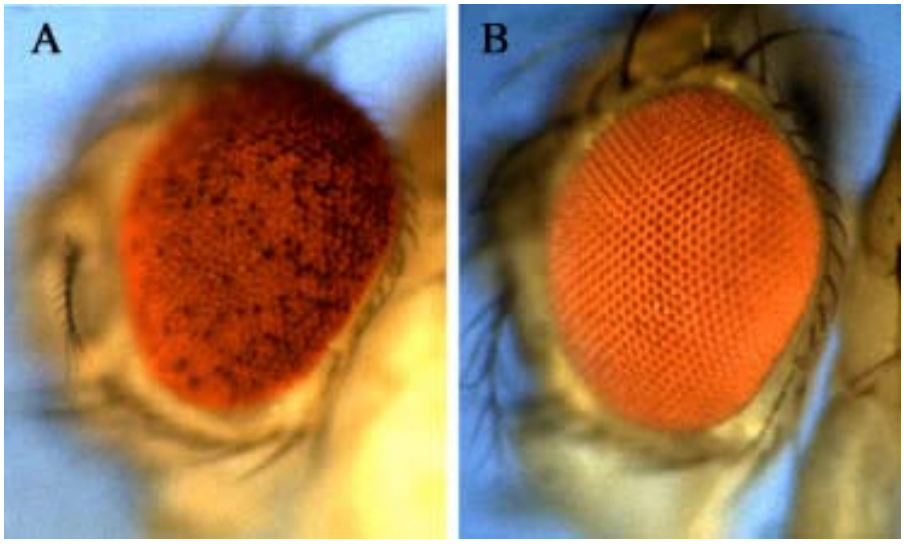


Figure 2. A) *roughy* homozygote male. B) *roughy* homozygote female. Note that females are almost indistinguishable from wild type. However, upon close examination female homozygotes often have a very slight roughening in the anterior part of the eye.

5- *odd ball*[1] (*ob*[1])
ORIGIN: Isolated from the stock *y v f* in August, 2012
PHENOTYPE: Mutant for *odd ball* males vigorously court *D. sechellia* females. Normally, *D. simulans* males are uninterested in *D. sechellia* females. Courtship towards *D. sechellia* usually lasts few seconds and is inhibited by proximity of the males to females. In contrast, *ob*[1] mutants sustain vigorous courtship for more than 30 minutes but are rejected by these females.
LINKAGE: unknown

6- *singed*[5] (*sn*[5])
ORIGIN: Isolated from Rincón de la Vieja in September, 2012
PHENOTYPE: Recessive, like *singed*[3]
LINKAGE: 1-30.2
NOTE: Alternative name *singed*[R]

7- *rough eye*[1] (*rey*[1])
ORIGIN: mapping cross
PHENOTYPE: Recessive, eyes modestly rough with bristles disorganized.
LINKAGE: Not yet determined

8- *body bristles miniaturized*[1] (*bbm*[1])
ORIGIN: Isolated from the stock Tabacón in September 2012
PHENOTYPE: Recessive, body bristles very reduced and thin. Male and female sterile.
LINKAGE: Not determined

9- *severe wing bubbles*[1] (*swb*[1])
ORIGIN: Isolated from the stock Cabuyal E in August 2012.
PHENOTYPE: Recessive, wings inflated like a balloon with liquid inside (Figure 3). No adhesion between wing surfaces. With age gas bubbles are formed inside and the liquid between the wings blackens. Emerging flies already have fully ballooned wings that make their exit from the pupal case difficult. Body color is darker than the wild type and with a subdued trident in the thorax. Young females have excellent fertility. Although coordinated, with age the flies become more immotile.

There is extensive lethality in larval stages. Only 33% of the homozygous flies from heterozygous parents reach adult stage.
LINKAGE: 2nd chromosome.

Figure 3. *severe wing bubbles*[1] mutant male. Note the inflated wings (arrowhead) and the trident in the thorax (arrow).

10- *Minute of Rincón* #4 (MR4)
ORIGIN: From *sn*[5].
PHENOTYPE: dominant. Macrochaeta thinner, late emergence and body color pale.
LINKAGE: Not yet determined

11- *blistered wings*[1] (*blw*[1])
ORIGIN: from *roy*[1] stock in November, 2012.
PHENOTYPE: Recessive, similar to *bl*. Semi-lethal. Survivors appear healthy.
LINKAGE: Autosomal.

12- *captain hook*[1] (*ch*[1])
ORIGIN: Tabacón stock in October, 2012.
PHENOTYPE: Tarsal segments t1-t5 of all six legs are fused. The distal most segment and claws are present indicating that the defect probably arises later than the Dpp/Wg cross regulation that sets up distal leg structures.
LINKAGE: Not yet determined

13- *Delta*[T] (*Dl*[T])
ORIGIN: Tabacón stock in November, 2012
PHENOTYPE: Dominant, deltas in the wing veins. Like *Delta*[R], the transmission of this mutation to the progeny is low.
LINKAGE: Not yet determined.


Figure 4. Wild type and a mutant leg of a *ch*[1] female. Note the fusion of tarsal segments in the mutant and the presence of claws (arrow).

14- *dark wood*[1] (*dw*[1])
ORIGIN: Cabuyal E stock in November, 2012
PHENOTYPE: body color darker than wild type. Soon after emerging, *db*[1] mutants appear darker than wild type but lighter than *ebony*, with a grayish/silver color. However, with age, this color does not mature into a black body color like in *ebony* or *black*. Instead it develops as a waxed brown.
LINKAGE: Not yet determined

15- *fine hairs* (*fh*[1])
ORIGIN: Rincón de la vieja stock in October, 2012
PHENOTYPE: Bristles thin like in Minute mutations, but recessive. Similar to *bbm*[1], except that males are fertile and females sterile. Females do not lay eggs.
LINKAGE: 4.

Figure 5. A *tnd*[1] mutant male. Note the extensive loss of macrochaeta in the thorax. Macrochaeta and microchaeta are removed and replaced by socket cells only (inset). Several cells fail to differentiate in any of the four types of mechanosensory cells and develop as cuticle (arrows). The tiny trichomes are preserved.

16- *totally naked*[1] *(tnd*[1]*)*
ORIGIN: *jgg*[3] stock in November, 2012
PHENOTYPE: Extensive loss of mechanosensory bristles. The loss appears to be partially due to transformation of the four cells that compose mechanosensory bristles (*i.e.*, glia, neuron, hair and socket) into socket cells. These defects affect coordination and the flies walk with their 5 tarsal segments in contact with a surface.
LINKAGE: Unknown

17-*hairy-like B*[1] *(hlB*[1]*)*
ORIGIN: Tabacón stock November. 2012
PHENOTYPE: Recessive, microchaeta in the lateral thorax below humerals similar to but less extreme *hl*[1]. Not tested for allelism with *hairy-like*[1].
LINKAGE: not yet determined

18- *Ubx of Cabuyal* (*Ubx*[CAB])
ORIGIN: Cabuyal stock in December, 2012.
PHENOTYPE: Dominant, like the *D. melanogaster Ubx*[130]carried in TM2.
LINKAGE: not yet determined

19- *disguised male killer A*[1] (*dmkA*[1])
ORIGIN: $w^{501}$ $sn^3$ $swg^1$ stock in December, 2012.
PHENOTYPE: 78% of the recombinants $w^+$ and $roy^+$ from $w^{501}$ $sn^3$ $swg^1$/ $roy^1$ mothers die, while the reciprocal recombinants $w^{501}$ and $roy^1$ survive. *dmkA*[1] behaves as part of a reciprocal lethal system involving two genes, *dmkA*[1] and *dmkB*[1]. Alone, *dmkA*[1] and *dmk*B[1] are viable. Lethality arises by the combination of *dmkA*[1] and *dmkB*[1] in a single chromosome. The genotypes $dmkB^+$ $dmkA^+$, $dmkB^+$ *dmkA*[1] and *dmkB*[1] $dmkA^+$ are viable, but *dmkA*[1]*dmkB*[1] are lethal. Lethal phase is unknown.
LINKAGE: 1-14.0.

20- *disguised male killer B*[1] (*dmkB*[1])
ORIGIN: *roy*[1]stock in December, 2012.
PHENOTYPE: described above in *dmkA*[1].
LINKAGE: 1, to the left of $w^+$.

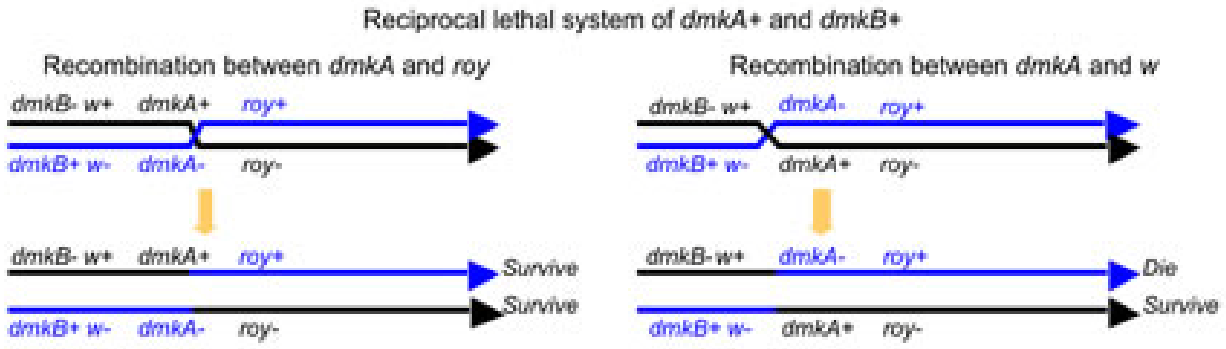


Figure 6. Schematic representation of the reciprocal lethal interaction between *dmkA*[1] and *dmkB*[1].

## Updates of Mutants Previously Described

*hairy-like*[1] *(hl*[1])
ORIGIN: *net pm*; *st e* stock
PHENOTYPE: microchaeta in the lateral thorax below humerals. The phenotype is fully penetrant but with variable expressivity. In the stock *swg*; *net pm*; *st e*; *hl* most animals have either four or two extra microchaetae on either side. In outcrosses, only one or two microchaeta and sometimes on one side only can be seen.
LINKAGE: 4

*cy* alleles
$cy^S$, $cy^R$ and $cy^{NC}$ are different alleles of the same gene. Although each of the alleles is viable, the combinations $cy^S/cy^R$ and $cy^S/cy^{NC}$ are mostly lethal, but escapers of this lethality have curled wings.

*Minute*$^{Rincón}$
Mutation is on the 4$^{th}$ chromosome and was renamed $M(4)M^R$. This mutation probably corresponds to the *D. melanogaster* M(4)M101, the only known Minute gene on the fourth chromosome. It was eventually lost due to the lack of a lethal to balance the mutation.

## Linkage map of *D. simulans.*

**Sousa-Neves[1], Rui, Emma Yang[2], and Youngmin Chu[2]**. [1]Department of Genetics and Genome Science, School of Medicine, Case Western Reserve University, Cleveland, OH, 44106; [2]Department of Biology, Case Western Reserve University, Cleveland, OH, 44106, USA.

The determination of genetic and physical positions of visible mutations is a valuable tool to map other mutations of interest. Over the past three years we have reported a number of spontaneous mutations isolated in *D. simulans* that serve for mapping purposes. Here we show a linkage map of some of these mutations in regard to other previously isolated mutations in this species. All genetic distances are corrected by the Kosambi function and as such may be different from previously uncorrected positions. Some distances were obtained from recombination between *D. simulans* and *D. sechellia* hybrids.

