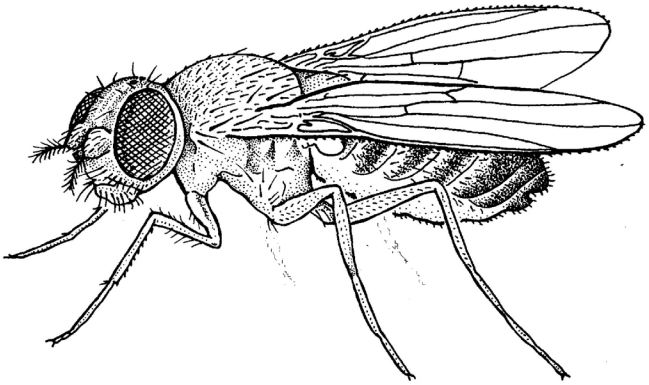
Drosophila Information Service



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Preface

Drosophila Information Service celebrates its 75th birthday with this issue. DIS 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 DIS 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." During the 75 years since that first issue, DIS has continued to promote open communication.

The production of DIS volume 92 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. Carol Baylor was also especially helpful in generating pdf copies of early articles in response to many dozens of individual researcher "reprint" requests. Beginning with volume 84 (2001), the official annual publication date is 31 December, with the contents including all submissions accepted during the calendar year. New issues are available for free access on our web page (www.ou.edu/journals/dis) soon after publication, and earlier issues are being archived on this site as resources permit.

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

Genotoxicity of Sevin in wing primordial cells of *Drosophila melanogaster* in vivo.

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Abstract

The genotoxicity of Sevin, a systematic carbamate pesticide, has been evaluated in the wing primordial cells of *Drosophila melanogaster* by the larval feeding method. Larvae (48 ± 4h/72 ± 4h) heterozygous for mutations were cultured on the Sevin-containing media for their remaining life. Emerged flies were screened for mosaics on their wings to know the genotoxicity of Sevin.

Introduction

With the increased production and application of pesticides all over the world, it is essential that maximum effort should be geared to generate the knowledge about the hazardous impact on non target organisms. The impact in non-targets may influence the genetic systems and cause mutations that ultimately influence the progeny by heredity. Carbaryl, a carbamate pesticide, causes behavioral and neurological (Moser, 1988; Sideroff and Santolucito, 1972; Dsi, 1974; Anger and Setzer, 1979; Branch and Jacqz, 1986), reproductive (Cantor, 1992; Zahm, 1988; Davis, 1993), and carcinogenic problems (Smalley *et al*., 1968; Whorton, 1979) in exposed systems. However, there are inconclusive reports on the genotoxicity of Carbaryl in *Drosophila* (Dey *et al*., 1987). Hence, the present investigation is an endeavor to explore the genotoxicity of Carbaryl in *Drosophila melanogster*, by wing mosaic assay.

Materials and Method

Most widely used Carbary, that is Sevin (CAS No.63-25-2), was tested. Two *Drosophila* stocks, *mwh*/*mwh* and flr^3/flr^3 (Lindsley and Grell, 1968) were used in the present studies. All the culture stocks are maintained on standard *Drosophila* medium at 25 ± 1°C and RH 60%. The larvae (48 ± 4h/72 ± 4h) obtained after crossing males of the *mwh*/*mwh* stock with the virgin females of the flr^3/flr^3 stock were collected by floating them in 20% NaCl (Graf *et al*., 1984). The same larvae were exposed to non-toxic doses (5, 10, 15 and 20ppm) of Sevin (LC_{50} = 150ppm) for their rest of their larval life. Wings of the emerged flies were screened for the induction of mosaics by the Sevin. Mosaics were analyzed statistically using the SMART program (Frei and Wurgler, 1988).

Results and Discussion

Sevin was assayed separately in somatic cells of *Drosophila* by wing mosaic test at two instars using four different concentrations (5, 10, 15, and 20ppm). The results of the experiment

(Tables 1 and 2) reveal the frequency according to number of mosaics counted. There is an increase in mosaics with the concentration of the chemical fed to the larvae. Among the mosaics, single spots were predominating and twin spots are completely absent. However, at all the doses tested, Sevin induced insignificant mosaics to control indicating nongenotoxicity in wing primordial cells of *Drosophila*.

The wing mosaic assay is based on the formation of homozygous mutant clones on the adult's wing cuticle due to exposure to a mutagen during larval stage (Auerbach, 1946; Becker, 1975) and is a tool to generate the structure activity relation of chemicals (Graf *et al*., 1992). Single spots arise due to gene mutation, gene conversion, aneuploidy, mitotic recombination, or nondisjunction, while twin spots are the result of mitotic recombination (Graf *et al*., 1984; Garcia-Bellido and Dapena, 1974).

Table 1. Data on wing mosaic assay after 48 ± 4h aged larval exposure to Sevin.

Larval age	Sevin (ppm)	No.of wings screened	Small single spots	Large single spots	Twin spots	Total spots
48 ± 4h	0.0(control)	40	3(0.07)	0(0)	0(0)	3(0.07)
	5	40	4(0.01)-	1(0.02)-	0(0)-	5(0.15)-
	10	40	4(0.01)-	2(0.05)-	0(0)-	6(0.15)-
	15	40	5(0.012)-	2(0.05)-	0(0)-	7(0.17)-
	20	40	6(0.15)-	3(0.07)-	0(0)-	9(0.22)-

Statistical diagnosis according to Frei and Wurgler (1988):
+ positive, - negative, i inconclusive.
Probability levels: α=β=0.05. One sided statistical test.

Table 2. Data on wing mosaic assay after 72 ± 4h aged larval exposure to Sevin.

Larval age	Sevin (ppm)	No.of wings screened	Small single spots	Large single spots	Twin spots	Total spots
72 ± 4h	0.0(control)	40	4(0.1)	0(0)	0(0)	4(0.1)
	5	40	5(0.12)-	2(0.05)-	0(0)-	7(0.17)-
	10	40	6(0.15)-	2(0.05)-	0(0)-	8(0.2)-
	15	40	6(0.15)-	3(0.07)-	0(0)-	9(0.22)-
	20	40	7(0.17)-	4(0.1)-	0(0)-	11(0.25)-

Statistical diagnosis according to Frei and Wurgler (1988):
+ positive, - negative, i inconclusive.
Probability levels: α=β=0.05. One sided statistical test.

The present study infers the nongenotoxicity of Sevin in wing disc cells of *Drosophila*. But Sevin had induced Sex Linked Recessive Lethal (Brzheskii, 1972) in *Drosophila*. However, the inconclusive results of Sex Linked Recessive Lethal (Dey *et al*., 1987) made it important to reevaluate the effect of Sevin. The microsomes of insects function similarly to those in mammalian liver (Vogel and Sobels, 1976). Reactions generating O_2 also generate H_2O_2 by dismutase reaction that results in -OH, a potent oxidant (Haber and Wiss, 1934). Dismutases, catalases, and peroxides

are maintained the homeostasis (Fridovich, 1975) that results in the integrity and fidelity of the DNA. Carbamates are known to inhibit proteins (Cornman, 1954; Rannug and Rannug, 1984). But from the present results of the wing mosaic assay, it is possible that the same mechanism may not be operating with Sevin in wing disc cells of *Drosophila*.

References: Anger, K.W., and J.V. Setzer 1979, J. Toxicol. Environ. Health 5: 793-808; Auerbach, C., 1946, Proc. Roy. Soc., Edin. 62: 211-222; Becker, H.J., 1975, Molec. Gen. Genet. 138: 11-24; Branch, R.A., and E. Jacqz 1986, Amer. J. Med. 80: 659-664; Brzheskii, V.V., 1972, Genetica. 8(6): 151-153; Cantor, K.P., 1992, Cancer Res. 52: 2447-2455; Cornman, I., 1954, *In*: Bourn, G.H., and J.F. Danielli, eds., Int. Rev. Cytol. 3: 113-128; Davis, J.R.., 1993, Arch. Environ. Contam. Toxicol. 24: 87-92; Dey, L, B. Majhi, N.K. Tripathy, and C.C. Das 1987, Current Science. 56(16): 848-850; Dsi, I., 1974, Toxicol. Appl. Pharm. 27: 465-476; Fridovich, I., 1975, *In*: Esmond, E., and Snerl, eds., Annu. Rev. Biochem. 44: 147-159; Frei, H., and F.E. Wurgler 1988, Mutation Res. 203: 297-308; Garcia-Bellido, A., and J. Dapena 1974, Mol. Gen. Genet. 128: 117-130; Graf, U., F.E. Wurgler, A.J. Katz, H. Frei, H. Joun, C.B. Hall, and P.G. Kale 1984, Environ. Mutagen. 6: 153-188; Graf, U., D. Wild, and F.E. Wurgler 1992, Mutagenesis. 7(2): 145-149; Haber, F., and J. Weiss 1934, Proc. Roy. Soc. (London), A. 147: 332-351; Lindsley, D.L., and E.H. Grell 1968, *Genetic Variations of* Drosophila melanogaster. Carnegie Inst. Wash. Publ. 627; Moser, V.C., 1988, Fund. Appl. Toxicol. 11: 189-206; Rannug, A., and U. Rannug 1984, Chem-Biol. Interac. 49: 329-340; Sideroff, S.I., and J.A. Santolucito 1972, Physiol. Behav. 9(3): 459-462; Smalley, H.E., J.M. Curtis, and F.L. Earl 1968, Toxicol. Appl. Pharm. 13: 392-403; Vogel, E.W., and F.H. Sobels 1976, *Chemical Mutagens. Principals and Methods for Their Detection. In*: Hollander, A., ed. 4: 93-142. Plenum Press, New York; Whorton, M.D., 1979, J. Toxicol. Environ. Health 5: 929-941; Zahm, S.H., 1988, Amer. J. Epidemiol. 128(4): 901.

Drosophila lowei collections from Mount Lemmon, Arizona, in 2009.

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The *Drosophila pseudoobscura* subgroup inhabits western North America and contains seven species (Lakovaara and Saura, 1982; Heed and O'Grady, 2000), but four of these species have reportedly not been collected in over a decade nor are there living cultures of them. The relationships of four of the species within the pseudoobscura subgroup are well supported by allozymes (Figure 1; Lakovaara *et al*., 1976), and a phylogeny reconstructed using the mitochondrial gene *cytochrome oxidase II* (Beckenbach *et al*., 1993) revealed that *D. lowei* diverged from *D. pseudoobscura* approximately 8.4 Mya (Aquadro *et al*., 1991; Beckenbach *et al.*, 1993). The next closest ancestors to *D. pseudoobscura*, species of the *D. affinis* subgroup, diverged approximately 17Mya (Aquadro *et al*., 1991; Beckenbach *et al*., 1993). Genomic sequence data and establishment of laboratory stocks of *D. lowei* may enhance comparative studies within the pseudoobscura subgroup. Scientific collections of *D. lowei*, however, have not been undertaken since those made for Beckenbach *et al*. (1993).

First collected in 1960 in Santa Catalina Mountains near Tucson, Arizona (Heed *et al*., 1962), *D. lowei* has also been documented in the Chiricahua Mountains and Mongollon Rim in Arizona and near Pikes Peak, Colorado (Heed *et al*., 1969). Like other southern species of the pseudoobscura subgroup (Heed and O'Grady, 2000), *D. lowei* is restricted to highlands and is found in highest

abundance in pine and fir forests at elevations greater than 7000ft in Arizona and 6000ft in Colorado in late summer to early fall (Heed *et al*., 1969). Males of *D. lowei* have two sets of sex combs, which are reduced in number (proximal sex comb: 4-6 teeth, distal sex comb: 3-4 teeth) and prominence as compared to *D. pseudoobscura* (Heed *et al*., 1969; Figure 2).

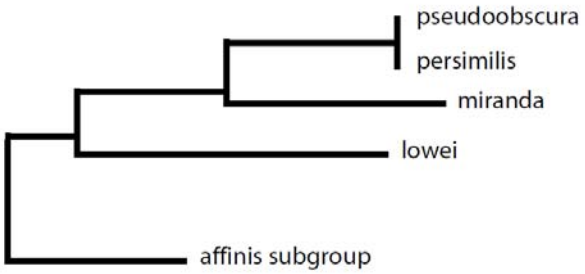

Figure 1. Schematic of *Drosophila pseudoobscura* subgroup in accordance with the phylogenetic reconstruction of Beckenbach *et al*. (1993) using the mitochondrial gene, *cytochrome oxidase II*.

Figure 2. *Drosophila lowei* sex combs. *D. lowei* is distinguished from other flies in the *pseudoobscura* subgroup by its sex combs. It has 3-4 teeth in the distal sex comb and 4-5 teeth on the proximal sex comb. *Drosophila pseudoobscura* from the Santa Catalina Mountains has 4-5 distal sex comb teeth and 6-8 proximal sex comb teeth.

Table 1. Counts of obscura group Drosophila captured on 30-31 August 2009 from banana traps set on Mount Lemmon near Tucson, AZ. Flies identified as *Drosophila macroptera* and *D. rubrifrons* were also found in qualitatively equal abundance to flies of the obscura group.

Location	Lat.	Long.	Elevation	lowei ♂	pseudoobscura ♂	*azteca* ♂
Box Camp Trail	32°25'8.184"N	110°44'28.637"W	7856 ft	25	1	1
Upper Solider Camp Rd	32°25'28.382"N	110°44'12.191"W	7963 ft	12		
Mount Bigelow Rd	32°24'54.479"N	110°43'42.273"W)	8323 ft	63	1	

From 26-31 August 2009, banana traps were set on Mount Lemmon near Tucson, AZ. Three main locations were used (Table 1), and collections were made by aspirating flies from hanging buckets and bottle traps. Flies were most abundant at dusk, but were also found near traps in early morning when the temperature was between 14.5-18.5°C. Of note, the more successful collections were made on two nights when storms were imminent. Male flies, morphologically concordant with the species description of *D. lowei*, were obtained, and the collection data are recorded here (Table 1). Sanger sequencing of two putative male *D. lowei* for a 543bp fragment of the mitochondrial gene *cytochrome oxidase II* (primers: COIIf: ACATGAGCTAATTTAGGTTTACAAGAT, COIIr: AATTAGTTTGGTTTAATCGTCCA) confirmed the morphological species identification (GenBank Accession number: GU060632). Of particular note is their extremely high abundance relative to other obscura-group *Drosophila* at these sites at this collection time (Table 1). This high abundance along with the detailed locality and collection information provided here should assist other investigators seeking to obtain new collections of this species.

One generation has successfully eclosed at room temperature (~23°C) in the lab. Larval development has been facilitated by providing a layer of instant media (Formula 4-24® Instant Medium, Carolina Biological Supply Company) over the standard sugar-yeast-agar media.

Acknowledgments: Beckenbach generously provided advice on field collecting and fly husbandry. Collections were made possible by NIH grant GM086445.

References: Aquadro, C.F., A.L. Weaver, S.W. Schaeffer, and W.W. Anderson 1999, Proc. Natl. Acad. Sci. USA 88: 305-309; Beckenbach, A.T., Y.W. Wei, and H. Liu 1993, Mol. Biol. Evol. 10: 619-634; Heed, W.B., D.W. Crumpacker, and L. Ehrman 1969, Ann. Entomol. Soc. Am. 62: 388-393; Heed, W.B., and P.M. O'Grady 2000, J. New York Entomol. Soc. 108: 98-105; Heed, W.B., J. Russell, and D. Harrington 1962, Dros. Inf. Serv. 36: 73-76; Lakovaara, S., and A. Saura 1982, In: *The Genetics and Biology of* Drosophila (Ashburner, M., H.L. Carson, and J.N. Thompson, jr., eds.), Vol. 3b, pp. 2-49, Academic Press, NY; Lakovaara, S., A. Saura, P. Lankinen, L. Pohjola, and J. Lokki 1976, Zool. Scripta 5: 1-18.

Sexual behavioral plasticity of *D. melanogaster* of Chamundi hill.

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Drosophila is a widely used and well suited model system for studying evolutionary response to extreme temperature (Hoffmann and Parsons, 1991). Phenotypic plasticity is the ability of an organism to alter its physiology, morphology, and behavior in response to changes in its environment. The capacity of a given genotype to produce different phenotypes in different environments is of growing interest among evolutionary biologists (David *et al.,* 2006). This emphasizes the fact that phenotypic plasticity is also a target of natural selection. Different aspects of sexual behavior, such as courtship latency, mating latency, and copulation duration, are good estimates of reproductive fitness of both the sexes. These behavioral traits are also genetically determined. Hence the obvious question that arises is, like phenotypic traits, whether these behavioral traits are also influenced by temperature or not. The author has tried to address this question using *D. melanogaster* flies collected from natural conditions.

To study the effect of different temperatures on sexual behavior, natural populations of *D. melanogaster* collected at domestic locality Chamundi hill, Mysore, were used. The flies were collected by keeping the quarter pint milk bottles containing mashed banana in kitchen and stores of a few houses at the top of the Chamundi hill, Mysore. Then the flies were sexed, and the females were individually placed in vials containing food so as to develop isofemale lines. When progeny appeared, equal numbers of them from each isofemale line were separately distributed to different culture bottles and reared under different temperature regimes: 12ºC, 22ºC, and 32ºC.

Sexual behavior acts, such as courtship latency, mating latency, and copulation duration, of 25 pairs involving each isofemale line were recorded (Hegde and Krishnamurthy, 1979). To identify the difference in sexual behavior at different temperatures, one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) were used. The data on qualitative sexual activities, such as courtship latency, mating latency, and copulation duration of *D. melanogaster* at three different temperatures, is reported in Table 1. The courtship latency was highest at 12ºC (119.2 ± 10.8). The courtship latency increased up to 22ºC and then decreased with increasing temperature. Mean courtship latency, mating latency, and copulation duration at different temperatures, such as

12ºC, 22ºC, and 32ºC, were statistically significant by one way ANOVA (F value = 28.63, 24.04, 35.45; P < 0.001; Table 1). Table 1 also shows that the mating latency was shortest at 22ºC (7.20 ± 0.85) and longest at 12ºC (16.64 ± 0.57). The mating latency at 22ºC was significantly different when compared to other temperatures. In contrast to this, the copulation duration was lowest at 32ºC (15.80 ± 1.84), which increased with the decreasing temperature. Thus the duration of copulation was longest at 12ºC (34.44 ± 1.05), which was significantly different (by ANOVA and DMRT) from all other temperatures (Table 1).

Table 1. Qualitative sexual behavior of *D. melanogaster* at different temperatures (values are Means ± SE).

Temperatures	Parameters		
	Courtship latency (in seconds)	Mating latency	Copulation duration
12 ± 1^{0}C	119.20 ± 10.80^c	16.64 ± 0.57^c	34.44 ± 1.05^d
22 ± 1^{0}C	31.56 ± 2.80^a	7.20 ± 0.85^a	19.76 ± 0.62^b
32 ± 1^{0}C	68.16 ± 6.25^b	7.84 ± 0.99^a	15.80 ± 1.84^a
F value	28.63*	24.04*	35.45*

Same alphabet as superscript in each column (for temperatures) is non-significant by DMRT. * P<0.001.

Sexually reproducing animals are endowed with special features, first to produce fertile offspring and second to adapt to a particular environment. The reproduction is preceded by a series of courtship acts, wherein males and females show unique rituals to attract each other, mate, and produce the offspring. The courtship and mating, although genetic, are also influenced by various factors.

In the present study an effort is made to study the effect of different temperatures on courtship and mating behavior of *D. melanogaster*. The courtship latency was shorter at 22ºC, than either at high or low temperatures (Table 1). The differences in courtship latency at different temperatures were also statistically significant Table 1. It is the period during which the pairs acclimatize to the mating chamber and then start the courtship activities. It actually indicates the vigor of males (Eastwood and Burnet, 1977). The shorter courtship latency noticed at 22ºC thus suggests that the males at this temperature have higher vigor and, therefore, are quickly attracted by the females (Markow, 1985).

The mating latency was also shorter at 22ºC compared to high or low temperatures (Table 1). Shorter mating latency indicates both vigor of males and receptivity of females required for males and females to initiate copulation (Spieth, 1968). A male with high vigor has to perform the same courtship act more times to a non-receptive female than to a receptive female. If she is receptive, only a few courtship acts are performed, leading to quick pairing.

Courtship activity of the male or female culminates in copulation. During copulation, sperm from the male are transferred to the female reproductive tract, and therefore the duration of copulation has a lot of significance in an animal's life. In the present studies, the copulation duration was longest at 12ºC than at other temperatures, and as temperature increased the copulation duration decreased. Thus, the copulation duration was shortest at 32ºC. At higher temperature perhaps the sperm transfer occurs more quickly than at lower temperatures. Longer duration of copulation permits the transfer of more sperm by a male to the female (Hegde and Krishna, 1997). Therefore, extension of copulation duration enhances the fitness of the male. However, the courtship latency and mating latency are longer at this temperature. Therefore, it is unlikely that the longer copulation

duration could enhance the fitness at 12ºC. Therefore, this confirms 22ºC is the optimum temperature for sexual activity of *Drosophila* flies.

References: David, J.R., H. Legout, and B. Moreteau 2006, J. Genet. 85(1): 9-23; Eastwood, and B. Burnet 1977, Beh. Genet. 7: 359-372; Hegde, S.N., and N.B. Krishnamurthy 1979, Aust. J. Zool. 27: 421-431; Hegde, S.N., and M.S. Krishna 1997, Animal Behavior 54: 419-426; Markow, T.A., 1985, Animal Behavior 33: 775-781; Spieth, H.T., 1968, *In*: *Evolutionary Biology*. (Dobzhansky, Th., M.K. Hecht, and W.C. Steere, eds.). pp. 157-193. Appleton- Century-Crofts, New York.

Spontaneous melanic mutant found in a *Drosophila neocardini* natural population.

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Introduction

The *Drosophila cardini* group is a Neotropical polymorphic group of species of the genus *Drosophila* (Heed, 1962; Heed and Russell, 1971). This group consists of 16 species inhabiting different areas of the Neotropical Region (Heed, 1962; Heed and Russel, 1971; Vilela *et al.*, 2002; De Toni *et al.*, 2005). Seven of these species (*D. antillea*, *D. arawakana*, *D. belladunni*, *D. caribiana*, *D. dunni*, *D. nigrodunni*, and *D. similis*) belong to the *dunni* subgroup, distributed in the Caribbean islands, while the other nine species (*D. acutilabella*, *D. bedichecki*, *D. cardini*, *D. cardinoides*, *D. neocardini*, *D. neomorpha*, *D. parthenogenetica*, *D. polymorpha*, and *D. procardinoides*) belong to the *cardini* subgroup, and are observed in an area that starts in Mexico and stretches to south Brazil, covering also the north of Argentina and Chile (Heed and Russell, 1971; Vilela *et al.*, 2002; De Toni *et al.*, 2005).

A series of previous studies reported the monomorphic or the polymorphic abdominal pigmentation state of the species of the *D. cardini* group. All but one of the species of the *D. cardini* subgroup are characterized by a highly polymorphic intraspecific abdominal pigmentation pattern that varies from almost completely pigmented to nearly unpigmented flies (Da Cunha, 1949; Da Cunha *et al*., 1953; Heed and Krishnamurthy, 1959; Heed and Blake, 1963; Martinez and Cordeiro, 1970). The exception is *D. procardinoides*, apparently restricted to the higher elevations in the Andes of Bolivia and Peru (Heed and Russell, 1971). The developmental control of abdominal pigmentation is variable in this subgroup as well (Da Cunha, 1949; Heed, 1963; Martinez and Cordeiro, 1970). *Drosophila neocardini* is one of the flies that displays this type of variation (Da Cunha, 1955). Its distribution covers Mexico, Panama, Colombia, Ecuador, Peru, and Brazil (Stalker, 1953; Heed and Russell, 1971), and occupies several kinds of environments with low abundance, except *cerrado* and *caatinga* Brazilian Biomes (Sene *et al.*, 1980). The abdominal pigmentation of *D. neocardini* is very similar to that of *D. neomorpha*, *D. parthenogenetica*, and *D. polymorpha*, but it is different in respect to the pattern of the abdominal black bands. In the middle of the sixth tergite

in *D. neocardini* there is a black square, which is absent in the other species (Da Cunha, 1955; De Toni *et al.*, 2001, 2005). Here, we report the discovery of a body melanic form in *D. neocardini* that has never been documented before for other *D. cardini* species.

Materials and Methods

Sample

The darker *D. neocardini* strain here analyzed was obtained from a collection performed in autumn of 2006 in a locality of Porto Alegre city, Brazil (30°07'S 51°10'W) (Garcia *et al.*, unpublished data) subject to a humid subtropical climate (Cfa according to Köppen) with average temperatures of 25°C in the summer and 14°C in the winter. The collection site is classified as an area of low urbanization level (over 40% vegetation cover) based on a classification system defined by Ruszczyk (1986) that considers the ratio between the green and built areas to differentiate the urbanization level.

Maintenance of color pattern test

To test whether the darker body color of the *D. neocardini* collected was influenced or not by the environmental conditions, we established two sub-strains from the darker F4 isofemale line. These two new strains were kept in chambers of controlled temperature and humidity, one of them at 17°C (± 1°C, 60% r.h.) and the other at 25°C (± 1°C, 60% r.h.) for 15 generations.

Crossing tests

The crossing tests were performed using the *D. neocardini* darker strain and a *D. neocardini* strain with light body color from Joinville city, Brazil (26º17'S 49º00'W). We carried out two experiments: (1) Cross 01: 10 virgin melanic females were crossed with 10 light-colored males, and (2) Cross 02: 10 virgin light-colored females were crossed with 10 melanic males, always with two vials for each cross. Control crosses were also done using 10 virgin melanic females and males, and 10 virgin light-colored females and males. All crosses were maintained in a controlled chamber at 25°C (± 1°C, 60% r.h.) in corn/flour culture medium. Photos of the specimens were taken with an Olympus stereophotomicroscope.

Cytological preparations

The salivary glands of third instar female larvae of each strain (dark and light) were prepared according to Ashburner (1989, p. 30) using Ringer's solution for dissection. Three larvae from each strain and three larvae from the F1 hybrid crosses were analyzed. The slides were examined under phase contrast Zeiss photomicroscope at 100× objective magnification.

Results and Discussion

In the analysis of the external characters of the three *D. neocardini* collected flies, we observed that they were darker than this species usually is, showing a shining light brownish-brown abdomen, mesonotum and scutellum (Figure 1). So, to confirm the initial identification we analyzed the terminalia of F1 offspring males, focusing on *aedeagus*. Using the available literature about the *cardini* group species (Val, 1982; Vilela and Bächli, 1990; Vilela *et al*., 2002; De Toni *et al.*, 2005), all the morphological characters indicated that the flies collected were conspecific of *D. neocardini*, but with darker body pigmentation, *i.e*., a melanic form.

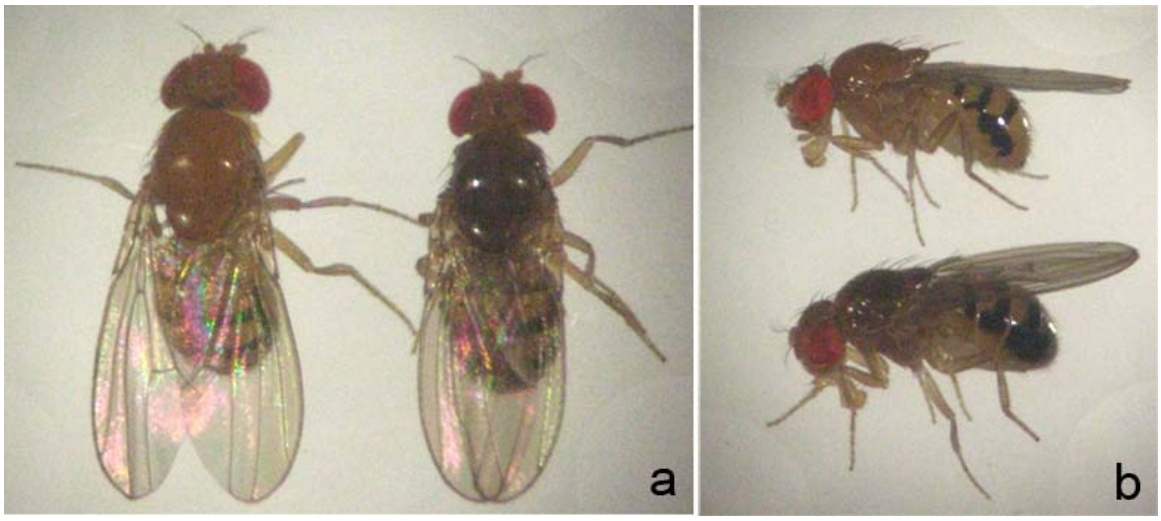

Figure 1. a, Dorsal view of the melanic (right) and light-colored (left) forms of *D. neocardini* individuals. b, Lateral view of the melanic (below) and light-colored

Confirming that the polymorphism detected is heritable, after 15 generations reared in different temperatures (17°C and 25°C) we verified that no different body color pattern became visible. This result indicates that: (1) this character is not environmentally controlled, and (2) all dark flies were possibly homozygous for the alleles that control body color pigmentation. Until now, after approximately 40 generations, we have not noticed any different pigmentation pattern in the melanic strain. We also observed that the lineages were better developed in the 25°C controlled chamber; this is why we used this temperature to conduct the crossing tests.

All the polytene chromosomes of the dark and light-colored strain, and also of the F1 hybrid offspring, presented the same banding pattern as in their reference photomaps (De Toni *et al*., 2001), and no rearrangements were detected in the hybrid chromosomes.

Table 1. Number of dark and light-colored offspring obtained in the crosses.

Offspring color	Cross 01		Cross 02	
	Vial A	Vial B	Vial A	Vial B
Light	58	41	24	24
Dark	11	05	06	09

In the crossing test all F1 offspring from crosses 01 and 02, resulted in a light color with yellowish-brown abdomen, mesonotum and scutellum. Table 1 shows the results obtained in the F2 offspring from crosses 01 and 02. In a first step, we tested our data for the χ^2 of heterogeneity for each cross, and it accepted to unite the data of the vials for each cross (cross 01 = 0.593 < $\chi^2_{0.05;1}$ = 3.84; cross 02 = 0.458 < $\chi^2_{0.05;1}$ = 3.84). Second, we tested our data for the hypothesis that the heritage pattern of the dark color observed behaves as a recessive autosomal characteristic; so, we tested the 3:1 proportion. The data from cross 01 did not confirm the H_0 hypothesis (cross 01 = 7.539 < $\chi^2_{0.05;1}$ = 3.84) that the data obtained did not differ statistically from the 3:1 proportion. However, the data from cross 02 supported the H_0 hypothesis (cross 02 = 0.047 < $\chi^2_{0.05;1}$ = 3.84). We observed that the melanic individuals had a less vigorous reproduction in the culture medium, sometimes resulting in the death of the larvae; in this way these individuals may have a less competitive ability when they are put together in the culture medium with the light-colored individuals. Considering this, we can assume that the dark individuals may have a reduced fitness, which would be reflected in the down deviations found in the statistical analysis of our crosses. Besides that, we also observed that the F2

melanic individuals produced a completely melanic F3 offspring, confirming that the dark *D. neocardini* form has a homozygous behavior.

Regarding the *D. neocardini* distribution (Heed and Russell, 1971), Porto Alegre city seems to be the southernmost limit of this species. At this latitude, the climate is subtropical with a mean temperature ranging from 2°C to 20.3°C in winter, although in this season temperatures as low as 0°C are common. In some *Drosophila* species, it is known that darker forms occur more commonly in samples collected in colder climates (Heed and Blake, 1963; Machado *et al.*, 2001).

So we can conclude that the *D. neocardini* melanic form corresponds to a recessive autosomal heritage pattern, and that the dark pattern of the individuals collected in Porto Alegre corresponds to a recessive homozygote condition for the dark allele.