Table 1. Recombination rate and corrected genetic distances of X-linked genes. The distance between *sn* and *jgg* may be overestimated due to the small sample size.

| Species | hybrids (sim/sec) | hybrids (sim/sec) | hybrids (sim/sec) | (sim and hybrids sim/sec) | sim | sim | sim | sim |
|---|---|---|---|---|---|---|---|---|
| Interval | *y to w* | *y to v* | *v to swg* | *v to f* | *w to sn* | *w to roy* | *sn to jgg* | *dmklA to roy+* |
| recombination rate | 0.056 | 0.356 | 0.066 | 0.248 | 0.228 | 0.098 | 0.432 | 0.016 |
| Total progeny | 1349 | 904 | 487 | 1391 | 473 | 193 | 146 | 193 |
| Corrected distance | 5.6 | 44.6 | 6.6 | 27.2 | 24.7 | 10.0 | 65.3 | 1.555 |

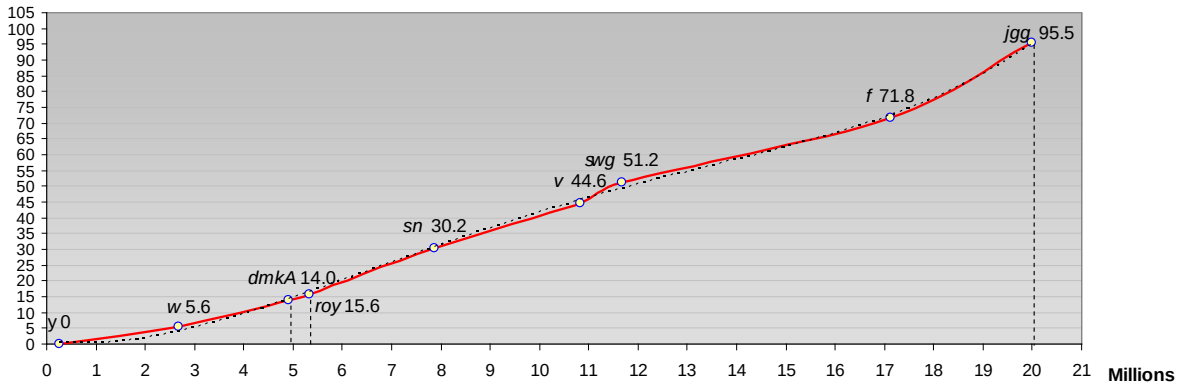


Figure 1. Genetic distances and physical distances of X- linked genes in *D. simulans*. Physical distances (in Megabases of the *D. melanogaster* genome) are calibrated by complementation tests with deletions or mutations in *D. melanogaster*. Vertical dashed lines indicate approximate expected physical positions, not tested yet. The distances are fit to a 4th order polynomial regression (dashed line following the red curve).

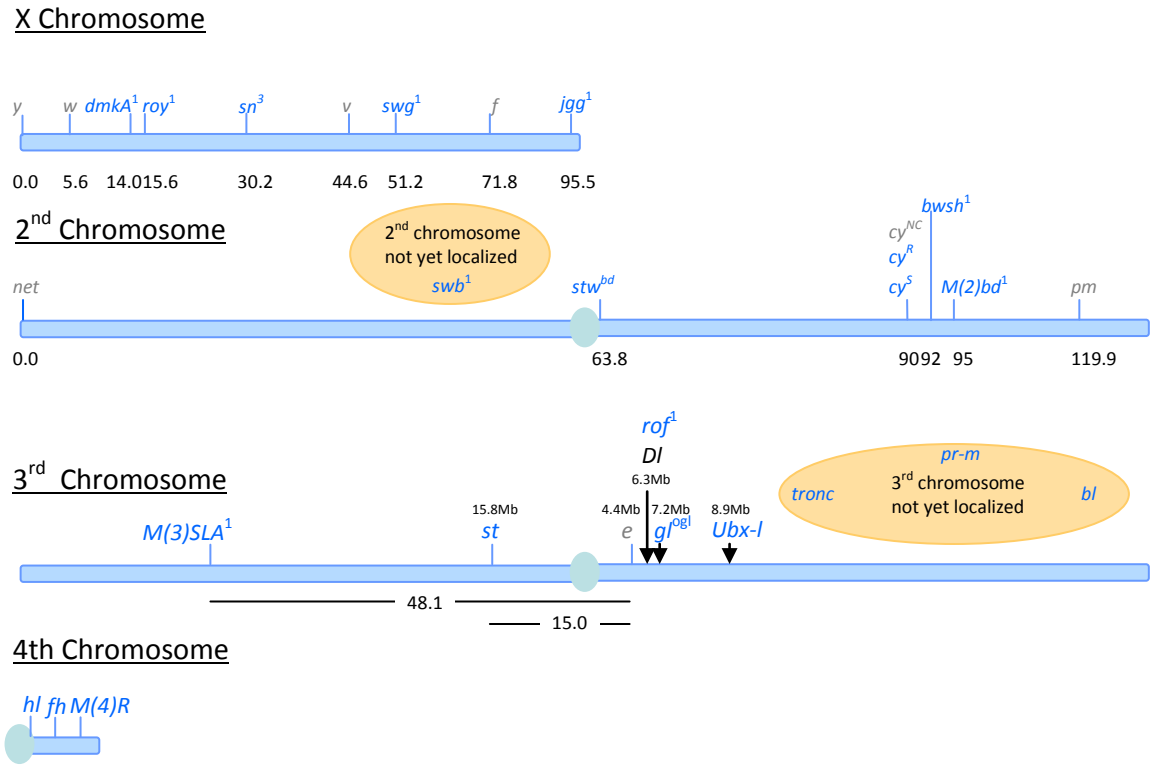


Figure 2. Position of several mutations recently described in relation to older mutations. In gray are indicated the older mutations and in blue the mutations described in the past three years. Below each chromosome are indicated the genetic distances corrected by the Kosambi function. In some cases, the distance shown is in Mb (above the chromosomes) to indicate that the physical position of this gene is known (*e.g.*, *st*, *gl*, and *Ubx*). The position of *rof* near *Dl* is based on the lack of recombination between *Dl*/*rof* [1] heterozygotes for several generations. The gene order of fourth chromosome mutations is arbitrary, since the lack of recombination does not allow establishing the gene order.

New Species

***Scaptodrosophila Mukherjee,* sp. nov., is a new member of the genus *Scaptodrosophila.***

**Chatterjee, Piyali, Arpita Rakshit, and R.N. Chatterjee*.** Genetics Research Unit, Department of Zoology, University of Calcutta, 35, Ballygunge Circular Road, Kolkata- 700019, India; *Author for Correspondence: chatterjee_rn@yahoo.co.in; chatterjeerncu@gmail.com

## Introduction

The genus *Scaptodrosophila* belongs to the family Drosophilidae. The genus includes over 200 species which are mostly endemic (Markow and O' Grady, 2006). This paper diagnosed and describes *Scaptodrosophila (Drosophila) mukherjee,* sp. nov.**,** as a new member of the *Scaptodrosophila* genus of the family Drosophilidae.

## Materials and Methods

The flies were collected from wild by using both trap-baits and net-sweeping methods. Collections were undertaken in July 2007 in the Ballygunge lake area, Kolkata.

In the laboratory, the flies were anaesthetized, diagnosed, and categorized as per Gupta (2005) and Markow and O' Grady (2006). The collected species from the wild were allowed to breed in vials containing standard *Drosophila* food medium. Cultures are maintained on *Drosophila* medium at 24º ± 1ºC in our laboratory.

## Results

*Taxonomy*

*Scaptodrosophila mukherjee* sp. nov.

*Diagnosis*

The external morphology of *Scaptodrosophila mukherjee* sp. nov. is more or less similar to *Scaptodrosophila paratriangulata* (Gupta, 1971) but the periphallic organs of the species is quite different, as shown in Figure 1, I and J.

*Description*

The morphological characters are described below. All type specimens have been deposited at the Genetics Research Unit, Department of Zoology, University of Calcutta, Kolkata, India.

*Holotype*

♂, Ballygunge, Kolkata, West Bengal, India.

*Paratype*

12♂, 15♀ (1♀ designated as "allotype") from Ballygunge, Kolkata, West Bengal, India, deposited at GRU, Department of Zoology, University of Calcutta, Kolkata, India.

*Distribution and Ecology*

This species is so far distributed in different parts of West Bengal, India.

*Etymology*

This species is named as *Scaptodrosophila mukherjee* in honor of Late Prof. A.S. Mukherjee, Department of Zoology, University of Calcutta, Kolkata, West Bengal, India.

**Morphological Characteristics**

*Average body length*: 3.5 mm (male) and 4.0 mm (female) (see Figure 1, A and B).

*Head*

Arista with 3 dorsal and 2 ventral branches in addition to the small terminal fork. Antennae with second segment dark brown; third segment little lighter. Frons including ocellar triangle brownish black. Orbital bristles ratio is 4:3:5. Vibrissae is single and strong. Palpus brownish black with 2 apical setae. Carina black and flat. Clypeus brownish black. Face and cheek is dark brown. Eyes are dark red.

*Thorax*

Acrostichal hairs in 6 regular rows between dorsocentrals, anterior and posterior scutellars convergent. Distance between anterior and posterior dorsocentrals 1/2 the distance between two anterior dorsocentrals. Postcellar setae are well-developed; proclinate orbital setae arises posterior to anterior reclinate; 1 pair of prescutellar acrostichal setulae present. Mesonotum and scutellum unicolorous, glossy black. Three katepisternal setae present. Sterno index = 0.57.

*Wings*

Clear, apically rounded (Figure 1, E and F). Wing-vein index: Costal index (C) = 3.33; Fourth vein index (4V) = 2.63; 4C- index = 0.94; 5X- index = 2.33. Halteres white. Wing length 2.5 mm. The indices are more or less similar to *Scaptodrosophila paratriangulata* (Gupta, 1971).

*Legs*

Coxa, femora and tibia of all legs are black and all tarsal segments of all legs are transparent in colour. No ornamentation like sex comb in males (Figure 1, G).

*Abdomen*

Tergites glossy black dorsally. 1T -6T with a medially interrupted black longitudinal band ventrally.

*Periphallic Organs*

Posterior parameres forming a triangular structure; epandrium dark brown, narrow, truncate below, with 15 bristles. Lower portion of genital arch with densely setose; anal plate with dense setae; especially at the apex; prensistae on surstylus arranged in a concave row. Surstylus long with

broader tip, having 13 closely placed black teeth and with 2 dorso-median setae. Cerci dark brown, triangular with 13 setae.

*Phallic Organs*

Aedeagus yellowish brown, bifid, long and narrowly curved apically (Figure 1, H). Basal apodeme of aedeagus usually long and broad basally. Anterior gonapophyses small with many sensilla. Posterior gonapophyses fused, broadened below.

*Oviscrapt*

Pale brown in colour, medially swollen, apically narrow and terminating into a large peg, with about 19 marginal ovisensillum (Figure 1, D). Anteroventral bridge short and narrow.

*Egg*

Eggs are small, ellipsoid, with 6 filaments (Figure 1, C). Its length varies from 0.39-0.42 mm in length and 0.15-0.17 mm in width. Filamentous length varies in each egg with maximum length of 0.35 mm.