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References: Ashburner, M., 1989, Drosophila: *A Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York; Da Cunha, A.B., 1949, Evolution 3: 239-251; Da Cunha, A.B. 1955, Rev. Bras. Biol. 15: 117-125; Da Cunha A.B., D. Brncic, and F.M. Salzano 1953, Heredity 2(7): 193–202; De Toni, D.C., C.B. Araujo, N.B. Morales, and V.L.S. Valente 2001, Dros. Inf. Serv. 84: 88-91; De Toni, D.C., J.A. Brisson, P.R.P. Hofmann, M. Martins, and H. Hollocher 2005, Dros. Inf. Serv. 88: 33-38; Heed, W.B., 1962, Univ. Texas. Publ. Stud. Genet. 6205: 173–206; Heed, W.B., 1963, Evolution 17: 502–518; Heed, W.B., and P.R. Blake 1963, Genetics 2: 217-234; Heed, W.B., and N.B. Krishnamurthy 1959, Univ. Texas. Publ. Stud. Genet. 5914: 155–178; Heed, W.B., and J.S. Russell 1971, Univ. Texas. Publ. Stud. Genet. 7103: 91–130; Hollocher, H., J.L. Hatcher, and E.G. Dyreson 2000, Evolution 54: 2046-2056; Machado, M. X., D.C. De Toni, and P.R.P. Hofmann 2001, Biotemas 14(1): 87-107; Martinez, M.N., and A.R. Cordeiro 1970, Genetics 64: 573–587; Ruszczyk, A., 1986, Rev. Bras. Bot. 9: 222–229; Sene, F.M., F.C. Val, C.R. Vilela, and M.A.Q.R. Pereira 1980, Pap. Avul. de Zool. 33: 315–326; Stalker, H.D., 1953, Ann. Ent. Soc. Am. 46: 343-358; Val, F.C., 1982, Pap. Avul. Zool. 34: 309-347; Vilela, C.R., and G. Bächli. 1990, Mitt. Schweiz. Ent. Ges. 63: 1-332; Vilela, C.R., A.F.G. Da Silva, and F.M. Sene 2002, Revta. Bras. Ent. 2 (46): 139-148.

The heterochromatin of *Drosophila inca, D. yangana,* and *D. huancavilcae* of the *inca* subgroup, *repleta* group.

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In 1982, Wasserman extensively studied the chromosome phylogeny of the *repleta* group when he reported the results of the analysis of the sequence of bands of polytenic chromosomes in 70 species belonging to five subgroups that existed then. Considering the morphological, ecological, geographical, and genetic information and based on the presence of certain inversions, he proposed the existence of a sequence Primitive I that would occupy a central place in both the phylogeny of the *repleta* group as in the *Drosophila* genus and at least three phyletic lines connected to the different subgroups. One of these phyletic lines could go from Primitive I to *mulleri-fasciola* subgroups; a

second could go from Primitive I to *repleta-mercatorum* subgroups; and a third could connect directly the Primitive I with *hydei* subgroup (Wasserman, 1982, 1992).

In Ecuador, there are three species that make up the subgroup *inca* proposed by Rafael and Arcos (1989). This sixth subgroup of *repleta* group clusters *Drosophila inca* Dobzhansky and Pavan, 1943 (this subgroup was considered in a miscellaneous group of no clustered species by Wasserman until 1982) with *Drosophila huancavilcae,* an Ecuadorian species belonging to *repleta* group, discovered in 1989; and with *D. yangana* Rafael and Vela, 2003, discovered in another exploration of Ecuadorian drosofauna.

We asked ourselves as follows: Which is the position of *inca* subgroup in the evolutionary scheme of the species of the *repleta* group? Do the three species form a phylogenetic unit?

Studies of species characterization allow us to determine that these species live in arid regions, they are cactophilics, and they have a restricted distribution: *D. huancavilcae* was recorded in three coastal provinces of Ecuadorian Pacific: Manabí, Guayas and El Oro; *D. inca* was recorded in two interandean provinces: Pichincha and Loja; and *D. yangana* was recorded only in Loja. For cytological characterization of these species we worked with individuals of *D. yangana* and *D. inca*, species which were collected in sympatry, in Loja province (4° 23 'S, 79° 11' W), and *D. huancavilcae,* that is allopatric to the previous species, was collected in the Manabí province (1° 2 '46"S 80° 40' 4"W 450m). The three species had been found in nature in the fruits and cladodes of cactus *Opuntia ficus indica, Opuntia soederstromiana* and some species of genus *Armatocerus* (Rafael and Arcos, 1989; Rafael and Vela, 2003), while in the laboratory these species have the best development enriching their cultures with chunks of fruit. Their biological cycles were studied in these conditions during 22 days in *D. yangana* and *D. inca,* and 23 days in *D. huancavilcae*. The values of the fitness components are in ascending order from 68% in *D. huancavilcae* to 88.7% in *D. yangana* and to 92.7% in *D. inca.* The fertility as well as the viability percentages were 64%, 81.2% and 86.6%. We had recently described the mitotic karyotypes of three species of *inca* subgroup (Mafla, 2005a, 2005b, 2008).

Traditionally the phylogenetic studies with species of diptera have used the advantages of exceptionally characteristic polytenic chromosomes, and they had relegated the mitotic chromosomes, because they have considered these as of limited information; however, White (1978) noted that in *Drosophila* the metaphasic chromosomes allow the detection of the heterochromatin rearrangements. Many authors call attention to analyze these sections of genetic material for different functions that it redeem: nucleolar organizer regions are heterochromatic parts; the NORs that not only form the nucleolus but also play a promoter role of meiotic mating; also fertility factors that are in the heterochromatic Y chromosome; as well as the indirect effect of Darwinian aptitude that has repetitive DNA of heterochromatin, and it indicates its importance in the evolutionary process (Hartl and Lozovskaya, 1995; Powell, 1997).

These reasons have conducted us to do a comparative analysis of percentage content of heterochromatin in mitotic chromosomes, with the goal to approach an explanation about the relationships between species subgroup as well as advance hypotheses about the position of the *inca* subgroup within the scheme of phylogeny proposed by Wasserman.

The cultures of species from *inca* subgroup were created with virgin females from nature. The types of these populations are codified with catalogue number QCAZ-1760 and QCAZ-1761 for *D. huancavilcae*; QCAZ-1757 and QCAZ-1758 for *D. yangana*; QCAZ-7808 (♀) for *D. inca* in the Invertebrate Section of the Museum of Zoology of the Pontificia Universidad Católica del Ecuador (QCAZ).

We reiterate that the plates of mitotic chromosomes of species belonging to the *inca* subgroup were tinged with Giemsa 5%, and in description of the karyotypes of the three species we used the nomenclature for the morphology of chromosomes recommended by (Levan *et al*., 1964). The

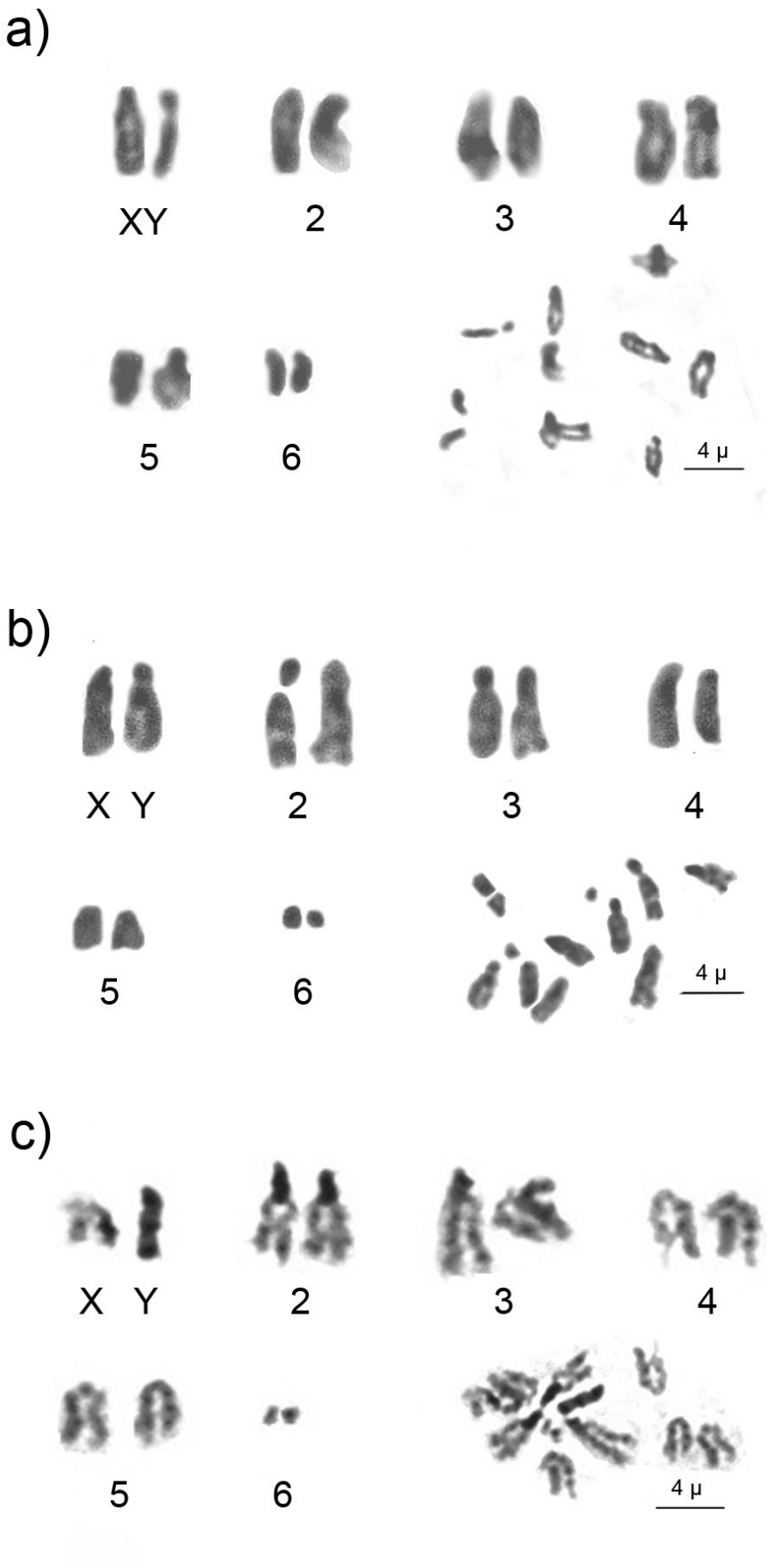

Figure 1. Karyotypes of: a) *Drosophila inca* with the par 6 sm, b) *D. yangana*, c) *D. huancavilcae* with the 6 chromosomes T and the Y chromosome bearer of secondary constriction.

Table 1. Relative length (RL) and morphology of chromosomes of the species of the *inca* subgroup.

Species	X	2	3	4	5	6	Y
Drosophila inca	11.35 st	9.92 St	9.07 st	8.32 sm	7.08 sm	5.08 Sm	9.21 sm
D. yangana [1]	10.19 sm	10.94 Sm	9.58 sm	8.90 sm	7.73 sm	2.58 T	10.06 sm
D. huancavilcae [2]	9.83 sm	11.89 St	10.08 st	8.64 st	7.41 st	2.21 T	9.31 st

[1]It can have one or two supernumerary chromosomes of RL 2.14.
[2]It can have one supernumerary chromosome of RL 3.25.

Table 2. Comparison of the heterochromatin percentages in the species of the *inca* subgroup.

Chromosome	*D. inca*	*D. yangana*	*D. huancavilcae*
X	2.46	2.66	2.64
2	4.30	5.64	5.76
3	4.40	4.74	3.68
4	4.36	4.62	3.40
5	3.86	4.22	3.32
6	10.16	5.16	4.42
Y	9.21	10.06	9.31
Total	38.75%	[1] 37.10%	[2] 32.53%

[1,2] No se considera la heterocromatina de los supernmerarios.

mitotic chromosomes were named as st, s, m or T according to the centromere position in subterminal, submedial regions, or at the end point, respectively (Mafla, 2005b).

The idiograms were built standardizing the values of 20 karyotypes (15 ♂♂, 5 ♀♀) of *D. inca*, 32 (27 ♂♂, 5 ♀♀) of *D. yangana*, and 27 (18 ♂♂, 9 ♀♀) of *D. huancavilcae* (Mafla, 2008). The percentage values of heterochromatin were obtained considering the relative lengths of short arms of chromosomes X, 2, 3, 4, 5 as well as the total length of the Y chromosome and chromosome 6.

In the three species of *inca* subgroup the most notable is that they coincide in their chromosome number 2n = 12 (Figure 1, Table 1). This fact allows us to consider these species like carrying the ancestral karyotype of genus (five bars and one dot), and this fact could guide us to relate the *inca* subgroup with the phyletic line that connects the Primitive I with *hydei* subgroup or *mulleri* subgroup, clusters where species with this karyotype are recorded.

However, the coincidence in chromosome number is only superficial, because each species has different amounts of heterochromatin in the short arms (See Table 2).

These rearragements are summarized by describing karyotypes: *D. inca* has three pairs of chromosomes st and three sm; the heterochromosome Y is sm, and it is completely heterochromatic.

The karyotype of *D. yangana* presents five pairs sm, the smallest couple is T; chromosome Y is sm, but it has a secondary constriction in its long arm. The karyotypes with one or two supernumerary chromosomes were found. The karyotype of *D. huancavilcae* has four couples st, one sm y one T; the chromosome Y is st, and highlighted by its heteropicnosis. In well extended nuclei is visible a secondary constriction in the long arm of Y chromosome; also karyotypes with one supernumerary chromosome had been registered.

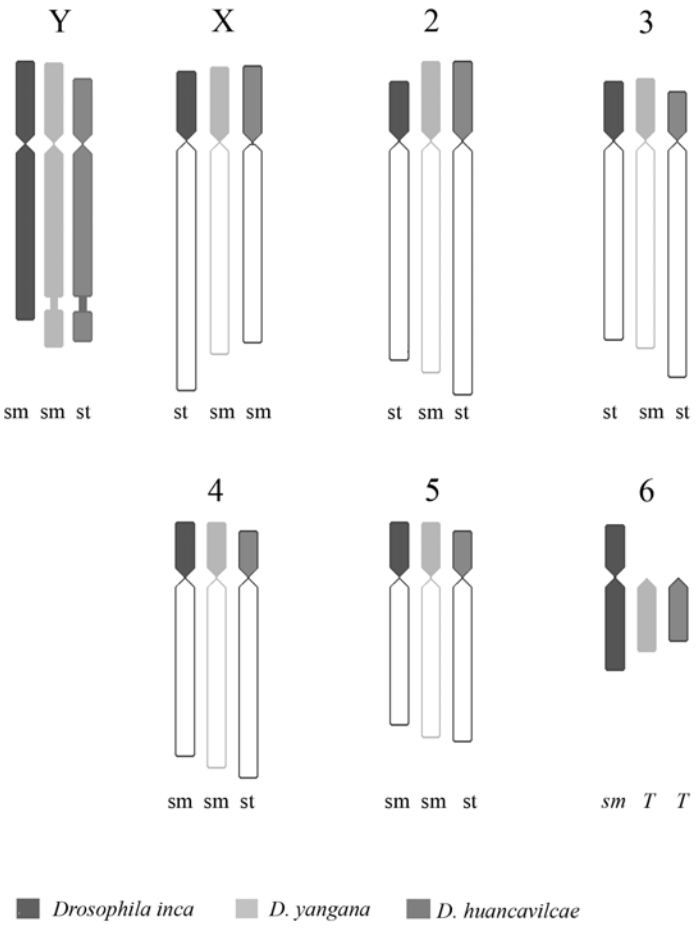

Figure 2. Idiograms showing the difference of morphology, relative length, and heterochromatin distribution.

The coincidence about the Y chromosome of *D. yangana* and *D. huancavilcae* having a secondary constriction, it could mean that they are more related between them than *D. inca*, whose Y chromosome lacks secondary constriction. But also the similarity in this marker with the reports in *D. uniseta* and *D. starmeri*, members of *mulleri* subgroup (Wasserman and Koepfer, 1979), provides a pathway that links the *inca* subgroup with phyletic line that connects the Primitive I with *mulleri* - *fasciola* subgroups rather than the *hydei* subgroup.

The presence of supernumerary chromosomes in both species, in *D. yangana* as in *D. huancavilcae*, is striking, because the extra chromosomes are considered exceptional in the genus, previously found only in two species: *D. nasuta albomicans* and *D. subsilvestris* (Powell, 1997). This isolated fact also could be unusual argument for proposing the closest relationship in this duo species.

The third aspect about similarity between *D. yangana* and *D. huancavilcae* is the presence of small telocentric 6, a characteristic that separates them from *D. inca*, which has metacentric 6 with double length.

The idiograms (Figure 2) simplify the morphological characteristics of chromosomes of these species and allow us to appreciate the heterochromatic portions. We can clearly discern that the short arms of subtelocentric chromosomes have less proportion than submetacentric chromosomes. As well as the great accumulation of heterochromatin in the Y chromosome and 6 chromosome, this latest property suggests a possible link between the origin of chromosome 6 metacentric of *D. inca* with supernumerary chromosomes of the other two species.

In Table 2 we compared the heterochromatin percentages of the three species of *inca* subgroup, highlighting the accumulation referred and the upward trend from *D. huancavilcae* to *D. yangana* and to *D. inca.* This gradient is similar to what was observed in the values mentioned in the introduction. This fact could be a confirmation of the correlation between accumulation of heterochromatin and acquiring greater biological fitness during the speciation process. Likewise, the extensive range of distribution that we had evidenced in *D. inca* could be interpreted like a stroke of major fitness of this species in comparison with their sisters that recently we had registered in the interandean province of Imbabura in the north of Ecuador Rafael, V., and Acurio, A. (2008, personal communication).

Consequently *D. inca* could be a derivative species and *D. huancavilcae* probably more ancient, and it could be related with *D. yangana.*

Polytene chromosomes of *D. huancavilcae* were analyzed, and we identified a new inversion called $2y^5$ and inversions: Xabc, 2ab, 3b of Hypothetic Primitive I sequence (Romero and Mafla, 2008 in press), while we are cultivating the two others species: *D. inca* and *D. yangana* to continue with the cytologic analysis of giant chromosomes.

Acknowledgments: We thank Dr. Clifford Keil, who helped in the revision of this article with appropriate comments about the content and wording, and also Dr. Violeta Rafael for her constant encouragement.

References: Hartl, D.L., and E.R. Lozovskaya 1995, *The* Drosophila *Genome Map: A Practical Guide*, R.G. Landes Company, Austin, 240 pp; Levan, A., K. Fredga, and A.A. Sandberg 1964, Hereditas 52 (2); Mafla, A.B., 2005a, Iheringia, Sér Zool, Porto Alegre, 95 (1): 89-91; Mafla, A.B., 2005b, Rev. Ecuat. Med. y C. Biológ. Vol XXVII N° 1 y 2: 21-25; Mafla, A.B., 2008, Rev. Ecuat. Med. y C. Biológ. 1 and 2 (XIX): 35-39; Powell, J.R., 1997, *The* Drosophila *Model*. Oxford University Press, New York, 562 pp; Rafael, V., and G. Arcos 1989, Evolución Biológica, Bogotá, 3 (3): 233-243; Rafael, V., and D. Vela 2003, Rev. PUCE Quito 71: 129-139; Wasserman, M., and H.R. Koepfer 1979, Genetics 93: 935-946; Wasserman, M., 1982, Evolution of the *repleta* Group. In: *The Genetics and Biology of* Drosophila (Ashburner, M., H.L. Carson, and J.N. Thompson, jr., eds.). Vol 3b, pp. 61-139. Academic Press, London; Wasserman, M., 1992, Cytological Evolution of the *Drosophila repleta* species group. In: Drosophila *Inversion Polymorphism* (Krimbas, C.B., and J.R. Powell, eds.), pp 455-552. CRC Press, Inc., Boca Raton; White, M.J.D., 1978, *Modes of Speciation*, W.H. Freeman and Company, San Francisco, 455 pp; Yoon, J.S., and R.H. Richardson 1978, Evolution 32 (3): 475-484.

Response to light and distribution of *Drosophila* larvae in a feeding environment.

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Introduction

Photoresponses are widespread between invertebrates. The sign of the photoresponse may adjust to variations in the external stimulus situation. In this way, the response to illumination

conditions of *Drosophila* larvae may act as to a token stimulus (Andrewartha and Birch, 1954). That is, the response to light may bring the larvae to places where there are ecological resources. For example, Godoy-Herrera (1977) and Godoy-Herrera *et al*. (1992) reported that *D. melanogaster* larvae burrowed deeper into medium in the dark than in the light gaining access to more food and space. Thus, the response to light may influence *Drosophila* spatial larval distributions in relationship to ecological resources as food and space. Photoreception in invertebrates is mediated in part by the inositol 1,4,5-trisphophate (IP_3) second messenger pathway, and phototransduction in *D. melanogaster* uses a G protein coupled phosphoinositide pathway (Hardie and Raghu, 2001) wherein photoisomerization of rhodopsin to metarhodopsin in the photoreceptor rhabdomeric microvilli activates heterotrimeric Gq. Gq- then activates a phosphatidylinositol (PI)-specific PLC, which hydrolyzes PI 4,5-bisphosphate (PIP2), generating inositol 1,4,5-triphosphate (IP3). This knowledge comes from genetic analysis using mutations that eliminate the light-evoked responses of photoreceptors. One of these genes is the *norpA* (no receptor potential; Hotta and Benzer, 1970). Here we used *D. melanogaster norpA*^{7} allele to investigate spatial distributional patterns of mutant and wild type larvae in a feeding environment. We want to understand how genes expressed in the nervous system of the larva may influence feeding strategies and use of space.

Materials and Methods

Subjects: The strains employed were: *norpA*^{7}, CS (wild type strain from which *norpA*^{7} was isolated; Hotta and Benzer, 1970), and Oregon R-c (wild type). All the stocks were kept in Burdick's medium (Burdick, 1954) at 24ºC.

Experimental design: We used 15 × 15 × 2 cm (width × length × height) plastic boxes filled with agar. On the agar surface was distributed a film of 4% yeast suspension in plain water. Each box was covered with a plastic lid. On the center of each lid, a thick, black paper disk of 8.5 cm in diameter was attached (details in Godoy-Herrera *et al*., 1992). The boxes were illuminated by a white cold bulb of 60 watts placed 25 cm above them. In this way, each box had two different visual environments: (a) a dark area produced by the shadow of the paper disk cast on the agar at the center of each box, and (b) a more highly illuminated annulus surrounding the center disk. Larvae find in the dark zone presumably prefer a less illuminated environment for feeding; those found eating on the more illuminated area were classified as larvae showing a photopositive behavior. We individually tested 35 third instar larvae of each of the *norpA*^{7}, CS, and Oregon R-c strains. Each individual was deposited onto the agar of the boxes between the dark area and the illuminated area, and the position of each larvae in each visual environment was recorded continuously for 5 min. In other experiments, we drew for 2 min the track made on agar by each one of 35 larvae of the same age for each one of the strains used. We measured the length of each track and the number of turns.

Results

Larvae of the *norpA*^{7} strain were distributed at random in the boxes (at the end of observation period 48.57% of the larvae were on the illuminated area, 45.71% were on the dark zone and 5.71% were on the limit between these two zones; *G*-test of independence, $\chi^2 = 1.56$, df = 2; NS). In contrast, Oregon R-c and CS larvae preferred the dark zone to feed (Oregon R-c: illuminated area, 25.71%; dark zone, 68.57%; limit between the two zones, 5.71%; *G*-test of independence, χ^2 =

12.38; $df = 2$; $P < 0.05$). In the case of CS larvae the percentages were, respectively, 24.69% (illuminated area), 72.21% (dark zone), and 3.10% (limit between the zones) (*G*-test of independence, $\chi^2 = 15.26$; $df = 2$; $P < 0.05$).

Larval locomotion of the Oregon R-c and CS strains was also greater than that of the *norpA*^{7} larvae (*t*-test, Oregon versus *norpA*^{7}, $t = 4.34$, $df = 58$; $P < 0.05$. CS versus *norpA*^{7}, $t = 5.25$, $df = 58$; $P < 0.05$). Larvae of the three strains did not show significant differences in number of turns made to crawl on agar (*t*-test).

Discussion

A variety of behavioral factors are involved in habitat choice in *Drosophila* (Powell, 1997). The present work indicates that *D. melanogaster* larvae may use their photoresponse to influence the direction of their movements. Differences in photoresponse between larvae of different genetic backgrounds indicate the importance of genotype in habitat selection in *Drosophila* larvae. On the other hand, the data suggest that neurological mutants that affect the sensory system of *Drosophila* larva by altering the signal transduction pathways could help us to link cell biology of nervous system with ecology of *Drosophila* breeding sites. Additionally, neurological mutants may also provide a means to understand how *Drosophila* larvae perceive and react to some environmental cues associated with the distribution and abundance of resources such us food. Little work has been done on the relationship between mutants that affect specific neural circuits and the ecology of larval stage. The altered response to light of *Drosophila* larva produced by the *norpA*^{7} mutation could serve as a model system to understand the functioning of their nervous system in relationship with the ecology of *Drosophila* larval stage.

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References: Andrewartha, H.G., and L.C. Birch 1954, *The Distribution and Abundance of Animals*. University of Chicago Press, Chicago; Burdick, AB., 1954, Dros. Inf. Serv. 28: 170; Godoy-Herrera, R., 1977, Behav. Genet. 7: 433-439; Godoy-Herrera, R., M. Alarcón, H. Cáceres, I. Loyola, I. Navarrete, and J.L. Vega 1992, Rev. Chilena Hist. Nat. 65: 91-101; Hardie, R.C., and P. Raghu 2001, Nature 413: 186-193; Hotta, Y., and S. Benzer 1970, Proc. Nat. Acad. Sci. 67: 1156-1163; Powell, J.R. 1997, *Progress and Prospect in Evolutionary Biology. The* Drosophila *Model*. Oxford University Press, Oxford.

First record of *Zaprionus indianus* (Diptera: Drosophilidae) in the Urucu Petroleum Province in Amazonas, Brazil.

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The genus *Zaprionus* Coquilet 1901 (Diptera Drosophilidae) is composed of two subgenera and 56 species. By now, only one species was found in other tropical areas of the world (De Toni *et al.*, 2000).

Zaprionus indianus Gupta, 1970 (Diptera: Drosophilidae) is from Africa, and their first record in the American continent was in 1999 in a metropolitan area in São Paulo city, State of São Paulo, Brazil (Vilela, 1999). Since this time the species has expanded quickly to Brazil (Santos *et al*., 2003; Tidon *et al*., 2003).

In May 2008 *Z. indianus* was registered for the first time in the URUCU Petroleum Province in the Amazon forest, Coari city, state of Amazonas, North of Brazil. The area is composed by a primary forest matrix with several clearings for wells and extraction, opened by the Petroleum Brazilian Company.

Drosophilids have been monitored in URUCU area since 2003 (Lima *et al.,* 2008). In this six years of monitoring were realized nine expeditions, with 476 samples in forest and clearing habitats. *Z. indianus* was collected with specific traps (Martins *et al.,* 2008) with banana baits exposed in clearings. Until now no *Zaprionus* was collected in traps in the interior of primary forest.

References: Tidon, R., D.F. Leite, and B.F.D. Leão 2003, Biol. Conserv. 112: 299-305; Santos, J.F., T.T. Rieger, S.R.C. Campos, A.C.C. Nascimento, P.T. Felix, S.V.O. Silva, and F.M.R. Freitas 2003, Dros. Inf. Serv. 86: 92-95; Kato, C.M., L.V. Foureax, R.C. Araujo, and M.P. Torres 2004, Ciênc. Agrotec., Lavras, v. 28, n. 2, p. 454-455; Lima, S.O.F.; M.B. Martins, A.L.C Prudente, L.F.A. Montag, and Monnerat (Org) 2008, Petrobras. Rio de janeiro.195p ils; Martins, B.M., R.N. Bittencourt, and J.A.N. Penna 2008, Dros. Inf. Serv. 91: 147; Van Der Linde, K., G.J. Steck, K. Hibard, J.S. Birdsley, L.M. Alonso, and D. Houle 2006, Florida Entomologist. 89: 3; Vilela, C.R., 1999, Dros. Inf. Serv. 82: 37-39.

First record of *Zaprionus indianus* (Diptera, Drosophilidae) in Fernando de Noronha, an Oceanic Island of Pernambuco State, Brazil.

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Introduction

Zaprionus indianus is an invader species in Brazil and America. It is the most common fly of the genus in the African continent, and it probably originated from the Afrotropical zone (Tsacas, 1985). *Zaprionus indianus* represents one of the most successful colonizing species of this genus (Chassagnard and Tsacas, 1993) and their first occurrence in Brazil was recorded in 1999, in São Paulo State (Vilela, 1999; Tidon *et al.*, 2003). Since then the species has been able to successfully colonize the whole Brazilian territory (review in Gottschalk *et al.*, 2008) and become one the most abundant species of drosophilids in open and disturbed areas (Tidon *et al.,* 2003; Silva *et al.,* 2005). In Pernambuco State, northeast of Brazil, this species was collected for the first time in April, 2000, in the cities of Recife and Bezerros (Santos *et al.*, 2003). Between June 2000 and August 2002 these authors also collected *Z. indianus* in seven other cities of Pernambuco, and in Paraiba and Bahia States. The aim of the present study is to contribute to the knowledge of the process of colonization of *Z. indianus* in north-eastern Brazil, where the drosophilid fauna is very poorly studied and only a few species have been recorded. We present here the first data of collections of *Z. indianus* in one

oceanic island (Fernando de Noronha) of Brazil, distant 360 km from the Natal city, and 525 km from Recife city, on the mainland.

Materials and Methods

In September 2009 fly samples were collected in five different environments of Fernando de Noronha Island (03º50'81.1"S/32º25'07.4"W), in Pernambuco State, Brazil. Fernando de Noronha is the most important island of the archipelago of the same name, whose area is 26 km^2. Fernando de Noronha island is the only inhabited island in the archipelago, and its area is 17 km². Part of it (60%) comprises the National Marine Park of Fernando de Noronha, which protects the only occurrence of oceanic mangroves of the South Atlantic Ocean, formed by the plant species *Laguncularia racemosa*.

The environments chosen to be sampled were the Mangrove of Sueste Beach (inside the National Park of Fernando de Noronha), three disturbed forests (one next to the Mangrove, one next to Morro do Pico, and one next to Estrada Velha do Porto), and an urbanized area in Vila dos Remédios, the most densely populated site of the island. Sixty retention traps with fermented banana were prepared according to Tidon and Sene (1988). On each collection site, traps were dispersed along the area 10 meters away from each other and were set on three days to provide a total of 72 h of sampling. After this time, the captured flies were transferred to small vials containing standard culture medium and transported to the laboratory of the *Universidade Federal de Pernambuco* (UFPE) for identification. Samples of the specimens analyzed were deposited in the Drosophilidae Collection of UFPE.

Results and Discussion

A total of 20,486 drosophilid flies were collected in Fernando de Noronha Island. Among these flies, 221 were *Zaprionus indianus* (Table 1), comprising 1.1% of the total samples. The relative frequencies of *Z. indianus* along the five collection sites ranged between 0.08% and 3.42%. Because of the low frequencies, our data probably indicate a very recent event of colonization of the island by *Z. indianus.* This is because our recent data indicate that *Z. indianus* is a very frequent species in collections carried out in open formations (as Caatinga biome for example), mangrove or in disturbed forests, in Pernambuco State in the mainland (unpublished results).

Table 1. Description of the five sites sampled in Fernando de Noronha Island and absolute abundance (N) and relative frequency (%) of *Zaprionus indianus* and other drosophilid species collected in each site.