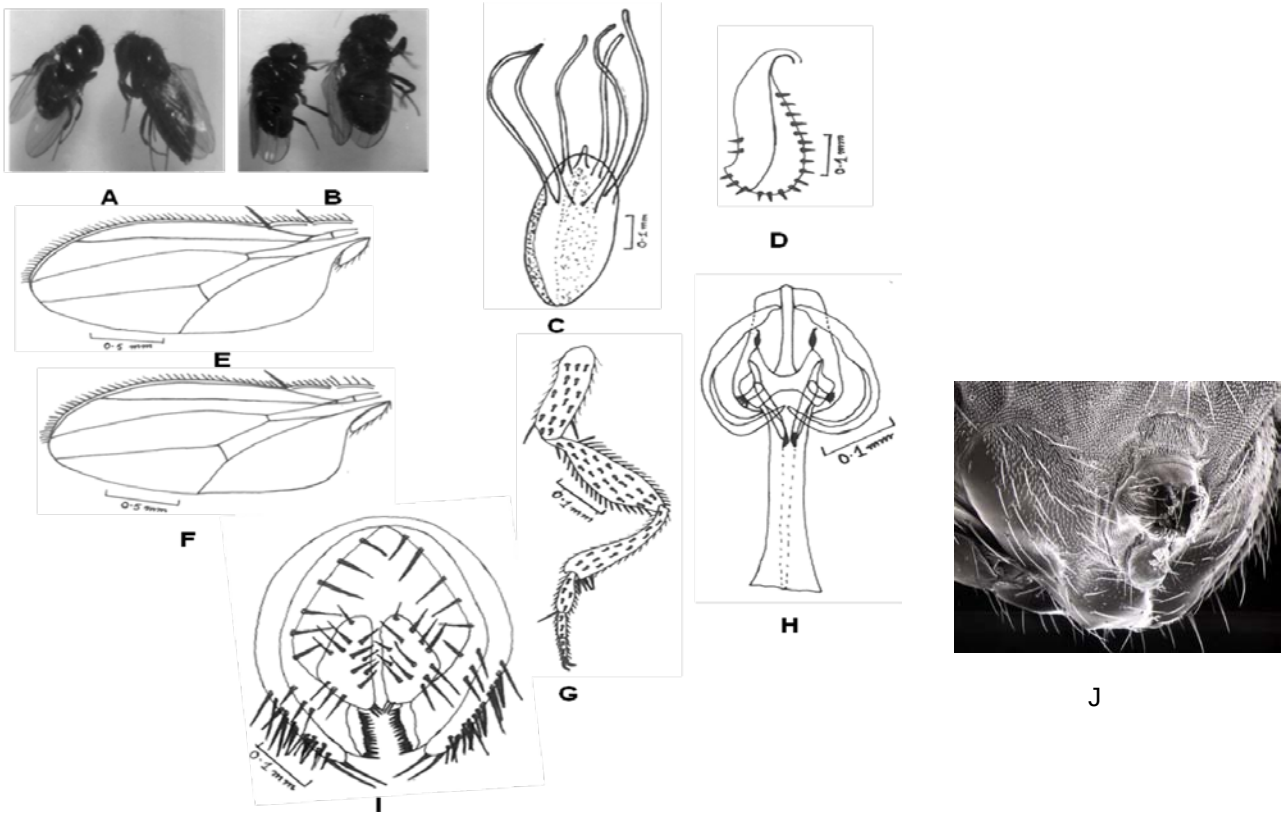


Figure 1. *Scaptodrosophila mukherjee* nov. sp.: Whole fly - dorsal view (A); ventral view (B); egg (C); oviscrapt (D); female wing (E); male wing (F); male fore leg (G); phallic organ (H); periphallic organ (I); SEM view of male terminalia (J).

## Discussion

This species superficially resembles ***Scaptodrosophila paratriangulata*** (Gupta, 1971) in having cross striped abdominal tergites, apically rounded wing, ocellar triangle brownish black, heel of the genital arch not pointed and the row of teeth of surstylus not convexed. However, this species distinctly differs from *S. paratriangulata* in having dark coloration, basal apodeme of aedeagus (usually long and broad basally), anterior gonapophyses (small with many sensilla) and white haltere. Since this species shows similarities in the main characteristic features of *Scaptodrosophila* and since it differs in many characters from the other species of the genus, it is diagnosed as new species of the genus *Scaptodrosophila.*

Acknowledgments: This work is financially supported by WB DST project 289 (Sanc.) ST/P/S & T/2G-30/2011 to RNC.

References: Gupta, J.P., 1971, Proc. of Zool. Soc. 22: 53-61; Gupta, J.P., 2005, J. Scientific Research 5: 1-252; Markow, T.A., and P.M. O' Grady 2006, Drosophila: *A Guide to Species Identification and Use*, Elsevier Academic Press, London.

## *Scaptomyza jadavpuri sp. Nov.* is a new member of picture wing Drosophilidae of the *Scaptomyza* complex.

**Rakshit, Arpita, Piyali Chatterjee, and R.N. Chatterjee.*** Genetics Research Unit, Department of Zoology, University of Calcutta, 35, Ballygunge Circular Road, Kolkata- 700019, India; *Author for Correspondence: chatterjee_rn@yahoo.co.in; chatterjeerncu@gmail.com

## Introduction

*Scaptomyza* is a very complex and poorly studied taxon. Over 150 described species of this taxon are endemic to the Hawaiian archipelago. The remaining 100 described species of *Scaptomyza* are placed in nine groups and are found elsewhere. Parascaptomyza is a relatively large, widespread group with species found on most of the world's major land masses. We describe here *Scaptomyza* (*Parascaptomyza*) *jadavpuri*, sp. nov.**,** as a new member of the subgenus *Parascaptomyza* of *Scaptomyza* genus of the family Drosophilidae.

## Materials and Methods

The species were collected from the wild by using both trap-baits and net-sweeping methods in August, 2008. Fermenting fruits, banana and guava, were used as baits in the wet sand.

In the laboratory, the flies were anaesthetized, diagnosed, and categorized as per Markow and O' Grady (2006) protocol. 56 individuals were collected from the wild and were allowed to breed in open food vials containing standard laboratory food medium within a plastic jar containing wet sand. Cultures were maintained at 24º±1ºC. However, the flies grow very poorly in the laboratory condition.

## Results

*Taxonomy*

*Scaptomyza* (*Parascaptomyza*) *jadavpuri* sp. nov.

*Diagnosis*

This species superficially resembles *Scaptomyza* (*Parascaptomyza*) *adusta* (Loew, 1862). But many organs of the species are quite different, as shown in Figure 1, A-I.

*Description*

The morphological characters are described below. All specimens have been deposited at Genetics Research Unit, Department of Zoology, University of Calcutta, Kolkata, India.

*Holotype*

♂; Jadavpur, South Kolkata, West Bengal, India, deposited at the GRU, Department of Zoology, University of Calcutta, Kolkata, India.

*Paratype*

10 ♂, 12 ♀ (1 ♀ designated as "allotype") from Jadavpur, Kolkata, West Bengal, India. Deposited at the GRU, Department of Zoology, University of Calcutta, Kolkata, India.

*Distribution and Ecology*

This species is so far known from Jadavpur areas, Kolkata, where it had been collected from fruits fallen on sand. This species is preserved in 70% ethanol and stored in the laboratory.

*Etymology*

This species is named as *Scaptomyza* (*Parascaptomyza*) *jadavpuri*, because it was only collected from Jadavpur, Kolkata, West Bengal, India.

## Morphological Characteristics

*Average body length*: 4.9 mm (male) and 5.3 mm (female) (Figure 1, A and B).

*Head*

Arista with 8 dorsal and 2 ventral branches with terminal fork. Antennae with second segment yellowish. Frons including ocellar triangle brown. Orbital bristles in ratio 3:2:4. Vibrissae single and strong. Palpus brown with 8 marginal setae. Carina yellowish and narrow. Clypeus yellowish brown. Face and cheek yellow. Eyes were red.

*Thorax*

Acrostichal hairs in 4 regular rows between dorsocentrals, Anterior scutellars divergent but posterior scutellars convergent. Distance between anterior and posterior dorsocentrals 1/3rd the distance between two anterior dorsocentrals. Prescutellum well developed. Mesonotum and scutellum dark yellow with brownish lower portion. Dark stripes present on mesonotum. Sterno index 0.58.

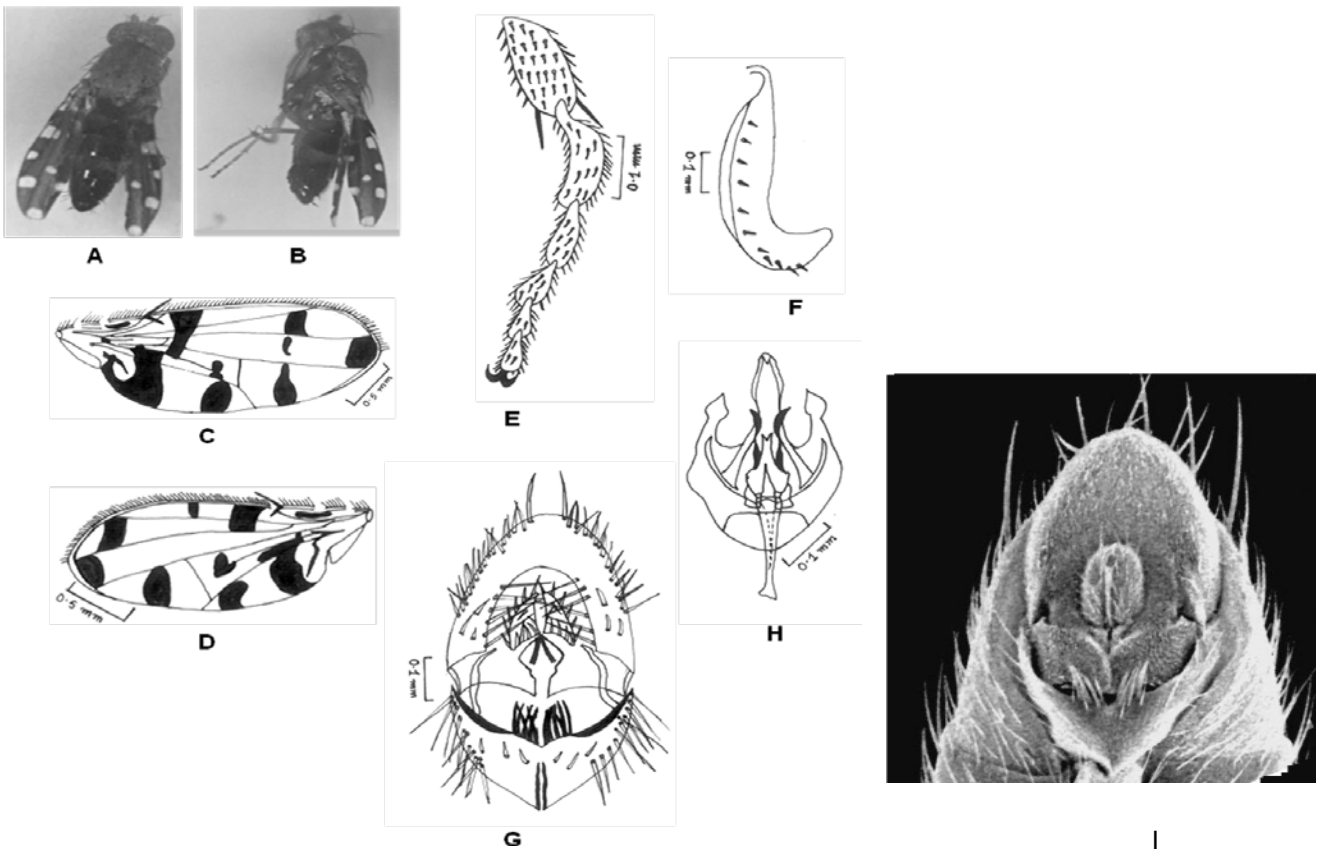


Figure 1. *Scaptomyza* (*Parascaptomyza*) *jadavpuri n.sp.:* Whole fly- dorsal view (A); side view (B); female wing (C); male wing (D)**;** male fore leg (E), oviscrapt (F**)**; periphallic organ (G); phallic organ (H); SEM view of male terminalia (I).