Sites of collection		Habitat	Traps	*Zaprionus indianus* N (relative frequency)	Other species N
National Park	Mangrove of Suape Beach	oceanic mangrove	25	5 (0.08 %)	5882
	Forest next to the mangrove	disturbed forest	10	9 (0.29 %)	3085
Protection Area	Morro do Pico	disturbed forest	10	54 (0.79 %)	6853
	Estrada Velha do Porto	disturbed forest	10	105 (3.42 %)	3065
(APA)	Vila dos Remédios	urbanized area	05	48 (2.99 %)	1601
			∑=60	∑=221	∑=20486

Also in Fernando de Noronha, there was a clear preference of *Z. indianus* for the environments of disturbed forests and urbanized area in relation to the environments of mangrove and adjacent forest, where only 14 individuals were collected. Our results are the first record of *Z. indianus* in an oceanic island in Brazil, 360 km far from the coast. The data reinforce the idea that *Z. indianus* has great dispersion and colonization capacities. The continuous analysis of drosophilids in Fernando de Noronha at this time of the process of colonization may clarify, in the future, the role of this invasive species in the dynamics of other exotic and native species already established on the island for a long time.

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References: Chassagnard, M.Th., and L.Tsacas 1993, Ann. Soc. Entomol. Fr 29: 173; Gottschalk, M.S., P.R. Hofmann, and V.L.S. Valente 2008, Check List 4(4): 485; Santos, J.F., T.T. Rieger, S.R.C. Campos, A.C.C. Nascimento, P.T. Félix, S.V.O. Silva, and F.M.R. Freitas 2003, Dros. Inf. Serv. 86: 92; Silva, N.M., C.C. Fantinel, V.L.S. Valente, and V.H. Valiati 2005, Neotrop. Entomol. 34: 363; Tidon, R., and F.M. Sene 1988, Dros. Inf. Serv. 67: 90; Tidon, R., D.F. Leite, and B.F.D. Leão 2003, Biol. Conserv. 112: 299; Tsacas, L., 1985, Ann. Soc. Entomol. Fr 21(3): 343; Vilela, C.R., 1999, Dros. Inf. Serv. 82: 37.

Diversity and geographical distribution of *Drosophila* (Diptera, Drosophilidae) in Ecuador.

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Introduction

Ecuador, with an area of only 256,370 km^2, is one of the ten most mega-diverse countries in the world (Coloma and Ron, 2001). The latitudinal position, the presence of the Andes Mountains, and the influence of ocean currents of the Ecuadorian coast have produced a wide variety of habitats with a unique flora and fauna.

The first records of the genus *Drosophila* in Ecuador were made by Becker (1919), who reported the results of the entomological collections made by Ribet in the early 18th century in Ecuadorian highlands or Sierra. In 1957, Wheeler recorded species of the *flavopilosa* group around the Cotopaxi Volcano. In 1983, Carson and colleagues made a taxonomic survey of *Drosophila* in the Galápagos Islands. Until then, *Drosophila* records were the result of sporadic collections.

In 1987, studies were initiated to evaluate the Ecuadorian *Drosophila* diversity with the work of G. Arcos (1989), Rafael and Arcos (1988, 1989), Rafael *et al*. (2000a, b), Rafael and Vela (2000, 2003), Vela and Rafael (2001, 2003, 2004a, b, c, 2005a, b), Acurio *et al*. (2002), Rafael (2007), and Acurio (2007). Currently, 16 provinces belonging to 4 geographical regions, Coast, Sierra, Amazon, and the Insular Region, have been sampled (Figure 1).

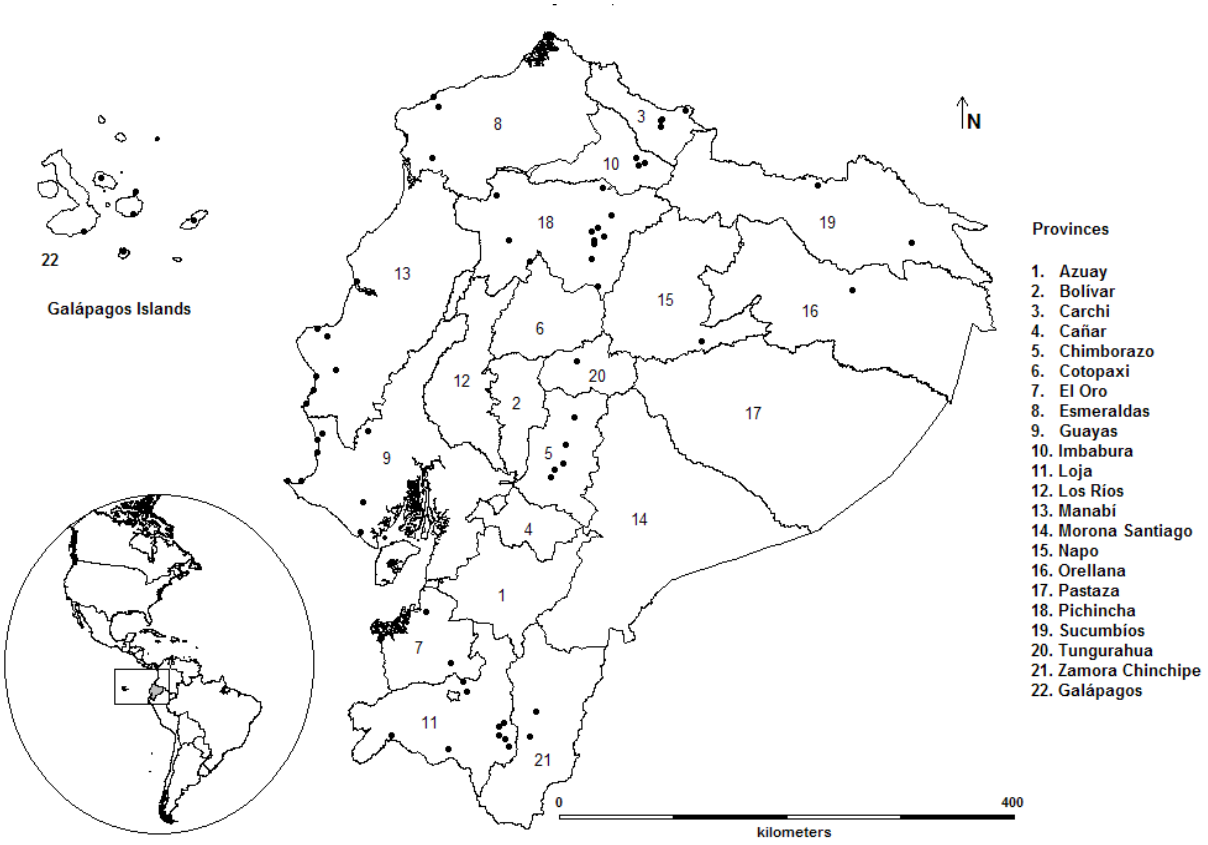

Figure 1. Geographic distribution of *Drosophila* species in Ecuador. The black dots show geographic coordinates of sampling sites.

In this paper, we synthesize what is currently known about the distribution and diversity of the genus *Drosophila* in Ecuador. Distributional data are important to understand population structure, speciation patterns, and community composition over time.

Methods

The data resources consulted were:

1. Literature review of all *Drosophila* records in Ecuador from 1919 to 2008.
2. Face review of the *Drosophila* specimens in Museo Ecuatoriano de Ciencias Naturales (MECN) and Museo de Zoologia de la Pontificia Universidad Católica del Ecuador (QCAZ).
3. Taxonomic surveys of *Drosophila* in different localities in Ecuador.
4. Consultation of on-line databases: Taxodros and Species 2000.

Results and Discussion

In Ecuador, 112 *Drosophila* species have been recorded (Table 1). These species are classified in 5 subgenera, *Drosophila*, *Dorsilopha*, *Phloridosa*, *Siphlodora*, *Sophophora;* and species without subgenus designation, *D. dilacerata, D. ferruginea*, *D. griseicollis* and *D. xerophila*.

Table 1. *Drosophila* diversity and provincial distribution in Ecuador.

Species group	Species, authority (first record in Ecuador) and provincial distribution
Drosophila subgenus: 92 species	
annulimana 3 species	*aracataca* Vilela & Val, 1983 (◙ R&Ve) in Loja; *araicas* Pavan & Nacrur, 1950 (A) in Orellana; *yana* Vela & Rafael, 2005 in Pichincha.
bromeliae 1 species	*bromelioides* Pavan & Cunha, 1947 (R&As) in Cotopaxi.
cardini 4 species	*cardini* Sturtevant, 1916 (R&As) in Cotopaxi, Guayas, Imbabura, Loja, Manabí, Orellana and Pichincha; *cardinoides* Dobzhansky & Pavan, 1943 (Ca) in Cotopaxi, Galápagos, Orellana; *neocardini* Streisinger, 1946 (◙R&Ve) in Loja; *polymorpha* Dobzhansky & Pavan, 1943 (H) in Esmeraldas, Guayas, Manabí and Pichincha;
canalinea 1 species	*canalinea* Patterson & Mainland, 1944 (W) in Pichincha.
flavopilosa 5 species	*korefae* Vela & Rafael, 2004 (†V&R) in Pichincha; *sisa* Vela & Rafael, 2005 (‡Ve&R) in Pichincha and Zamora; *suni* Vela & Rafael, 2005 (‡Ve&R) in Pichincha; *taxohuaycu* Vela & Rafael, 2005 (‡Ve&R) in Pichincha
guaraní 7 species	*cuscungu* Vela & Rafael, 2005 (‡Ve&R) in Pichincha; *ecuatoriana* Vela & Rafael, 2004 (╬Ve&R) in Pichincha; *griseolineata* Duda, 1927 (Mo) in Pichincha; *ornatifrons* Duda, 1927 (‖R&VE) in Pichincha; *pichinchana* Vela & Rafael, 2004 (╬Ve&R) in Pichincha; *quitensis* Vela & Rafael, 2004 (╬Ve&R) in Pichincha; *urubamba* Vilela &Pereira, 1993 (Mo) in Imbabura and Pichincha.
immigrans 1 species	*immigrans* Sturtevant, 1921(Ca) in Chimborazo, Galapagos, Guayas, Imbabura, Loja, Pichincha.
mesophragmatica 7 species	*amaguana* Vela & Rafael, 2004(♦Ve&R) in Pichincha; *gasici* Brncic, 1957(R&As) in Pichincha; *gaucha* Jaeger & Salzano, 1953(‖R&Ve) in Pichincha, *mesophragmatica* Duda, 1927(‖R&Ve) in Chimborazo, Cotopaxi, Loja, Pichincha and Tungurahua; *pavani* Brncic, 1957(‖R&Ve) in Pichincha; *ruminahui* Vela & Rafael, 2004(♦Ve&R) in Pichincha; *shyri* Vela & Rafael, 2004(♦Ve&R) in Pichincha.
morelia *1 species*	*ogradi* Vela & Rafael, 2004(†Ve&R) in Pichincha.
onychophora 2 species	*asiri* Vela & Rafael, 2005 (■Ve&R) in Pichincha; *margarita* (NR) Hunter, 1979 in Pichincha.
pallidipennis 1 species	*pallidipennis* Dobzhansky & Pavan, 1943(Mo) in Pichincha.
repleta 26 species	*aldrichi* Freire-Maia & Pavan, 1950 (‖R&Ve) in Esmeraldas, El Oro, Loja, Manabí, Guayas, Imbabura and Pichincha; *ellisoni* Vilela, 1983(Vi) in Cotopaxi and Pichincha; *guayllabambae* Rafael & Arcos, 1988 (R&As) in Chimborazo, El Oro, Imbabura, Loja, Pichincha and Tungurahua; *huancavilcae* Rafael & Arcos, 1989 (◊R&As) in Guayas and Manabí; *huayla* Suyo, Pilares & Vasquez (NR) in Manabí; *hydei* Sturtevant, 1921 (Ca) in Galápagos, Guayas, Imbabura, Loja, Pichincha and Tungurahua; *inca* Dobzhansky & Pavan, 1943(◊R&As) in Chimborazo, Loja, Pichincha in Tungurahua; *longicornis* Patterson & Wheeler, 1942(R&As) in Chimborazo, Imbabura and Pichincha; *mayaguana* Vilela, 1983(W&W) in Manabí; *mercatorum* Patterson & Wheeler, 1942 (R&Co) in Cotopaxi, Orellana, Pichincha and Tungurahua; *meridiana* Patterson and Wheeler, 1942 (NR) in Manabí and Imbabura; *meridionalis* Wasserman, 1962 (*R&Co) in Guayas and Manabí; *neohydei* Wasserman, 1962 (‖R&Ve) in Pichincha and Tungurahua; *neorepleta* Patterson & Wheeler, 1942 (A&Co) in Pichincha; *nigricruria* Patterson & Mainland,1943 (‖R&Ve) in Pichincha and Tungurahua; *nigrohydei* Patterson &Wheeler, 1942 (R&As) in Imbabura, Loja and Pichincha; *nigrospiracula* Patterson & Wheeler, 1942(R&As) in Pichincha; *paranaensis* Barros, 1950(‖R&Ve) in El Oro, Esmeraldas, Guayas, Loja, Manabí and Orellana; *parisiena* Heed & Grimaldi, 1991(W&W) in Manabí; *peninsularis* Patterson & Wheeler, 1942 (A) in Orellana; *pseudorepleta* Vilela & Bächli, 1990 (A&Co) in Orellana and Pichincha; *repleta* Wollaston, 1858(Ca) in Galapagos, Pichincha and Tungurahua; *shuyu* Vela & Rafael, 2005(‡Ve&R) in Pichincha; *straubae* Heed & Grimaldi, 1991(W&W) in Manabí; *vicentinae* Vilela, 1983(◙R&Ve) in Loja and Orellana; *yangana* Rafael &Vela, 2003(◙R&Ve) in Loja.
tripunctata 19 species	*arcosae* Vela & Rafael, 2001(£Ve&R) in Pichincha; *bandeirantorum* Dobzhansky & Pavan, 1943(Mo) in Imbabura, Loja and Pichincha; *carlosvilelai* Vela & Rafael, 2001(‖R&Ve) in Pichincha; *fontdevilai* Vela & Rafael, 2001(‖R&Ve) in Pichincha; *ichubamba* Vela & Rafael, 2005(‡Ve&R) in Pichincha; *machachensis* Vela & Rafael, 2001(‖R&Ve) in Pichincha; *mesostigma* Frota-Pessoa, 1954(‖R&Ve) in Pichincha; *metzii* Sturtevant, 1921(Ca) in Galapagos; *ninarumi* Vela & Rafael, 2005(‡Ve&R) in Pichincha; *paraguayensis* Duda, 1927(‖R&Ve) in Pichincha; *pasochoensis* Vela & Rafael, 2001(‖R&Ve) in Pichincha; *patacorona* Vela & Rafael, 2005(‡Ve&R) in Pichincha; *pilaresae* Vela

	&Rafael, 2001(‖R&Ve) in Pichincha; *quillu* Vela & Rafael, 2005(‡Ve&R) in Pichincha; *roehrae* Pipkin & Heed, 1964 (NR) in Cotopaxi and Pichincha; *surucucho* Vela & Rafael, 2005(‡Ve&R) in Pichincha; *tomasi* Vela & Rafael, 2001(‖R&Ve) in Pichincha; *urcu* Vela & Rafael, 2005(‡Ve&R) in Pichincha; *valenciai* Vela & Rafael, 2001(‖R&Ve) in Pichincha.
virilis 1 species	*virilis* Sturtevant, 1916(R) in Esmeraldas and Manabí.
willistoni 7 species	*bocainensis* Pavan & Cunha, 1947(Mo) in Pichincha; *equinoxialis* Dobzhansky, 1946(A) in Orellana; *nebulosa* Sturtevant, 1916(Ca) in El Oro, Esmeraldas, Galápagos, Guayas, Manabí, Napo, Orellana; *paulistorum* Dobzhansky & Pavan, 1949(D&S) in Orellana and Pichincha; *sucinea* Patterson & Mainland, 1944(‖R&Ve) in Loja, Orellana and Pichincha; *tropicalis* Burla & da Cunha, 1949(S) in Pichincha; *willistoni* Sturtevant, 1916(C) in El Oro, Esmeraldas, Galápagos Guayas, Loja, Manabí, Orellana, Pichincha and Sucumbios.
Not grouped 7 species	*apag* Vela & Rafael, 2005(■Ve&R) in Pichincha; *condormachay* Vela & Rafael, 2005(‡Ve&R) in Pichincha; *mellea* Becker, 1919(B) in Carchi; *neochracea* Wheeler, 1959(NR) in Pichincha; *pugyu* Vela & Rafael, 2005(‡Ve&R) in Pichincha; *rumipamba* Vela & Rafael, 2005(■Ve&R) in Pichincha; *runduloma* Vela & Rafael, 2005(■Ve&R) in Pichincha.
Dorsilopha Subgenus : 1 species	
busckii 1 species	*busckii* Coquillett, 1901(Mo) in Imbabura, Loja, Orellana and Pichincha.
Phloridosa Subgenus : 1 species	
Not grouped 1 species	*lutzii* Sturtevant, 1916(Mo) in Pichincha;
Siphlodora Subgenus : 1 specie	
Not grouped 1 species	*flexa* Loew, 1866(Vi&B) in Chimborazo and Pichincha.
Sophophora Subgenus : 13 species	
melanogaster 5 species	*ananassae* Doleschall, 1858(Ca) in Cotopaxi, El Oro, Esmeraldas, Galápagos, Guayas, Loja, Manabí, Orellana; *kikkawai* Burla, 1954(A) in Orellana and Pichincha; *malerkotliana* Parshad & Paika, 1964(R&Co) in El Oro, Esmeraldas, Guayas, Loja, Manabí, Orellana, Pichincha and Sucumbios; *melanogaster* Meigen, 1830(Ca) in Chimborazo, Esmeraldas, Galápagos, Guayas, Imbabura, Loja, Manabí, Orellana, Pichincha and Sucumbios; *simulans* Sturtevant, 1919(Ca) in Chimborazo, El Oro, Galápagos, Guayas, Loja, Manabí, Orellana, Pichincha and Sucumbios;
obscura 1 species	*tolteca* Patterson & Mainland, 1944(NR) in Cotopaxi and Pichincha.
saltans 7 species	*emarginata* Sturtevant, 1942(Ma) in El Oro, Guayas, Loja, Manabí, Orellana and Pichincha; *neocordata* Magalhaes, 1956(A) in Orellana; *parasaltans* Magalhaes, 1956(‖R&Ve) in Orellana and Pichincha; *prosaltans* Duda, 1927(R&Co) in Loja and Orellana; *saltans* Sturtevant, 1916(R&Co) in El Oro, Guayas, Loja, Napo; *sturtevanti* Duda, 1927(‖R&Ve) in El Oro, Esmeraldas, Loja, Manabí, Orellana, Pichincha and Sucumbios; *subsaltans* Magalhaes, 1956(A) in Orellana.
Without subgenus: 4 species	
	dilacerata Becker, 1919(B) in Carchi; *ferruginea* Becker, 1919(B) in Chimborazo and *griseicollis* Becker, 1919(B) in Carchi; *xerophila* Val, 1983(Ca) in Galapagos.

A= Acurio (2007); A & Co = Acurio, *et al*. (2002); B = Becker (1919); Ca= Carson, *et al*. (1983); C = da Cunha, *et al*., (1959); D & S = Dobzhansky & Spassky (1959); H= Heed (1963);MA = Magalhaes (1962); (Mo)= Morán *et al*., (1999); R & As = Rafael & Arcos (1988); ◊ R & As = Rafael & Arcos (1989); R & Co = Rafael *et al*., (2000a); *R & Co = Rafael *et al*., (2000b); ‖ R & VE = Rafael & Vela (2000); ◙ R & VE =Rafael & Vela (2003);R = Rafael (2007); S = Spassky, *et al*. (1971); £ Ve & R = Vela & Rafael (2001); ♦ Ve & R= Vela & Rafael (2004a); † VE & R = Vela & Rafael (2004b);╬ VE & R= Vela & Rafael (2004c); ■ VE & R= Vela & Rafael (2005a); ‡ VE & R = Vela & Rafael (2005b);(Vi) Vi = Vilela (1983); Vi & B = Vilela & Bachli (2000); W & W = Wasserman & Wasserman (1992); W = Wheeler (1957); (NR) new records to Ecuador.

The *Drosophila* subgenus is represented by 15 species: *annulimana*, *bromeliae*, *cardini*, *canalinea*, *flavopilosa*, *guarani*, *immigrans*, *mesophragmatica*, *morelia*, *onychophora*, *pallidipennis*, *repleta*, *tripunctata*, *virilis*, *willistoni* and 7 species not grouped. The dominant species groups are *repleta* and *tripunctata* with 26 and 19 species, respectively.

Three *Drosophila* subgenera are represented by 1 species each: the subgenus *Dorsilopha* with *D. busckii*, subgenus *Phloridosa* with *D. lutzii*, and the subgenus *Siphlodora* with *D. flexa*. The *Sophophora* subgenus is represented by three species groups: *melanogaster*, *obscura*, and *saltans*.

In this work we record five new country records: *D. margarita, D. huayla, D. meridiana, D. roehrae,* and *D. neochracea*. These species were collected in different surveyed localities using banana baited traps and were identified using their terminalia and morphological characters. Male and female specimens were pinned, labeled, and deposited as voucher at the Invertebrate Section of QCAZ.

Drosophila latifasciaeformis that was previously classified in the subgenus *Scaptodrosophila* and *Drosophila pictiventris*, previously classified in the subgenus *Hirtodrosophila*, were promoted to the genus level by Grimaldi (1990). Therefore, these species have not been included in Table 1.

Although distributional records were gleaned from disparate sources, the results show that due to both historical and ecological factors the *Drosophila* fauna is not evenly distributed within Ecuador. Despite the small geographical area, Ecuador has a large *Drosophila* species richness demonstrated by a high proportion of endemic *Drosophila* species. There are 41 species only recorded in Ecuador, more than 36% of the total of 112 species.

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References: Arcos, O.G., 1989, Microdistribución de las especies del grupo de *Drosophila repleta* (Diptera, Drosophilidae) en un cuadrante Guayllabamba, Ecuador, con la descripción y Biología de una nueva especie. Tesis de Licenciatura de la Pontificia Universidad Católica del Ecuador; Acurio, A., C. Carrera, V. Rafael, and D. Vela 2002, Resum. XXVI Jorn. Ecuat. Biología. Pág.51; Acurio, A., 2007, Diversidad del género *Drosophila* (Diptera, Drosophilidae) en seis diferentes hábitats del Parque Nacional Yasuni y Clave Taxonómica Multimedia de *Drosophila* para Ecuador. Tesis de Licenciatura Pontificia Universidad Católica del Ecuador; Becker, T., 1919, Mission du Service Géographique de l.Armée pour la mesure d.un arc de Méridien Equatorial en Amérique du Sud, 1899-1906, 10(2): 208-209; Carson, H.L., F.C. do Val, and M.R. Wheeler 1983, Int. J. Entomol. 25: 239-248; Coloma, L., and S. Ron 2001, Ecuador megadiverso. Serie de Divulgación del Museo de Zoología. Centro de Biodiversidad y Ambiente, P.U.C.E. 1: 140 pp.; da Cunha, A.B., Th. Dobzhansky, O. Pavlovsky, and B. Spassky 1959, Evolution13: 389 –404; Dobzhansky, Th., and B. Spassky 1959, Proc. Natl. Acad. Sci., USA 45: 419-428; Grimaldi, D.A., 1990, Bull. Am. Mus. Nat. Hist. 197: 1-139; Heed, W.B., 1963, Evolution 17: 502-518; Magalhaes, L.E., 1962, Univ. Texas Publs. 6205: 135-154; Morán, T., B. Larrea, D. Vela, and V. Rafael 1999, Resum. XXIII Jorn. Ecuat. Biología. Pag.148-150; Rafael, V., and G. Arcos 1988, Evolución Biológica 2: 167-176; Rafael, V., and G. Arcos 1989, Evolución Biológica 3: 233-243; Rafael, V., G. Arcos, and L.A. Terán 2000ª, Revta. Pont. Univ. Católica Ecuador. 65: 130-155; Rafael, V., G. Arcos, and L. Arcos Terán 2000b, Revta. Pont. Univ. Católica Ecuador. 65: 156-175; Rafael, V., and D. Vela 2000, Dros. Inf. Serv. 83: 85-88; Rafael, V., and D. Vela 2003, Revta. Pont. Univ. Católica Ecuador. 71: 129-139; Rafael, V., 2007, Revta. Ecua. Med. Cienc. Biol. 28 (1,2): 30-43; Spassky, B., R.C. Richmond, S. Perez-Salas, O. Pavlovsky, C.A. Mourao, A.S. Hunter, H. Hoenigsberg, Th. Dobzhansky, and F.J. Ayala 1971, Evolution. 25: 129-143; Vela, D., and V. Rafael 2001, Revta. Pont. Univ. Católica Ecuador. 66: 92-120; Vela, D., and V. Rafael 2004a, Iheringia, Ser.Zool 94 (3): 295- 299; Vela, D., and V. Rafael 2004b, Revta Ecua. Med. Cienc. Biol. 26(1,2): 7-13; Vela, D., and V. Rafael 2004c, Revta. Pont. Univ. Católica Ecuador 26: 14-21; Vela, D., and V. Rafael 2005a, Revta. Pont. Univ. Católica Ecuador. 75: 69-80; Vela, D., and V. Rafael 2005b, Revta. Ecua. Med. Cienc. Biol. 27(1,2): 27-41; Vilela, C.R., 1983, Revta. bras. Ent. 27: 1-114; Vilela, C.R., and G. Bachli 2000, Mitteilungen der Schweizerische Entomologischen Gesellschaft [Bulletin de la Societe

Entomologique Suisse] 73: 23-47; Wasserman, M., and F. Wasserman 1992, Evol. Biol. 26: 357-381; Wheeler, M.R., 1957, Taxonomic and distributional studies on Nearctic and Neotropical Drosophilidae. University of Texas Publications. Studies in Genetics: 5721: 79- 114.

Olfactory response of *Drosophila* flies for different fruits.

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The fruit fly, *Drosophila*, is equipped with a sophisticated olfactory sense system that permits it to recognize and discriminate hundreds of discrete odorants. The olfactory response of these odorants is essential for the animal to identify relevant good sources and suitable sites for egg laying. *Drosophila* and related genera occupy a wide variety of habitats including rotting fruit and plant parts in deserts and tropical forests, slime fluxes, fungi, flowers, ferns, and crabs. A major problem in rearing such species is inducing females to oviposit. Different species may show different distribution patterns and seasonal frequencies ranging from a tight nuclear distribution at a food source to a uniform low-density distribution (Carson *et al.,* 1970; Dobzhansky and Pavan, 1950). A variety of behavioral factors are involved in habitat choice, and it is clear that different species rely on different sensory modalities to varying degrees in deciding their direction of movement. The present study was made to study the preference of adults to different fruits.

For the present investigation *D. melanogaster, D. simulans, D. ananassae, D. malerkotliana, D. yakuba,* and *D. mauritiana* were collected from University of Mysore, Mysore. These flies were used to study the olfactory response of flies in different fruits, such as banana, sapota, apple, and papaya. The sliced fruits were placed inside the four culture bottles. They were kept in each corner of the experimental transparent glass box (35 × 25 cm). 300 flies are released to the box and closed, and then the boxes were kept without any disturbance. The flies were observed, and the numbers of flies attracted towards specific fruits were recorded carefully for an interval of one hour. Three replicates were carried out at ambient temperature of 25 ± 2˚C. The data were analyzed by Chi-Square test.

Table 1 shows the percentage of *Drosophila* flies attracted to various fruits. Sapota was found to be a good attractant in all the species. All the species were attracted at maximum to sapota (observation I-III), except banana, which attracted more *D. simulans* flies at the end of the third observation, whereas the minimum attraction of different species of flies varies in the remaining three fruits. *D. melanogaster* and *D. bipectinata* were attracted minimum to papaya in all the observations, except the third observation of *D. simulans* (papaya). *D. yakuba* and *D. malerkotliana* were attracted least to banana and apple, respectively. The Chi-Square test reveals that the differences of attraction of flies between different fruits are found to be highly significant at all the three observations (χ^2 - 233.75, χ^2 - 209.93, and χ^2- 239.75, df = 20).

In the present study, the percentage of olfactory responses of various species of *Drosophila* varies in all the fruits analyzed. It is evident that all the species prefer maximum sapota, whereas *D. melanogaster, D. simulans* and *D. bipectinata* prefer minimum for papaya (10.1%, 5.8%, and 5.6%,

respectively), *D. yakuba* (8.3%) to banana, *D. ananassae* and *D. malerkotliana* to apple (9% and 6.2%) in the first observation.

Table 1. Mean ± SD of olfactory response of different species of *Drosophila* flies on various fruits (Figures in parentheses are % of preference).

	Ist observation			
Species	Sapota	Apple	Banana	Papaya
D. melanogaster	112.1±15.5 (37.3%)	36 ± 4.38 (12%)	69.3 ± 22.2 (23.1%)	30.3 ± 4.1 (10.1%)
D. simulans	18.6 ± 8.93 (22.5%)	32 ± 5.9 (9.7%)	53.8 ± 17.1 (14.6%)	37.3 ± 16.4 (5.8%)
D. yakuba	52.5 ± 20.6 (17.4%)	49.1 ± 12.1 (16.3%)	25.1 ± 14.2 (8.3%)	30.8 ± 42.1 (10.2%)
D. ananassae	101.3 ± 33.6 (33.7%)	27.1 ± 4.7 (9%)	36.3 ± 12.4 (12.1%)	35 ± 8.8 (11.6%)
D. bipectinata	61.8 ± 14.3 (20.6%)	15 ± 2.1 (6%)	34.1 ± 5.1 (9.6%)	16.8 ± 3.3 (5.6%)
D.malerkotliana	53.8 ± 17.1 (17.9%)	18.6 ± 8.9 (6.2%)	37.3 ± 16.4 (12.4%)	32 ± 5.9 (10.6%)
	IInd observation			
Species	Sapota	Apple	Banana	Papaya
D. melanogaster	112.5 ± 10.7 (37.5%)	32.8 ± 6.2 (10.9%)	62 ± 5.9 (20.6%)	27.1±7.0 (1.1%)
D. simulans	10.8 ± 4.5 (16.77%)	25 ± 6.2 (6.8%)	70.5 ± 23.3 (13.5%)	45.6 ± 21.4 (11.9%)
D. yakuba	61.5 ± 20.3 (20.5%)	52.5 ± 13.4 (17.5%)	25.3 ± 9.0 (8.4%)	26.3 ± 40 (8.7%)
D. ananassae	104.6 ± 36.7 (34.8%)	25 ± 8.6 (8.3%)	36.3 ± 11.5 (12.1%)	32.1 ± 4.7 (10.7%)
D. bipectinata	62.8 ± 23.6 (20.9%)	17.5 ± 3.3 (5.8%)	62.1 ± 18.0 (20.7%)	13 ± 3.2 (3.6%)
D. malerkotliana	70.5 ± 23.3 (23.5%)	10.8 ± 4.5 (3.6%)	45.6 ± 21.4 (15.2%)	25 ± 6.2 (8.3%)
	IIIrd observation			
Species	Sapota	Apple	Banana	Papaya
D. melanogaster	116.1 ± 20.3 (38.7%)	33 ± 3.03 (11%)	60.3 ± 12.8 (20.1%)	30.5 ±1 3.4 (10.1%)
D. simulans	13.8 ± 4.0 (17.1%)	30.1 ± 9.08 (12.2%)	63 ± 31.2 (18.1%)	52 ± 21.9 (4.6%)
D. yakuba	68.6 ± 27.8 (22.8%)	65.8 ± 20.9 (21.9%)	14.5 ± 4.6 (4.8%)	25.3 ± 45.4 (8.4%)
D. ananassae	103.3 ± 33.6 (33.4%)	35.1 ± 2.8 (11.7%)	35.6 ± 10.2 (11.8%)	36 ± 11.4 (12%)
D. bipectinata	63.5 ± 30.6 (21.1%)	18.6 ± 5.7 (6.2%)	61.3 ± 25.6 (20.4%)	24 ± 7.8 (8%)
D. malerkotliana	63. ± 31.2 (21%)	13.8 ± 4.0 (4.6%)	52. ± 21.9 (17.3%)	30.1 ± 9.0 (10%)

Tureli *et al.* (1984) reported that *D. melanogaster* and *D. simulans* showed significant, but very weak, choice than *D. pseudoobscura* in habitat selection in different fruit trees. In the present study, the flies of *D. melanogaster* and *D. simulans* prefer maximum for sapota in all the trials. The natural fruits, such as banana, sapota, papaya, and apple contain different compositions of chemicals and nutrients (Abdul Khader *et al.,* 1985). *Drosophila* is sensitive to a wide variety of odorants and is capable of odour discrimination. It is likely that olfactory response plays an important role in the selection of food and in the identification of hazardous substances (Fuyama, 1978).