*Wings*

Clear. Approximate wing-vein index: C index - 2.47; 4V- index 1.50; 4C- index 0.75; 5X- index 1.72. Halteres pale yellow. Wing length 2.8 mm. Wings were sexually dimorphic (Figure 1, C and D).

*Legs*

Yellow in color. Preapicals on all three tibiae; apical on first and second tibiae. No ornamentation like sex comb in males (Figure 1, E).

*Abdomen*

1T yellowish, 2T brown dorsally and yellowish ventrally, 3T-6T with complete black bands.

*Periphallic Organs*

Epandrium brownish black, narrow above and below and broad in the middle. Upper portion with about 24-28 setulae and lower portion with about 36-40 setulae. No setae on secondary clasper on anal plate. Surstylus large, flatter, with only two regions of prensistae, arched and fused to epandrium with about a row of 6 bristle like teeth present in lower margin of surstylus. A pair of brownish paralobes present just above the Cerci. Cerci dark brown, triangular with 10 setae.

*Phallic Organs*

Aedeagus elongated, pale yellow, apparently bifid, pubescent ventro-apically and slightly curved dorsally at tip (Figure 1, H). Anterior gonapophyses long, apically narrowly pointed and curved. Posterior gonapophyses dorsally dilated and proximally with a small conical process. Novasternum with lateral processes, median processes very small with a pair of submedian spines. Ventral fragma triangular, broader than long.

*Oviscrapt*

Lobe pale brown in color, rounded apically, robust with about 11 marginal ovisensillum (1F); no discal teeth present; basal isthmus short.

**Discussion**

This species superficially resembles *Scaptomyza* (*Parascaptomyza*) *adusta* (Loew, 1862) in having four rows of acrostichal setulae between antero dorsocentral setulae, presence of picture wing, presence of paralobes in periphallic organ, surstylus flatter, with only two regions of prensistae. However, this species distinctly differs from *S. adusta* in dark coloration of the body, spots in wings in males and females, and absence of setae on secondary clasper on anal plate. For these reasons, it is diagnosed as distinct to other species of the genus *Scaptomyza.*

Acknowledgments: This work is financially supported by WB DST project 289 (Sanc.) ST/P/S & T/2G-30/2011 to RNC.

References: Loew, H, 1862, Berliner Entomologische Zeitschrift. 6: 185-232; Markow, T.A., and P.M. O' Grady, 2006, *A Guide to Species Identification and Use*, Elsevier/AP: 1-259.

**_Samoaia bengalensis_ sp. nov., is a new member of the picture wing Drosophilidae of the genus _Samoaia._**

**Chatterjee, R.N*, and Mausumi Bhattacharyya.** Genetics Research Unit, Department of Zoology, University of Calcutta, 35, Ballygunge Circular Road, Kolkata- 700019; India; *Author for Correspondence: chatterjee_rn@yahoo.co.in; chatterjeerncu@gmail.com

**Introduction**

Picture wing Drosophilidae are mainly reported from Hawaiian islands. Wing pigment spots occur in highly reproducible, species-specific, two dimensional patterns, and their genetics and development are beginning to be understood. Therefore, *Drosophila* wing is an attractive target for evolutionary biologist. *Samoaia* is a small genus of seven described species endemic to the islands of Samoa. In this report, we diagnosed and describe *Samoaia bengalensis* sp. nov.**,** as a new member of the genus *Samoaia* of the family Drosophilidae from West Bengal, India.

## Materials and Methods

The species were collected from the wild by using net-sweeping methods in September, 2012. In the laboratory, the flies were anaesthetized, diagnosed, and categorized as per Markow and O'Grady (2006) protocol. 36 individuals were collected from the wild. However, we were not able to culture in the laboratory condition.

## Results

*Taxonomy*

*Samoaia bengalensis* sp. nov.

*Diagnosis*

This species superficially resembles *Samoaia leonensis*, but many organs of the species and body coloration are quite different, as shown in Figure 1, a-d. The detailed description will be communicated elsewhere. All specimens have been deposited at Genetics Research Unit, Department of Zoology, University of Calcutta, Kolkata, India.

*Holotype*

♂; Uttarpara, Hooghly, West Bengal, India, deposited at the GRU, Department of Zoology, University of Calcutta, Kolkata, India.

*Paratype*

14 ♂, 16 ♀ (1 ♀ designated as "allotype") from Uttarpara, Hooghly, West Bengal, India. Deposited at the GRU, Department of Zoology, University of Calcutta, Kolkata, India.

*Distribution and Ecology*

This species is so far known from only Uttarpara and Tarakeswar areas, Hooghly, where it had been collected from bushy areas. This species is preserved in 70% ethanol and stored in the laboratory.

*Etymology*

This species is named *Samoaia bengalensis*, because it was only collected from Hooghly, West Bengal, India.

## Morphological Characteristics

*Average body length*: 5.1 mm (male) and 5.3 mm (female) (Figure 1, a-d).

*Wings and thorax*

Wings and thorax with distinct pattern of tan, gray, and white (Figure 1a-d). Wings are sexually dimorphic.

*Legs*

Yellow in color, not banded. No ornamentation like sex comb in males.

*Abdomen*

1T – 7T dorsally yellowish with distinct pattern of tan, gray, and white, and yellowish ventrally (Figure 1, b-d). The detail description will be communicated elsewhere.

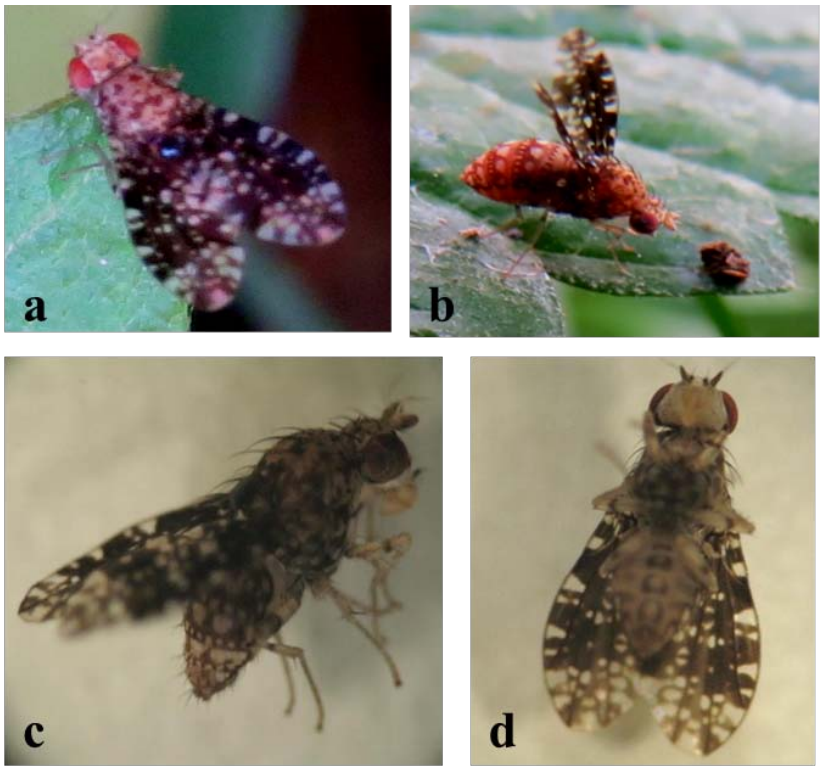


Figure 1. *Samoaia bengalensis n.sp.:* (a) A fly resting in a leaf; (b) one fly chasing for food in a leaf; (c) fly- side view; and (d) ventral view of a female fly**.**

**Discussion**

This species superficially resembles *Samoaia leonensis* with distinct pattern of tan, gray, and black spot. However, this species distinctly differs from *Samoaia leonensis* with coloration of the body, spots in wings in males and females, and other characters. For these reasons, it is diagnosed as distinct to other species of the genus *Samoaia.*

Acknowledgments: This work is financially supported by WB DST project 289 (Sanc.) ST/P/S & T/2G-30/2011 to RNC.

References: Markow, T.A., and P.M. O' Grady, 2006, *A Guide to Species Identification and Use*, Elsevier/AP: 1-259.

# Guide to Authors

Drosophila Information Service prints short research, technique, and teaching articles, descriptions of new mutations, and other material of general interest to *Drosophila* researchers. The current publication schedule for regular issues is annually, with the official publication date being December. The annual issue will include material submitted during the calendar year. To help us meet this target date, we request that submissions be sent by 15 December, but articles are accepted at any time. A receipt deadline of 31 December is a firm deadline, due to printer submission schedules. Electronic submissions are encouraged, and may be required for lengthy or complex articles.

Manuscripts, orders, and inquiries concerning the regular annual DIS issue should be sent to James Thompson, Department of Zoology, University of Oklahoma, Norman, OK 73019. Telephone (405)-325-2001; email jthompson@ou.edu; FAX (405)-325-7560.

**Submission**: Articles should be submitted electronically, if possible. Alternatively, we ask that a diskette be included with an article mailed to us. MS Word or Rich Text Formats are preferred. To help minimize editorial costs, proofs will not be sent to authors unless there is some question that needs to be clarified or they are specifically requested by the authors at the time of submission. The editor reserves the right to make minor grammatical, spelling, and stylistic changes if necessary to conform to DIS format and good English usage. If the article contains tables, complex line figures, or half tones, we may ask that a printed copy be mailed to us after seeing the electronic version if we have questions about content or presentation. Color illustrations will appear black and white in the printed version but will be in color in the electronically-accessible version on our web site (www.ou.edu/journals/dis).

**Citation of References**: Citation should be by name and date in the text of an article (Smith, 1989; Jin and Brown, 1990; Waters *et al*., 1990). At the end of the article, references should be listed **alphabetically** by senior author, listing all authors with initials, date, journal, volume and page numbers. Titles will not be included except for books, unpublished theses, and articles in press. An example format is:

Green, R.L., 1998, Heredity 121: 430-442.

Waters, R.L., J.T. Smith, and R.R. Brown 1990, J. Genet. 47: 123-134.

Note the initials are before each name except for the senior author.

Teaching Notes


**Effect of genetic background on viability of dominant visible mutations that are also recessive lethals in *Drosophila melanogaster*.**

**Grimm, Abbey J., Ashley F. Kuchcinski, and R.C. Woodruff.** Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403.

The genetic background can influence the activity of genes. For example, Wright (1988) pointed out:

> "Most early geneticists thought of the phenotype as if it were a mosaic of unit characters, each determined by a single locus, with effects as conspicuous as those that they used in their experiments. They thought of alleles as having constant relative selective values. … This early viewpoint changed with the demonstration … that quantitative variation usually depends on the total effect of multiple minor factors. This implied that numerous superior combinations could exert more or less similar effects and that the selective value of any gene depends on the rest of the genome."

It is clear that genetic backgrounds can influence phenotypes. For example, genetic backgrounds alter viability associated with visible and lethal mutations in *Drosophila melanogaster* (Polivanov, 1964; Anderson, 1969), the lifespan and olfactory behavior of *D. melanogaster* (Leips and Mackay, 2000; Anholt *et al*., 2003), the expression of a knockout mutation that affects behavior in mice (Brown *et al*., 1996), blood and bone traits in mice and rats (Shao *et al*., 2008), the response to selection and growth in plants (Ungerer *et al*., 2003; Alcazar *et al*., 2009), ovarian cancer in humans with mutations in the BRCA1 gene (Tagliaferri *et al*., 2009), and human genetic diseases (Badano

and Katsanis, 2002), including X-linked adrenoleukodystrophy (X-ALD) (Moser *et al*., 2005; Lewis, 2012).