A microgeographic genetic difference between habitats, which may be inconsistent, has been found in *D. persimilis* and *D. melanogaster* (Taylor and Powell, 1979). Taylor and Powell (1978) and Tureli *et al.* (1984) studied habitat choice by mark release recapture method in *D. melanogaster, D. simulans*, *D. pseudoobscura,* and *D. persimilis* in canopy woods and different fruit trees. They proposed that the difference in habitat choice behaviours were adaptive responses to patchy environment, and the habitat choice depends on the physiological conditions of the flies. Present study reveals that flies of the different species of *Drosophila* selected sapota maximum, despite all the odors of different fruits kept at each corner of the experimental box.

References: Abdul Khader, J.B.M. Md, K.P. Callappan, O. Pllai, and P.K. Chattopadhaya 1985, *Fruits, Tropical and Subtropical*. (Bose, T.K., ed.). pp. 140-161; Carson, H.L., D.E. Hardy,

H.T. Spieth, and W.S. Stone 1970, In: Essays in Evolution and Genetics in Honor of Theodosius Dobzhansky. pp. 437-543; Dobzhansky, Th., and C. Pavan 1950, J. Anim. Ecol. 19: 1-14; Fuyama, Y., 1978, Behav. Genet. 8: 399-414; Taylor, C.E., and J.R. Powell 1978, Oecologia 37: 69-75; Taylor, C.E., and J.R. Powell 1979, Genetics 85: 681-695.

Fine-scale crossover rate and interference along the XR-chromosome arm of *Drosophila pseudoobscura.*

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Although broad-scale variation in recombination rates across *Drosophila* genomes is well established, recent studies have identified that fine-scale crossover rate variation also exists within the *Drosophila pseudoobscura* genome. The first study to explore such fine-scale variation focused on a 2-MB region of the XL chromosome arm and identified where crossovers fell among 370 progeny known to have had a crossover event between two phenotypic mutant markers (Cirulli *et al.*, 2007). Fine-scale recombination rates ranged from 1.4 to 52 cM/megabase. A later work examined variation across the second chromosome in crossover rate and correlated fine-scale recombination rate with patterns of nucleotide diversity within species and divergence between species (Kulathinal *et al.*, 2008). This latter study employed an illumina bead genotyping approach, but assumed that crossovers in adjacent windows likely represented an erroneous genotype (or a gene conversion event) in one of the windows.

Both of these studies assumed that crossover interference exists within *D. pseudoobscura*, an assumption that has not been tested in this species. The first study would have missed all double-crossover events within the 2-MB window because of the methods employed, while the second study would have erroneously excluded adjacent crossovers, hence underestimating overall crossover rates. One can best address this deficiency by examining many closely linked markers in a very large panel of backcross progeny.

Here, we directly measured crossover rate between markers within a 3 MB region on the XR chromosome arm of *Drosophila pseudoobscura* in a very large panel of backcross progeny. We identified fine-scale crossover rate variation in this region of the genome. We also used these results to obtain estimates of the coefficient of coincidence and interference in this species.

Materials and Methods

We crossed two strains of *Drosophila pseudoobscura*, Flagstaff 1993 and Mather 17, and backcrossed the F_1 females to males from the Flagstaff 1993 line. To achieve a sufficiently large sample size of F_2 backcross progeny, we performed two such crosses identically. We extracted DNA from and genotyped 1208 F_2 backcross individuals from the first cross and 2057 F_2 backcross individuals from the second cross, totaling 3265 F_2 backcross individuals, and we characterized crossover rate in a 3 MB region on the XR chromosome arm in these backcross progeny. Because here we examined this region exclusively, our further use of the terms "recombinant" and "non-recombinant" refers only to crossover events between XR_group8 position 5,051,027 and position 7,973,182 (Richards *et al.*, 2005).

We used a two-step process to characterize the crossover rate. First, we identified recombinants in the backcross sample using four microsatellite markers dispersed across the region (hence splitting it into three 1-megabase regions dubbed "A" through "C") as well as one additional marker in the center of region "B". We then genotyped the recombinant sample using six additional markers. Because we identified only two double recombinants in the initial scan of the first cross, we used the two outermost flanking markers, DPSX037N and DPSX021B1 (previously used in Ortiz-Barrientos *et al*., 2004), to differentiate recombinants from non-recombinants in the second cross for further genotyping with all of the other microsatellite markers. We assessed whether the distribution of crossovers deviated from a random distribution by bootstrapping (accounting for the sizes of the regions- see Cirulli *et al.,* 2007).

PCR was performed in 10 μL reactions containing 1 μL of DNA (Gloor and Engels, 1992), using the following touchdown cycling protocol: 1 min at 95°C, 3× (95° for 30s, 56°C for 30 s, 72°C for 30 s), 3× (95°C for 30 s, 53°C for 30 s, 72°C for 30 s), 30× (95°C for 30 s, 50°C for 30 s, 72°C for 30 s). PCR products were visualized on a polyacrylamide gel using LiCor 4300 DNA sequencer/ analyzers. The complete list of markers surveyed and primer sequences are available upon request.

Results and Discussion

We found significant crossover rate heterogeneity along three megabases of the XR chromosome arm ($p < 0.0001$, see Figure 1), a result consistent with studies that reported such heterogeneity along the XL and 2-chromosome arms of this species (Cirulli *et al*., 2007; Kulathinal *et al.*, 2008). Recombination rates in this XR region ranged from 1.8 to 19.8 cM/megabase. This heterogeneity was significantly associated with GC-content and simple repeats (multiple regression $r = 0.81$, overall $p = 0.02$, $P_{GC} = 0.04$, $P_{repeats} = 0.0087$), again confirming findings for the XL chromosome arm (Cirulli *et al.*, 2007).

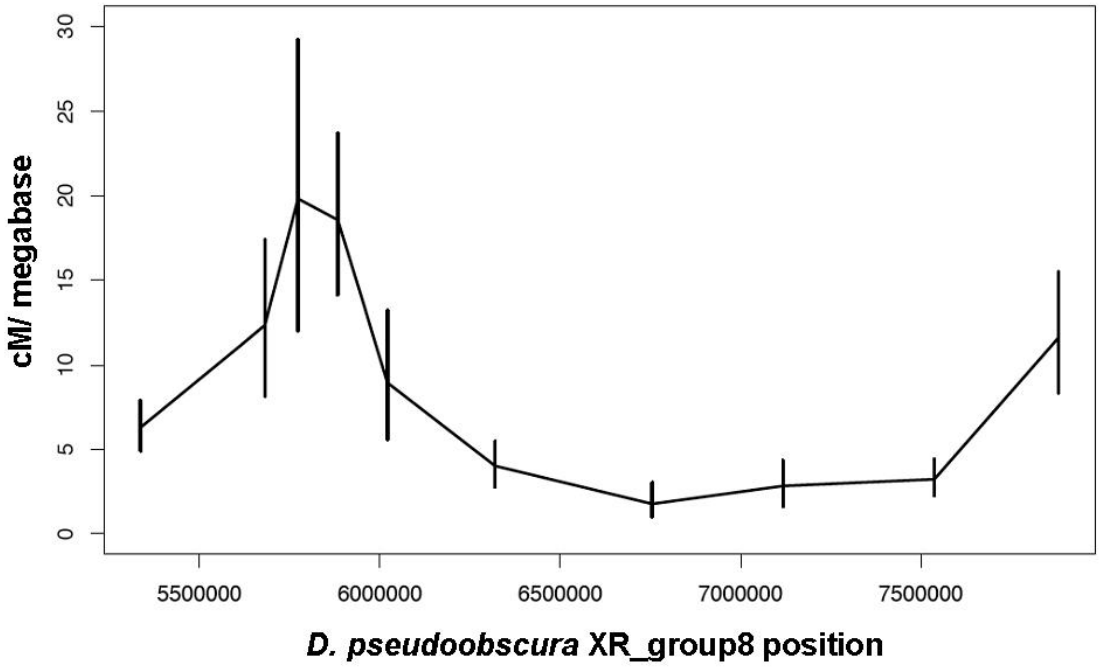

Figure 1. Fine-scale recombination rate across 3MB of the *D. pseudoobscura* XR chromosome arm.

Interference (I) is defined as I = 1-coefficient of coincidence (c.o.c.), where the c.o.c. reflects the ratio of observed to expected double crossover frequencies. The expected frequency of double crossovers between two endpoints was calculated from the RF of individual regions. Any interval completely lacking double crossovers will have a c.o.c. equal to 0 and can, therefore, be characterized as having complete crossover interference (I = 1). Because we only observed double crossovers between intervals A and C, we present the calculation of interference for that pair only. All other closer windows (2 megabases or less) exhibited complete crossover interference.

Of the 1208 flies that we genotyped at all 5 markers in the first scan of backcross progeny, 230 were recombinant. This recombinant frequency of 0.19 yields an estimated probability of double crossovers of 0.036 and, with no interference, predicts 43.8 double crossovers between the two outermost markers, DPSX037N and DPSX021B1. However, we only observed two double crossovers in that region. Therefore, the c.o.c. = 0.046 and I = 0.954 for markers three megabases apart.

Overall, these results demonstrate fine-scale crossover rate variation along a 3-MB region of the XR chromosome arm, strong crossover interference in this region, and complete (or nearly complete) interference in closer windows. Many questions remain about the molecular mechanism underlying this phenomenon, and the strength of interference may well vary throughout the genome. If this is the case, a more thorough characterization of genomic interference patterns will strengthen the overall understanding of recombination.

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References: Cirulli, E.T., R.M. Kliman, and M.A.F. Noor 2007, J. Mol. Evol. 64**:** 129-135; Gloor, G.B., and W.R. Engels 1992, Dros. Inf. Serv. 71**:** 148-149; Kulathinal, R.J., S.M. Bennett, C.L. Fitzpatrick, and M.A.F. Noor 2008, Proc. Natl. Acad. Sci. USA 105**:** 10051-10056; Ortiz-Barrientos, D., B.A. Counterman, and M.A.F. Noor 2004, PLoS Biol. 2**:** e416; Richards, S., Y. Liu, B.R. Bettencourt, P. Hradecky, S. Letovsky et al., 2005, Genome Res. 15: 1-18.

Sperm storage and nuptial gifts in *Drosophila paulistorum.*

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Theodosius Dobzhansky, doctoral mentor, and colleague for some three decades, used to speculate that sperm entering the two pigmented sophophoran spermathecae could not exist. The single ventral receptacle, he maintained in our routine Saturday morning chats, was the primary sperm storage organ. So perhaps spermathecal sperm were digested, constituting protein-rich nuptial gifts granted needy gravid females.

Many male insects donate nuptial gifts to help insure successful copulation and offspring, a form of paternal investment. While nuptial gifts come in various forms in arthropods, evolutionary origins are obscure. Gifts range from inanimate objects to balls of silk to sacrificing their own life, all to insure copulation with the females and the production of progeny. Greater numbers or masses of a nuptial gifts seem to correlate with greater numbers of offspring (…success for fruit flies, 2009).

He pointed out, correctly, that sperm entering transparent receptacles oriented themselves toward egg micropyles, while sperm entering spermathecal stalks clustered irregularly, resembling knotted hair. Doby specifically cited *D. prosaltans* spermathecae as possessors of funnel-like barriers at the distal tip of spermathecal stalks

Herewith we present a survey of this storage organ, present twice, transparent and light yellow in *D. paulistorum,* a neotropical *willistoni* group cluster of semispecies. (See Ehrman *et al.,* 1995, and Ehrman and Powell, 1982, for literature reviews.) Note especially the top part of the stalk inside the bulb constituting 'thecal storage areas.

We are reporting our observations here, hoping for assistance from those better informed: Do the spermathecal parts depicted here have formal entomologic names (unknown to us despite searches)? Is the Dobzhansky anecdote even partially true? Could moribund 'thecal sperm exist only when an ejaculate fully fills the ventral receptacles first? (See Figures 1, 2a, 2b, and 3.)

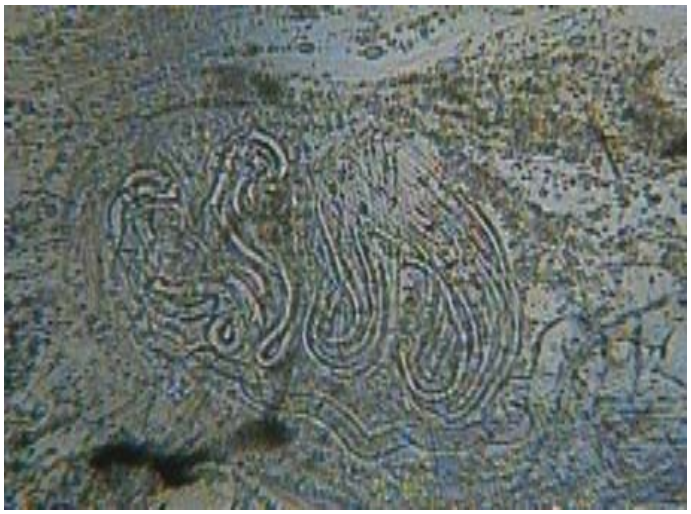

Figure 1. Ventral receptacle of *D. paulistorum* (unstained, 400×, in physiologic saline).

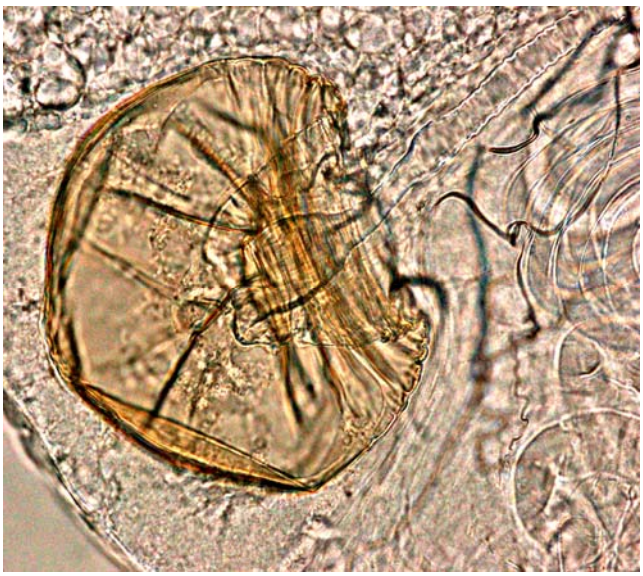

Figure 2a. Spermathecae of *D. paulistorum* (unstained, 400×, in physiologic saline). 2b. One spermatheca from an Amazonian female, 400×, with part of her ventral receptacle visible to the right. Note the centrally located funnel-like structure at the internal end of the spermathecal stalk

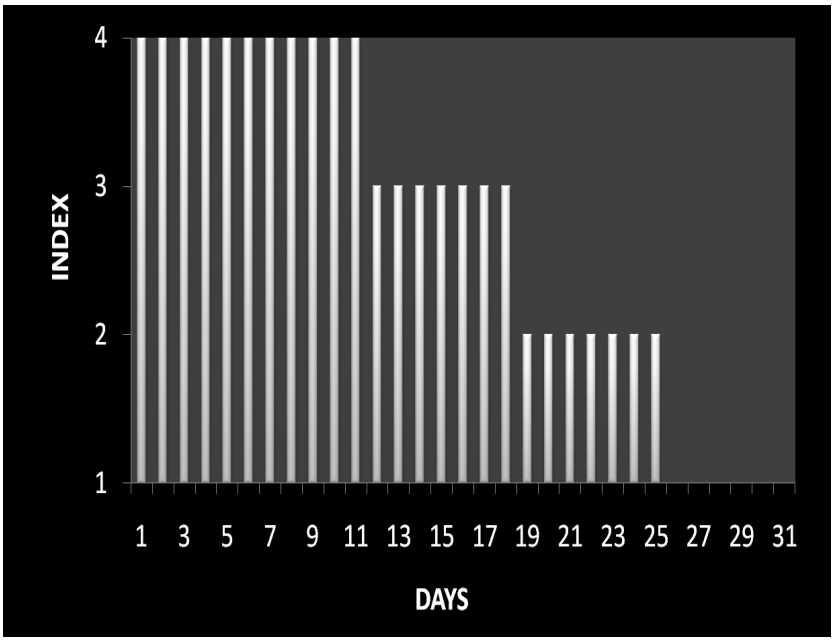

Figure 3. Exhaustion of *D. paulistorum* ventral receptacles over time (4 = full; 3 = half full; 2 = quarter full; 1 = empty).