It is the objective of this study to attempt to identify the influence of genetic background on viability associated with dominant visible mutations that are also recessive lethals in *D. melanogaster*. Examples of the crossing schemes used in this study are shown in Figure 1 and Figure 2. The mutations tested were *Dr* $^{Mio}$ (Drop small eye defect, third chromosome) and a combination of the two mutations *Gl Sb* (Glued, small eye, and Stubble, short bristles, third chromosome). The inbred lines used for genetic backgrounds with *Dr* $^{Mio}$ were yIB251, PCIB24, PPIB34, and IB1&2-11, whereas the inbred lines used for genetic backgrounds with *Gl Sb* were yIB254, PCIB27, PPIB37, and IB1&2-11. The number in each inbred line designation is the number of generations of single brother/sister matings for each line at the time of their use. The C(1)DX, *y w f* chromosome is two X chromosomes attached to a single centromere and contains the recessive markers *y* (yellow, yellow body color), *w* (white, white eyes), and *f* (forked, short bristles). TM6, *Ubx* and LVM are balancer third chromosomes (see Lindsley and Zimm, 1992).

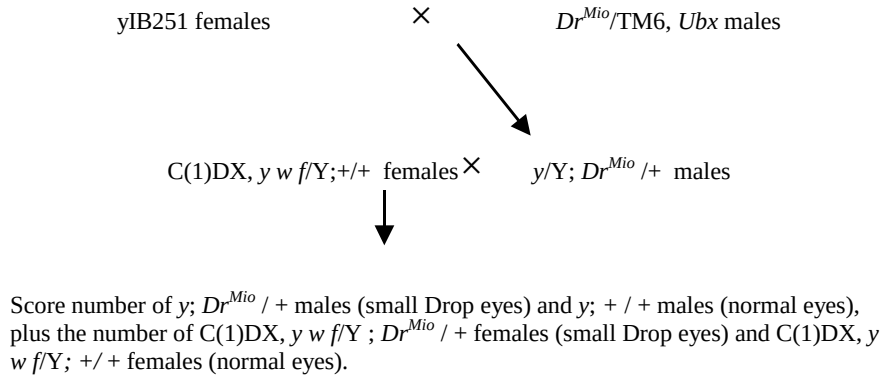


Figure 1. Mating scheme used to measure the effect of genetic background on viability of the $Dr^{Mio}$ dominant visible/recessive lethal mutation in *D. melanogaster*.

The crosses of Figure 1 were repeated with the PCIB24, PPIB34, and IB1&2-11 inbred lines. It is our hypothesis that the proportion of *Dr* $^{Mio}$ and $Dr^{+}$ progeny will vary for each cross, due to interactions of *Dr* $^{Mio}$ and $Dr^{+}$ with different alleles of other genes in the genetic backgrounds.

The same crosses as in Figure 1 were repeated using *Gl Sb* / LVM males and yIB254, PCIB27, PPIB37, and IB1&2-11 inbred lines. An example of these crosses is shown in Figure 2.

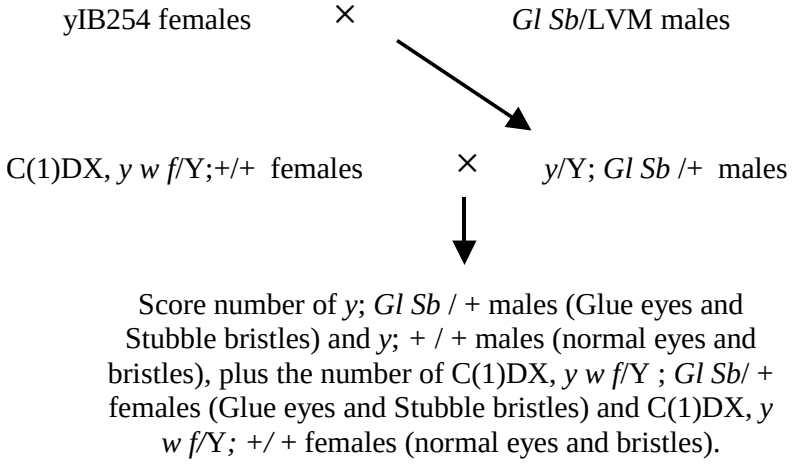


Figure 2. Mating scheme used to measure the effect of genetic background on viability of the *Gl Sb* dominant visible/recessive lethal mutations in *D. melanogaster*.

It was our hypothesis that the proportion of *Gl Sb* and $Gl^{+}$ $Sb^{+}$ progeny will vary for each cross, due to interactions of *Gl Sb* and $Gl^{+}$ $Sb^{+}$ with different alleles of other genes in the genetic backgrounds.

We also recorded the number of triplo-X females that were recovered in each cross of Figures 1 and 2 to determine if the genetic background influences the survival of XXX females. These triplo-X females are easily identified because they are wild type for the yellow, white, and forked genes, plus they have crumpled wings and deformed rear legs (Lindsley and Zimm, 1992).

## Results

The results of the effect of genetic backgrounds on the viability of the *Dr* $^{Mio}$ and *Gl Sb* dominant visible and recessive lethal mutations are shown in Tables 1-3 and Figures 3-5. As shown in Table 1 and Figure 3, the frequencies of recovered *Dr* $^{Mio}$/+ and +/+ males and females in the four genetic backgrounds were significantly different (P = 0.036).

Table 1. Effect of genetic backgrounds on viability of $Dr^{Mio}$ / + and +/ + progeny.

| Genetic Background from: | Number of +/ + females | Number of $Dr^{Mio}$ / + females | Number of +/ + males | Number of $Dr^{Mio}$ / + males |
|---|---|---|---|---|
| yIB251 | 251 | 268 | 379 | 454 |
| PPIB34 | 339 | 309 | 518 | 511 |
| PCIB24 | 412 | 383 | 707 | 650 |
| IB1 & 2-11 | 431 | 420 | 622 | 616 |
| P = 0.036 | | | | |

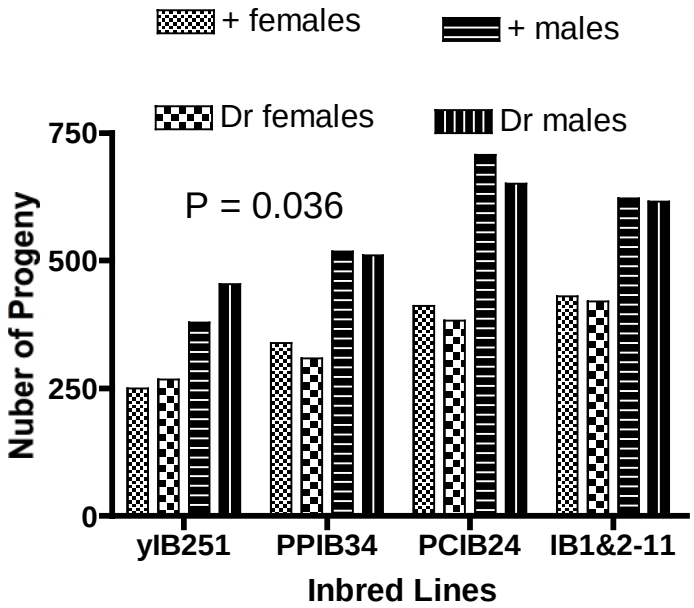


Figure 3. Effect of genetic backgrounds on viability of *Dr* $^{Mio}$ / + and +/ + progeny.

Table 2. Effect of genetic backgrounds on viability of *Gl Sb* and $Gl^{+}$ $Sb^{+}$ progeny.

| Genetic Background from: | Number of + +/+ + females | Number of *Gl Sb* /++ females | Number of + +/+ + males | Number of *Gl Sb* /+ + males |
|---|---|---|---|---|
| yIB254 | 271 | 229 | 344 | 340 |
| PPIB37 | 336 | 308 | 530 | 465 |
| PCIB27 | 278 | 257 | 410 | 432 |
| IB1 & 2-11 | 391 | 365 | 583 | 552 |
| P = 0.48 | | | | |

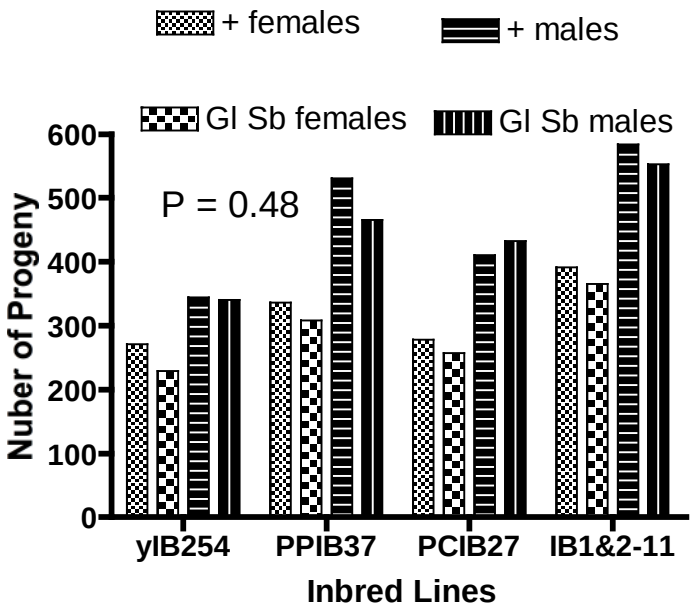


Figure 4. Effect of genetic backgrounds on viability of *Gl Sb* and $Gl^{+}$ $Sb^{+}$ progeny

Table 3. Effect of genetic backgrounds on viability of XXX (triplo-X) and XX females.

| Genetic Background from: | Number of XXX (triplo-X) females | Number of XX females | % XXX (triplo-X) females |
|---|---|---|---|
| yIB251-257 | 6 | 1108 | 0.54 |
| PPIB34-37 | 78 | 1292 | 5.69 |
| PCIB24-27 | 40 | 1320 | 2.94 |
| IB1 & 2-11 | 21 | 1607 | 1.29 |
| P < 0.0001 | | | |

The significant difference in progeny in Table 1 and Figure 3 was due to differences in the recovery of $Dr^{Mio}$ / + and +/ + males (P = 0.03), but not females (P = 0.54).

In contrast to the $Dr^{Mio}$ crosses, there was a non-significant difference (P = 0.48) in the recovery of *Gl Sb*/+ + and + +/+ + progeny, as shown in Table 2 and Figure 4.

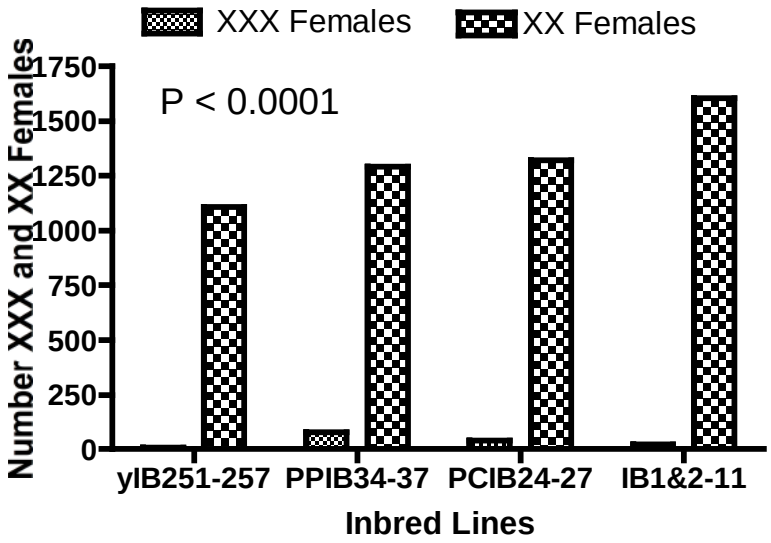


Figure 5: Effect of genetic backgrounds on viability of XXX (triplo-X) and XX females.

There was also a significant difference ($P < 0.001$) in recovery of triplo-X females with different genetic backgrounds, as shown in Table 3 and Figure 5. The numbers of females in Table 3 and Figure 5 were taken from both the *Dr* $^{Mio}$ and the *Gl Sb* experiments. It should be noted that 13 of these triplo-X females were tested and found to be sterile, as expected.

In summary, the genetic background did influence the recovery of *D. melanogaster* with the mutation *Dr* $^{Mio}$ (which is a dominant visible and a recessive lethal mutation) and the recovery of triplo-X females. In relation to the latter observation, it might be of interest for students to discuss if the frequencies of humans with trisomy-21 (Down syndrome), XO (Turner syndrome) and XXY (Klinefelter syndrome) would be expected to vary in different parts of the world, based on the assumption that genetic backgrounds are not the same in humans. Students could also go to the World Wide Web and see if frequencies of humans with extra or missing chromosomes are given for different parts of the world.

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**The identification of unequal crossing-over events at the Bar (*B*) locus of *Drosophila melanogaster*.**

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Unequal crossing over at meiosis can lead to an extra copy of a gene that evolves into a new function or one of the tandem duplicated genes mutates into a pseudogene that is not functional (Chen *et al*., 1990; Lynch and Conery, 2000; Zhang *et al*., 2003; Hurles, 2004). For example, in humans the five beta globin genes (and one pseudogene) arose by duplication events (Hurles, 2004), as did the genes giving humans red-green color vision (Botstein, 1986). In addition, unequal crossing-over events can lead to human genetic disorders (Emanuel and Shaikh, 2001). For example, Charrot-Marie-Tooth disease and red-green color blindness are caused by unequal recombination events (Purandare and Patel, 1997; Nathans *et al*., 1986; Drummond-Borg *et al*., 1988; Jagla *et al*., 2002).

The classical example of a change in phenotype associated with unequal crossing-over is the X-linked Bar (*B*) locus of *Drosophila melanogaster*. A duplication of the 16A region of the X chromosome gives the Bar-eye mutation, where the eye is reduced in size to a narrow vertical bar (Tice, 1914; Sturtevant and Morgan, 1923; Sturtevant, 1925; Bridges, 1936; Muller, 1936; Lindsley and Zimm, 1992). Unequal crossing over at the Bar locus is shown in Figure 1 and the Bar and wild-type structure of the eye are shown in Figure 2.

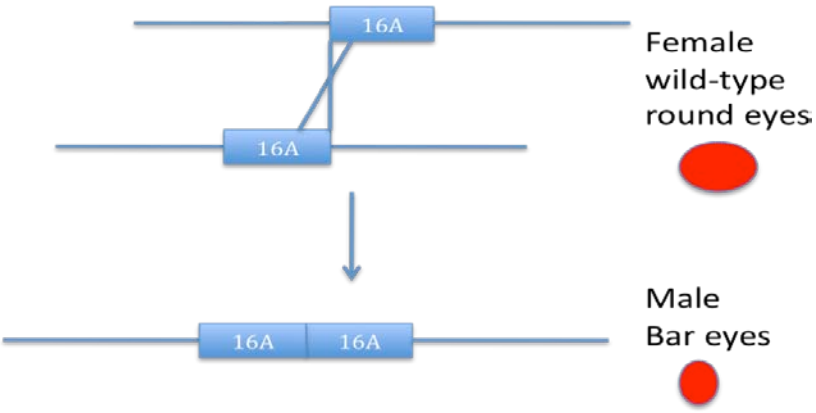



Figure 1. Unequal crossing over leading to a tandem duplication of the 16A region of the X chromosome of *Drosophila melanogaster* and to the Bar eye phenotype.

In this teaching exercise, we will attempt to verify the hypothesis that the narrow-eye Bar mutation reverts to wild type (*B* to $B^+$) by unequal crossing-over, as shown in Figure 3.

First, we demonstrated that reversion of the Bar mutation to wild type (*B* to $B^+$) only occurs in females; *D. melanogaster* males do not undergo recombination (Morgan, 1912). Second, we

confirmed that these Bar reversion events in females are always associated with recombination of outside genetic markers on either side of the *B* locus on the X chromosome.


Figure 2. Bar (*B*) eye (left) and wild type ($B^+$) eye (right) of *D. melanogaster*.

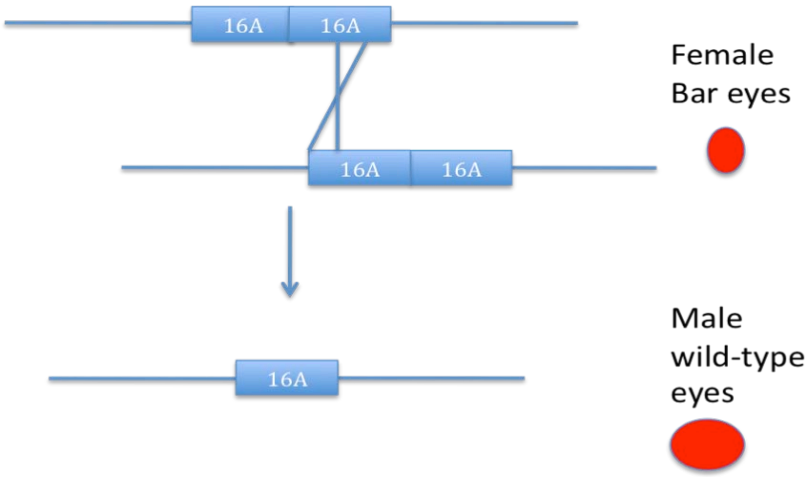



Figure 3. Unequal crossing over in *B*/*B* females can lead to reversion of *B* to $B^+$.

*Reversion of B only occurs in females*

We began this study by measuring the occurrence of $B^+$ revertants in a *f B* stock (*f* = forked bristles, X linked). We used the *f* marker to eliminate wild-type contaminants in this cross. Any true $B^+$ revertants will also be *f* and have forked bristles. We recovered two $B^+$ revertants out of 7,917 total flies (0.00025 or about one revertant in 3,959 flies). One of the revertants was a female and one was a male; both had forked bristles, *i.e.*, they were *f* $B^+$. We also tested each of these $B^+$ revertants and they bred true for the $B^+$ phenotype. This frequency of observed $B^+$ revertants (1/3,959) is similar to the previous report of about one revertant in 2,500 progeny (Sturtevant, 1925). To verify that these $B^+$ revertants came from unequal crossing over, we also measured the frequency of $B^+$ revertants from males in the following cross. The C(1)DX, *y f* chromosome is two X chromosomes attached to a single centromere and contains the recessive markers *y* (yellow, yellow body color), and *f* (forked, short bristles). In this cross, the X chromosomes in the F1 male progeny come from the parental males. If $B^+$ reversions were caused by unequal crossing over, there would be no expected $B^+$ revertants recovered in the male progeny of this cross.

C(1)DX, *y f* / Y females × *f B*/Y males

↓

Score the *f B* / Y patroclinous male progeny for $B^+$ revertants

We observed no $B^+$ revertants out of 15,849 F1 male progeny from this cross. Although the frequencies of revertants from females (2/7,917) and males (0.15,8459) are not significantly different (P = 0.21), the results support our hypothesis that $B^+$ reversion events only occur in females.

*Reversions of B are associated with recombination*

To confirm the hypothesis that $B^+$ revertants occur by unequal crossing over, we also determined if $B^+$ revertants from females were always associated with recombination. In the cross of this experiment (shown below), *f* (map position 56.7, distal from the centromere of the X chromosome) is located to the left of *B* (map position 57.0) and $Bx^3$ (a dominant marker that causes narrow, excised wings, map position 59.4, proximal to the centromere of the X chromosome) is located to the right of *B*. We measured the map distance between *f* and $Bx^3$ in f + / + $Bx^3$ females and got 1.5% recombination, which is similar to the reported distance of 1.7% (Lindsley and Zimm, 1992). The females in this cross were mated with $w^{1118}$/Y males that have a white eye due to a deletion of part of the white gene (http://flybase.org/reports/FBal0018186.html).

*f B* + / + *B* $Bx^3$ females × $w^{1118}$ / Y males

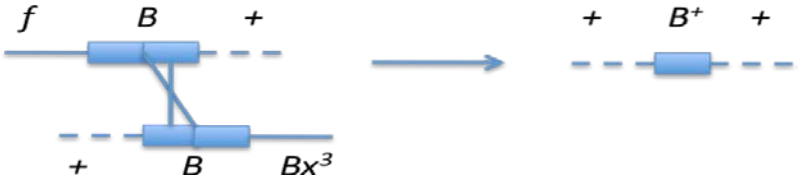
↓

Female and male F1 progeny were scored for $B^+$ revertants
and for the *f* and $Bx^3$ outside markers.

All presumptive revertants were mated to make sure that the $B^+$ revertant phenotypes bred true and that the F1 $B^+$ revertant females were heterozygous for the $w^{1118}$ containing X chromosome.

From this cross, we recovered eight $B^+$ revertants out of 23,064 progeny. Six of these $B^+$ revertants were females, which all gave some F2 white-eyed male progeny, and two were males, which bred true as F2 $B^+$ males when mated with C(1)DX, *y f* / Y females. Hence, all eight were true $B^+$ revertants.

All eight of the $B^+$ revertants were also recombinant for outside markers, supporting the hypothesis that $B^+$ reversions are caused by unequal crossing-over events. All eight of these $B^+$ revertants were + $B^+$ + recombinants, *i.e.*, they were wild type for the *f* focus, $B^+$ and wild type for the $Bx^3$ locus. The unequal crossing-over event that gave rise to the + $B^+$ + chromosomes are shown below.




We do not know why the reciprocal *f* $B^+$ $Bx^3$ revertants were not recovered in this experiment.

In summary, the results from two experiments supported the hypothesis that reversions of the *B* mutation occur by the mechanism of unequal crossing over. 1) Bar reversions only occurred in females and 2) $B^+$ revertants were always associated with exchange of closely linked outside markers.

Class discussions may include the following. 1) About 8% of males and 0.5% of females of European origin have red-green color vision defects (Drummond-Borg et al., 1988). Why is there such a higher frequency of color blindness in males? The reason is that this is a sex-linked trait and hemizygous (XY) males are expected to have a higher frequency than females who must be homozygous (XX) for the defective region. 2) If the red-green sequences are in Hardy/Weinberg equilibrium and the frequency of the defect is 8% in males, what is the expected frequency in females? The answer is 0.08 × 0.08 = 0.6%, close to the reported 0.5%. 3) The assumption that humans are in Hardy/Weinberg equilibrium for the red-green sequences also assumes that females and males that have red-green color blindness are as fit as humans without color blindness, *i.e*., humans with and without the red-green defect have about the same number of offspring. One might ask students if they think this would be true in prehistoric and modern times. What is known is that a higher frequency of red-green color blindness is found in more advanced societies than in some primitive societies (Malhotra, 1978; Narahari, 1993). This may suggest relaxed natural selection for red-green color blindness in modern societies.

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## White eye phenotypes and their genetic analysis.

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An interesting case for undergraduate students of general Genetics is to consider that different genes can produce the same or similar phenotypes. We present here an experiment to discover that the same phenotype could be produced by different genes, and then, to carry out the genetic analysis of these genes. For this laboratory study we have used the following *Drosophila melanogaster* strains: *white* (white eyes) and *scarlet – brown* (white eyes).

Initially, students have a couple of strains (named mutant 1 and mutant 2) showing the same phenotype (white eyes) and the first question is, are they mutations from the same gene or from different genes? The classical approach is to carry out reciprocal crosses between them. The crosses and results that would be obtained are:

(P) ♂ mutant 1 × ♀ mutant 2

↓

($F_1$) All individuals present normal eyes

(P) ♂ mutant 2 × ♀ mutant 1
↓
($F_1$) ♂ white eyes + ♀ normal eyes

Thus, it is possible to deduce that mutant 1 and mutant 2 do not complement, and thus mutant 1 and mutant 2 affect different genes.

For the particular analysis of each mutant strain, reciprocal crosses have to be carried out between individuals from the mutant and normal strains. Thus, for the case of **mutant 1 strain**:

**A)** (P) ♂ mutant 1 × ♀ normal and **B)** (P) ♂ normal × ♀ mutant 1

In the first cross, all $F_1$ individuals presented normal eyes. However, in the reciprocal cross all males have white eyes whereas all females are normal. Flies from both $F_1$ (derived from **A** and **B** parental crosses) have to be independently intercrossed to obtain the respective $F_2$. In this generation and for the parental **A** cross, the proportions of individuals are approximately: 1/2♀ normal + 1/4 ♂ normal + 1/4 ♂ white eyes. The $F_2$ obtained from the parental cross B is composed of 1/4♀ normal + 1/4 ♀ white eyes + 1/4 ♂ normal + 1/4 ♂ white eyes. These results are according to a sex-linked inheritance pattern. Checking the *D. melanogaster* essential genetic maps (for instance Gardner *et al*., 1991; Russell, 1992; Griffiths *et al*., 1996; Klug and Cummings, 1997; Pierce, 2009), mutant 1 strain corresponds most likely to the *white* gene.

For analyzing **mutant 2**, the reciprocal crosses carried out are:

**C)** (P) ♂ mutant 2 × ♀ normal and **D)** (P) ♂ normal × ♀ mutant 2

In both cases (crosses **C** and **D**), the $F_1$ was constituted by normal individuals. Thus, reciprocal crosses **C** and **D** are equivalents. Males and females of $F_1$ have to be intercrossed to obtain the $F_2$. The phenotypes obtained and their proportions are approximately: 9/16 normal + 3/16 bright eyes + 3/16 brown eyes and 1/16 white eyes. Thus, a couple of genes are controlling this trait and new phenotypes (not presented in the parental individuals) arise in the $F_2$ generation. This is due to a gene interaction between two genes presenting independent transmission. Studying the *D. melanogaster* genetic maps (Gardner *et al*., 1991; Russell, 1992; Griffiths *et al*., 1996; Klug and Cummings, 1997; Pierce, 2009), it is possible to deduce that the genes producing this interaction are likely *scarlet* (located in chromosome III) and *brown* (in chromosome II).

We consider that this *Drosophila* experiment is very useful to students, because it allows working the complementation concept, to study the segregation of a sex-linked gene (*white* gene) and to introduce the fundamentals of gene interaction (*scarlet* and *brown* genes). If a basic *Drosophila* laboratory is available, logistic for the experiment is not difficult. The number of generations (and thus, the weeks needed for the whole experiment) is restricted. Furthermore, the number of weeks can be reduced if, after the first cross (complementation test), some students carry out the genetic analysis of mutant 1 strain, whereas others study mutant 2 strain. Finally, only a basic statistical level is required by the students, because all statistical analyses can be carried out using the $\chi^2$ test.

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**An undergraduate laboratory exercise aimed to demonstrate regulation of eukaryotic gene expression using the GAL4-UAS system in *Drosophila melanogaster*.**

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## Abstract

The aim of this laboratory exercise is to demonstrate the genetic aspects of gene regulation using transgenic approaches in *Drosophila melanogaster*. During this exercise the students learn about the life cycle and morphology of *Drosophila* and aspects of Mendelian genetics, while testing effects of mis-expression of genes by means of observing phenotypes. The students are directed to set up simple genetic crosses and analyze the effects of mis-expression of genes that cause cell death or are responsible for eye determination. This exercise uses the GAL4-UAS system, a well-established system in flies, to express genes in a tissue or cell-specific manner. This exercise is convenient for an undergraduate lab set up owing to the short generation time of the model organism, ease of manipulation, and amplification for both large and small group set ups. At the end of this exercise the students learn about the mechanisms involved in spatial (domain specific) and temporal regulation of different genes during development. More specifically, they study the role of regulatory sequences (cis-acting elements) and transcription factors (trans acting elements) in defining tissue specificity. These aspects of eukaryotic gene regulation are elucidated by means of reverse genetics that involve mis-expression of genes involved in cell death and cell fate determination.

## Introduction

An important aspect of most undergraduate genetics courses is the study of prokaryotic and eukaryotic gene regulation. We devised a laboratory exercise to demonstrate the role of regulatory elements in specific tissues for our Genetics Laboratory course. We designed this lab exercise using the GAL4-UAS system (Brand and Perrimon, 1993) that allows ectopic expression of genes. The exercise involved over-expression (gain-of-function) of specific genes that caused observable phenotype. All cells gain their identity based on the characteristic expression profile of genes. Our knowledge of gene function has traditionally depended on analysis of null phenotypes caused by loss of function of genes. However, sometimes loss of function has no apparent phenotype due to genetic redundancy or it causes lethality. In these scenarios, gain-of-function approach is also an informative tool to study the effects of gene expression in a cell (Phelps and Brand, 1998). Consequently, gain-of-function approaches have also provided important insights into gene function and regulation. Thus, both loss-of-function and gain-of-function methodologies provide important insights into gene regulation and function that generate specific cell fates.

In the lab exercise, we use the bipartite GAL4-UAS system, where one transgenic fly harbors the GAL4 gene under the control of a specific promoter, while the other transgenic fly harbors a target gene fused to GAL4 binding sequences called the Upstream Activator Sequence (UAS) (Figure 1). In the system generated by Brand and Perrimon, the gene of interest is cloned into the polylinker (multiple cloning site, MCS) of the vector p[UAST] downstream of the five optimized GAL4 binding

sites. This construct is microinjected into *Drosophila* embryos to generate transgenic flies that harbor Upstream activating Sites (UAS) and the gene of interest. Both the GAL4 and the UAS transgenic flies are viable as the genes are not constitutively active in individual flies. This helps to maintain lethal mutations and study their function in tissue specific manner. When these transgenic lines are crossed to each other then the Gal4 and the UAS transgenes are both expressed in trans-heterozygous flies of the F1 generation (Figure 1). The GAL4 protein dimerizes and binds to the UAS sites and drives the expression of the downstream gene. If the mutation is not lethal, then both the GAL4 and the UAS sequence can be recombined into one fly.

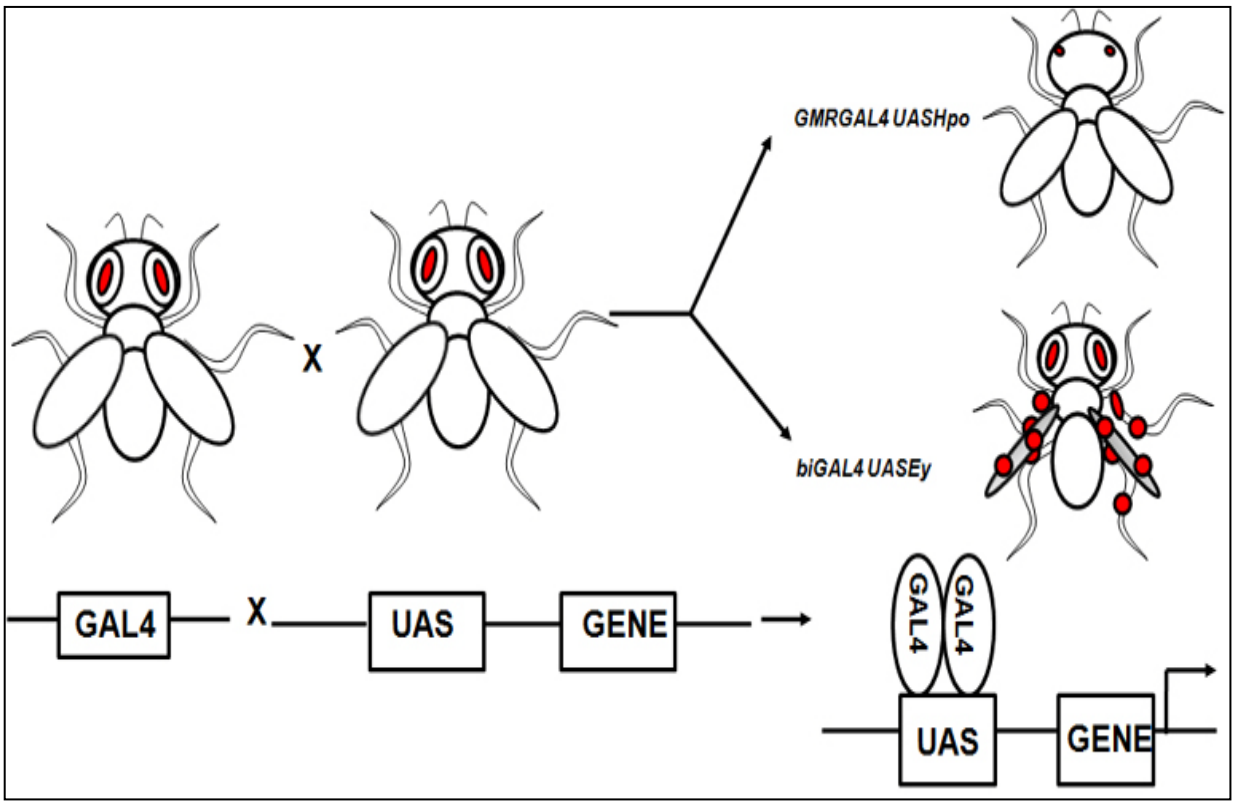


Figure 1. A model of the GAL4-UAS system: The fly harboring the GAL4 gene linked to a tissue-specific promoter is crossed to a fly harboring the gene of interest downstream of the UAS sequence. The progeny for *GMRGAL4 UASHpo* show small necrotic eyes, while the progeny for *biGAL4 UASEy* show ectopic eyes on legs, halteres, and wings as demonstrated in the figure.

Prior to proceeding to the lab session, the students are expected to know the general structure of DNA, chromosomes, and concepts related to transgenes, transcription, and translation in eukaryotes. The students are given brief introduction to the genetics laboratory that covers the theoretical aspects and the experimental design. Two sets of experiments are performed, one with the Hippo transgene (UAS-Hpo) (Udan *et al.*, 2003) and the second with the *eyeless* transgene (UAS-Ey) (Halder *et al*., 1995; Kango-Singh *et al*., 2003). Over-expression of *hippo* is known to cause cell death (Udan *et al*., 2003; Verghese *et al.*, 2012). This experiment utilizes the GMR-Gal4, which directs the expression of UAS-linked transgenes in the region posterior to the morphogenetic furrow where photoreceptor cells differentiate (Figure 2c). The over-expression of UAS-Ey is done using

the bi-GAL4 (Figure 2a), which is expressed in the leg, halteres, and wings and can be used for domain specific expression of transgene (Tare *et al*., 2012). The over-expression of Eyeless leads to development of ectopic eyes on legs, halteres, and wings of the flies (Halder *et al*., 1995; Kango-Singh *et al*., 2003).

## Experimental Design

These experiments span a period of roughly three weeks (see Table 1). During the first lab session, the students learn to distinguish between the males and females on the basis of the anatomy of the fly. They are also taught to collect virgins of the required genotypes and set the cross. Typically, the teaching assistant amplifies the required stocks to hasten the progress of this step.

On an average the ratio of females to males per cross is 8:6. For the next two weeks, the students regularly flip the flies and incubate them at room temperature until they have about 5 tubes with healthy cultures growing in them. During the third week, the students observe the progeny under dissection microscopes.

Table 1.

Week 1:
(1) Learn to distinguish between male and female flies. Observe fly anatomy.
(2) Set crosses (below), and transfer to fresh food vials
  (a) GMR GAL4 females × UAS *Hpo* males
  (b) bi GAL 4 females × UAS *Ey* males

Week 2: Flip flies into fresh food vials
Week 3: Observe phenotypes of the progeny in F1 generation, score phenotypes, estimate ratios of flies that are wild-type versus the ones showing phenotype, and document effects by taking images of the adult flies in an apotome.

The students record the following data points:

1. Counts from the F1 progeny for each phenotypic class.
2. Are all expected classes observed; is there lethality, and if so what is the explanation for the observed lethality?
3. In case of the F1 progeny from the GMRGAL4 flies crossed to UAS-Hpo flies, pharate lethals are observed. The students are shown how to dissect the pharates and observe the ectopic eye phenotype.
4. The data for each experiment are recorded by taking images of adult flies (Figure 2) with the over-expression phenotype.

## Conclusions

We have successfully carried out this laboratory exercise for three semesters now. The students have shown interest and appreciated how genes influence pattern or cell fate determination through the bi-GAL4 UAS-Ey experiment (Figure 2a, b). The cytotoxic effects of Hpo over-

expression (GMR-GAL4 UAS-Hpo) also illustrate the effect of normal *versus* abnormally high levels of protein expression in a cell (Figure 2c, d). The stark contrast in the outcomes of the two experiments illustrates the importance of normal regulation of genes during development and how mis-expression or over-expression of genes alters the pattern or causes inappropriate patterning. At the end of this exercise students learn about the mechanisms of regulation of gene expression, specifically the role of different promoters in generating tissue-specific gene expression profile along the temporal axis during development. They also study the role of transcription factors, DNA binding sites, and the effects of mis-expression of genes. These exercises provide students the hands-on experience that align theoretical knowledge with actual wet-bench experimentation and reinforce the concepts learned in class.

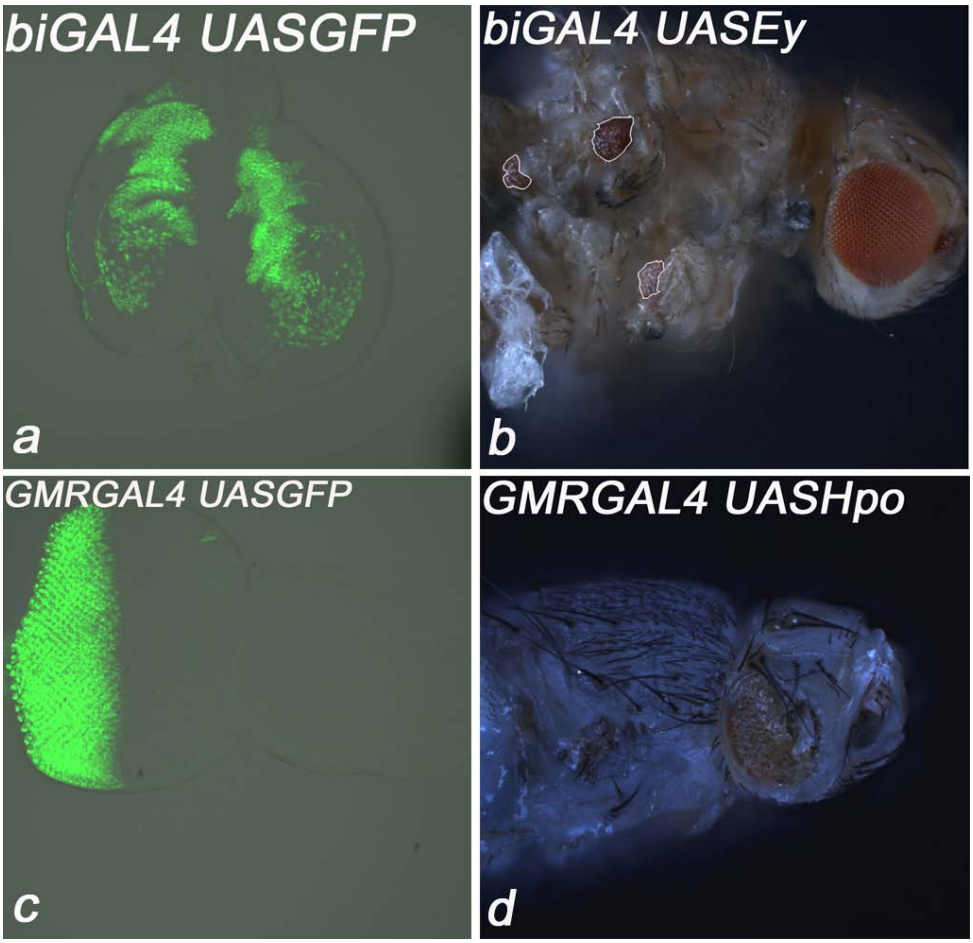


Figure 2. Effects of mis-expression of Ey and Hpo: a) Over-expression of UAS-GFP using bi-GAL4. GFP expression (green) is observed in the dorsal domain the leg and haltere disc. b) Over-expression of UAS-Ey using bi-GAL4 produces ectopic eyes on legs, wings, and halteres (marked by white lines). c) Over-expression of UAS-GFP using GMR-GAL4. GFP expression (green) is seen in the photoreceptor neurons. d) Over-expression of UAS-Hpo using GMR-GAL4 produces small necrotic eyes in the adult.

References: Brand, A.H., and N. Perrimon 1993, Development 118: 401-415; Halder, G., P. Callaerts, and W.J. Gehring 1995, Science 267: 1788-1792; Kango-Singh, M., A. Singh, and Y. Henry Sun 2003, Dev. Biol. 256: 49-60; Phelps, C.B., and A.H. Brand 1998, Methods 14: 367-379; Tare, M., O.R. Puli, M.T. Moran, M. Kango-Singh, and A. Singh 2012, Genesis; Udan, R.S., M. Kango-Singh, R. Nolo, C. Tao, and G. Halder 2003, Nat. Cell Biol. Oct, 5(10): 914-920; Verghese, S., S. Bedi, and M. Kango-Singh 2012, Cell Death Differ. Oct, 19(10): 1664-1676.

# 53rd Annual *Drosophila* Research Conference

The 53rd Annual *Drosophila* Research Conference was held on 7 – 11 March 2012 at the Sheraton Chicago Hotel & Towers, Chicao, IL. The 2012 Organizing Committee was Celeste Berg, Steve Crews, Erika Matunis, and Kevin White. The conference was sponsored by The *Drosophila* Board in association with the Genetics Society of America, 9650 Rockville Pike, Bethesda, MD 20814-3998. The Program and Abstracts Volume lists 892 presentations, including 156 platform session talks and 736 posters (www.drosophila-conf.org).

**Future Advances in Drosophila Research,** moderated by Steve Crews (University of North Carolina, Chapel Hill)

**Featuring**: Hugo Bellen, Ross Cagan, Dan Kiehart, and Trudy Mackay

**Plenary Lectures** (In Presentation Order)

Carl S. Thummel (Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT). Regulation of energy metabolism in *Drosophila*.

Julie H. Simpson (HHMI, Janelia Farm Res. Campus, Ashburn, VA). Behavioral and anatomical analysis of the neural circuits that drive fly grooming.

Stephen DiNardo (Department of Cell & Developmental Biology, Perelman School of Medicine, Institute for Regenerative Medicine, University of Pennsylvania, Philadelphia, PA). News from the niche.

Suzanne Eaton (The Max Planck Institute of Molecular Cell Biology and Genetics, Germany). Lipoproteins in human and *Drosophila* Hedgehog signaling.

John Tower (Molecular and Computational Biology Program, University of Southern California, Los Angeles, CA). 3D videotracking of *Drosophila* behavior and GFP expression and predictive biomarkers of aging.

Steven Reppert (UMass Medical School, Worcester, MA). The neurobiology of Monarch Butterfly migration.

Denise J. Montell (Department of Biological Chemistry, Center for Cell Dynamics, Johns Hopkins School of Medicine, Baltimore, MD). Oogenesis: where physiology and development meet.

Manyuan Long (Department of Ecology & Evolution, University of Chicago, Chicago, IL). Evolution and phenotypic effects of new genes in *Drosophila*.

Julie A. Brill (Cell Biology Program, The Hospital for Sick Children, Toronto, ON). PIPs control cell morphogenesis in *Drosophila*.

Chris Q. Doe (Inst. Neuroscience, University of Oregon, Eugene, OR). Spindle orientation in neural stem cells.

Eileen E. Furlong (Genome Biology, EMBL, Heidelberg, Germany). Deciphering the *cis*-regulatory code.

Thomas B. Kornberg (Cardiovascular Research Institute, University of California, San Francisco, CA). A mechanism of morphogen protein dispersion mediated at points of direct contact.

**Workshops** (in order of presentation)

Ecdysone Workshop
Organizers: c/o bashirullah@wisc.edu; robward@ku.edu

Organelles in the *Drosophila* Ovary
Organizers: Ji-Long Liu (Oxford University) and Lynn Cooley (Yale University)

Apoptosis, Autophagy, and Other Cell Death Mechanisms
Organizers: Andreas Bergmann (University of Massachusetts Medical School) and Michael Brodsky (University of Massachusetts Medical School)

Chromosome Pairing and Dynamics in Meiotic and Somatic Cells
Organizers: Giovanni Bosco (University of Arizona) and Sharon Bickel (Dartmouth College)

Undergrad Researcher Workshop
Organizers: Karen Hales (Davidson College) and Beth Ruedi (GSA)

Everything You Ever Wanted to Know About Sex
Organizers: Artyom Kopp (University of California, Davis), Michelle Arbeitman (University of Southern California), Mark Siegal (New York University), and Mark Van Doren (Johns Hopkins University)

*Drosophila* Research and Pedagogy at Primarily Undergraduate Institutions (PUI)
Organizers: Justin DiAngelo (Hofstra University), Jason Duncan (Wilamette University), and Hemlata Mistry (Widener University, Chester, PA)

modENCODE
Organizers: Susan Celniker (Lawrence Berkeley National Laboratory) and Kevin White (University of Chicago)

# The North American *Drosophila* Board

The Board's duties include: overseeing community resource centers and addressing other research and resource issues that affect the entire *Drosophila* research community. The Board also administers the finances for the annual North America *Drosophila* Research Conference and its associated awards, and it chooses the organizers and the site of the annual meeting. The Board consists of eight regional representatives and four international representatives, who serve 3-year terms. The three elected officers are President, President-Elect, and Treasurer. In addition, the Board has *ex officio* members who represent *Drosophila* community resources or centers. For more information about the Board and the summaries of the annual Board meetings, see: the FlyBase web site: flybase.bio.indiana.edu.

## *Drosophila* Board Membership as of 53rd Annual *Drosophila* Research Conference March 2012

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Karen Hales (Davidson College, Davidson, NC)

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Jim Thompson (Drosophila Information Service; University of Oklahoma)
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