Correlations scored between primary and secondary sperm storage sites proved inversely proportional. Females dissected after sperm exhaustion in the production of embryos, had no sperm in ventral receptacles, but sperm were present in spermathecae. Females dissected shortly after insemination had sperm in the ventral receptacle but no sperm in the spermathecae; however, as post mating time progressed, the spermathecae would contain sperm while the ventral receptacle was emptied in egg fertilization.

In the venerable 1947 (#4720) *University of Texas Publication: Studies in the Genetics of Drosophila V. Isolating Mechanisms,* M. Wheeler wrote of the *D. paulistorum* sibling species, *D. equinoxialis* (and *D. willistoni*, pp 84 and 85):

> These flies were then left together overnight and the females dissected early the following morning. Two inseminations were secured. In both specimens the ventral receptacle was teeming with sperm while the spermathecae contained only a few.

Professor Wolfgang Miller, Laboratory of Genome Dynamics, Medical University of Vienna, has recently stained *D. paulistorum* intersemispecific hybrid females (supplied by the Ehrman Purchase laboratory and checked for hybridity there) with DAPI (for DNA), plus fluorescent dye Alexa 488 (for *Wolbachia*), and found intense concentrations of this microorganism in all spermathecae. Could the *D. paulistorum* endosymbiotic *Wolbachia* be exploiting a reproductive storage organ as a "bacteriocyte"? If so this would constitute a newly evolved nutritional strategy, suggested by Dr. Miller who also notes that sperm stored in *D. paulistorum* spermathecae degenerate. We are certainly grateful to him for his ongoing insightful collaboration.

References: Ehrman, L., I. Perelle, and J.R. Factor 1995, Endosymbiotic Infectivity in *Drosophila paulistorum* Semispecies. *The Continuing Influence of Theodosius Dobzhansky.* Chap. 18: 241-261. New York: Columbia University; Ehrman, L., and J. Powell 1982, The *Drosophila willistoni* species group. In: *The Genetics and Biology of Biology of Drosophila.* (Ashburner, M., H.L. Carson, and J.N. Thompson, jr., eds.). Vol 3b, pp. 193-225. New York: Academic Press; Vomiting is path to romantic success for fruit flies. Retrieved March 5, 2009, from http://planetearth.nerc.ac.uk/news/story.aspx?id=344.

Temperature sensitive gene expression: Two rules.

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Gene expression is composed of *two* words: *gene* and *expression*.

Gene: a gene is defined as the basic unit of the transmission of a biological trait from parents to their children (or in molecular terms, a segment of deoxyribonucleic acid (DNA), composed of a transcribed region and a regulatory sequence that makes transcription possible).

Expression: the word expression, *in genetics*, is defined as the process of expressing a gene.

Each gene functions like a book that stores the information. Consider several books in an academic library. It is evident that all the books are not alike in subject matter making some more popular than others. Most books are read a lot once taken off from the shelf while others are for *consultation* or *reference* purpose *only* and thus are used *very rarely*. Each book, in turn, contains different information and has a different function and purpose. Similarly, each gene stores the genetic information and has different genetic expression effect.

The gene is found in the cells of all living organisms, from bacteria to humans. For a molecular geneticist, a *gene* is a segment of DNA of which some is transcribed and that includes a promoter (a specific DNA sequence to which RNA polymerase binds and signals where RNA synthesis (transcription) occurs), coding sequences (the sequence of nucleotides in DNA or RNA that determines the specific amino acid sequence in the synthesis of proteins), and a signal for ribonucleic acid polymerase (RNA polymerase) to stop. The rationale of genes is to store the genetic information. Each gene contains the information required to make a protein, or in some cases a non-coding RNA (ribonuclease). Genes are expressed to produce functional RNA and protein molecules in the cell.

Gene expression occurs in two steps (Figure 1). The *first* step is *transcription*. In this process, the gene is copied to produce a RNA molecule (a primary transcript) with essentially the same sequence as the gene. Most genes (humans) are divided into *exons* (any segment of an interrupted gene that is represented in the mature RNA product. The protein-coding sequences of a gene) and the *introns* (the DNA base sequences interrupting the protein-coding sequences of a gene). These sequences are transcribed into RNA but are cut out of the message before it is translated into protein. Only the exons carry information required for protein synthesis. Most primary transcripts are therefore processed by *splicing* to remove intron sequences and generate a mature transcript or mRNA (messanger RNA) that only contains exons. The *second* step

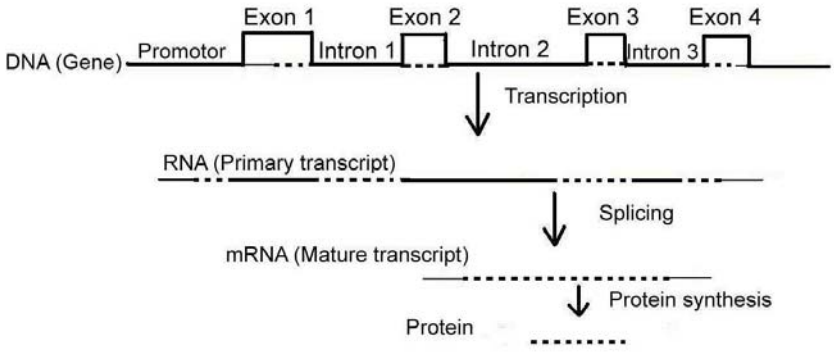

Figure 1. A model on gene expression: transcription and translation process.

is *translation*. Three nucleotides are required to produce one amino acid and the chain of amino acids must fold up to fabricate the final tertiary structure of a protein.

Some genes are expressed in each cell of an individual all of the time (known as housekeeping genes) and are essential for very basic cellular functions. Other genes are expressed in particular cell types or at particular stages of development. For example, in case of an individual human the genes that encode muscle proteins such as actins and myosin are expressed only in muscle cells, not in brain cells. Still other genes can be activated or inhibited by signals circulating in the body, such as hormones. This differential gene expression is achieved by regulating transcription and translation. All genes are surrounded by DNA sequences that control their expression. Proteins called transcription factors bind to these sequences and can switch the genes ***on*** or ***off***. Gene expression therefore is controlled by the availability and activity of different transcription factors.

As transcription factors are protein themselves, they must also be produced by genes, and these genes must be regulated by other transcription factors. In this way, all genes and proteins can be linked into a regulatory chain of command starting with the *transcription factors* present in the *egg* at the beginning of the development. A number of human diseases, such as diabetes II that appears in time and space at a later age of an individual's life due to the high carbohydrate intake (environmental stimulus), are known to result from the absence or malfunction of transcription factors and the disruption of gene expression thus caused. A classical example for the demonstration of gene regulation in humans is the variation in the human skin color from one individual to another and that varies from light to dark black. Skin color is determined by the amount and type of the pigment in the skin termed as melanin. Individuals close to sun are darker (more pigment) than those far away from the sun (less pigment) and is an example of gene expression effect. The structural gene for example forms the hair on a human body, while the regulatory gene regulates the intensity of coloration of human hair ranging from white to dark black. Such studies, however, do not provide any information on how a contribution of an interaction of genes with the environment affects the phenotypic variation from one individual to another. That is whether a gene is turned "***on***" or it is turned "***off***" during the development of a phenotypic trait (how the gene expression effect is altered through the environmental stimuli).

In humans, upon fertilization of the mother's egg by a father's sperm, the genes are turned "***on***" and or turned "***off***" at each stage of the developmental process depending upon the environmental conditions through the transcription-translation process, thereby forming different parts of the body depending upon the size and shape.

Thus, the development of a phenotypic trait is a product of gene expression (genes interacting with each other and with the environment). There are innumerous studies on gene regulatory systems in plants, on *Drosophila*, bacteria and humans. Such investigations demonstrate only whether a given gene product is present (turned "***on***") or absent (turned "***off***") under a specific environmental condition. For example, Gupta (1983) investigated the presence and/or the absence for an enzyme adult acid phosphatase-6 (AP-6) in backcross progeny of *Drosophila pseudoobscura* and *D. persimilis*. AP-6 is present or absent in *D. pseudoobscura* but always absent in *D. persimilis*. Gupta and Lewontin (1982) published a paper that explains the influence of the environment stimuli on genes and vice-versa. Other examples are the markings on a cat's fur, and the shape of a tree's leaf.

The present paper deals with the importance of gene expression effect in developing the two rules.

As it is impossible to carry out an experiment on humans, Gupta (1978) used *Drosophila* to prove how the process of transcription and translation leads to the two hypotheses: 1) whether the genes are turned "***on***", and 2) the genes are turned "***off***" for a phenotypic trait under different environmental conditions. That is how the gene expression plays an important role in the development of a morphological trait from one environment to another.

Experimental Procedure

Eight iso-chromosomal lines for the second chromosome of *Drosophila pseudoobscura* from Santa Cruz Island, at sea level, California, were used for the experiment. These lines were maintained in half-pint milk bottles on Carpenters medium at 24ºC before they were used for the experimental work. Heterozygotes (F_1's) between lines were created by mating pairs of iso-chromosomal strains at random (1 × 2, 3 × 4, 5 × 6 ... 7 × 8) so as to reconstitute the variety of genotypes present in nature (The results for heterozygotes are not discussed in this paper). For our purpose, we have considered the data on viability % *only* in *parents*. [*Viability* is defined as the percent of fertilized eggs placed in a vial that reached adulthood (eclosion from the pupal case)] to provide the experimental evidence for the occurrence of gene expression phenomenon. The fertile eggs collected varied from 6 to 14 hours of age. For each parental homozygote, eggs were collected and cultured at two densities (40 and 140 eggs/vial) at the specified temperature. Ten replicates were made at two egg densities and at three temperatures (14º, 21º, and 26ºC). Each vial contained 10-12 ml of Carpenters medium. The results obtained at 40 eggs/vial are not discussed here as both the egg densities showed the same pattern from gene expression view-point.

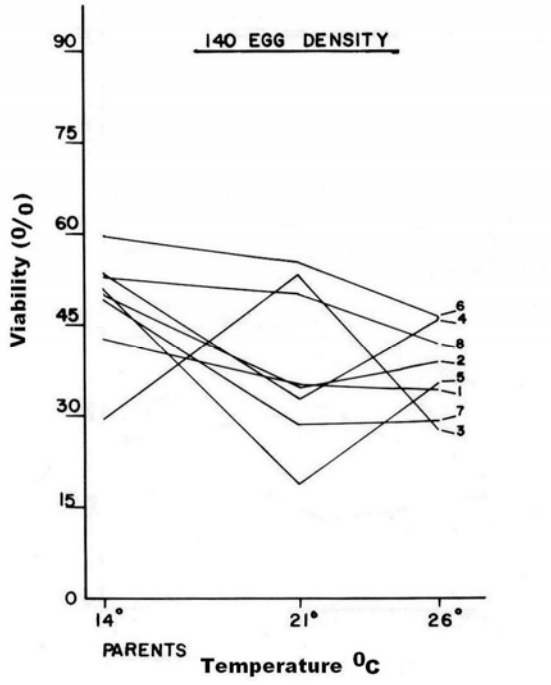

Figure 2. Viability of eight genotypes of *D. pseudoobscura* (numbered from 1 to 8), Santa Cruz, California. Each raised at three temperatures (14ºC, 21ºC, and 26ºC). Each genotype interacts differently with the temperature and provides evidence for the gene expression effect (genes turned **on** and genes turned **off)**.

Results and Discussion

Figures 2, 3, and 4 shows on the x-axis the three different temperatures (14º, 21º, and 26ºC) while on y-axis is the viability (%) in parental strains of *Drosophila pseudoobscura*.

In Figure 2, there are eight different parental genotypes numbered from 1 to 8, each raised at the same time at three different temperatures. The genotypes numbered 6 and 8 produce more progeny at 14ºC but less at the other two temperatures (21º and 26ºC); genotype 3 produces highest viability at 21º but low at the extremes 14º and 26ºC; genotype 5 yields highest viability at 14º but lowest at 21ºC; genotype 4 produces more viable progeny at 14º and 26ºC but lowest at 21ºC; genotype 7 yield the highest viability at 14º but lower at 21º and 26ºC. The Figure leads to the three important evolutionary approaches: 1) the norm of genotype 5 is of 'V' shaped; 2) while the norm of genotype 3 is of inverted 'Λ' shaped; 3) the two genotypes 3 and 5 *cross each other* when nurtured at a temperature range between 14º and 21ºC; and between 21º & 26ºC. It demonstrates the phenomenon

of genotype × temperature interaction. Based on such facts one cannot predict the outcome of the phenotype developed from a genotype when moved from one temperature to another. Thus, the development of a phenotypic trait of phenotype from a given genotype is temperature sensitive. These data provide the gene expression effect on the development of an individual fruit-fly. This signifies that each genotype is different genetically and the genes differ in their expression from one temperature to another. That is how the genes are expressed considering and analyzing the viability data for a genotype in question from one temperature to another. Thus this figure demonstrates the presence of turning "***on***" for *higher* viability at one temperature, and turning "***off***" the genes for *lower* viability when raised at other temperatures for a given genotype.

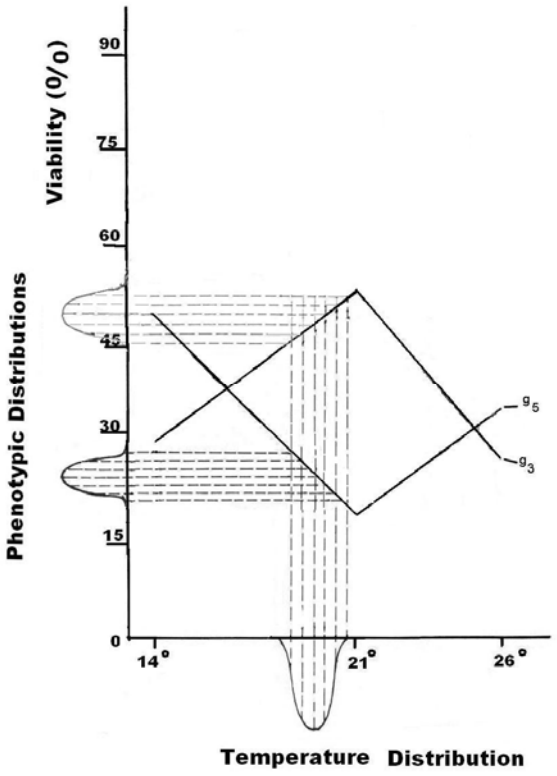

Figure 3. **Rule 1: Two phenotypic Distributions**: The viability data, illustrating the ***two*** phenotypic distributions, for two genotypes g_3 and g_5 taken from figure 2 at the temperature range of 18.5ºC and 20.6ºC provides the evidence of genes **turned on** (mostly gene expression effect).

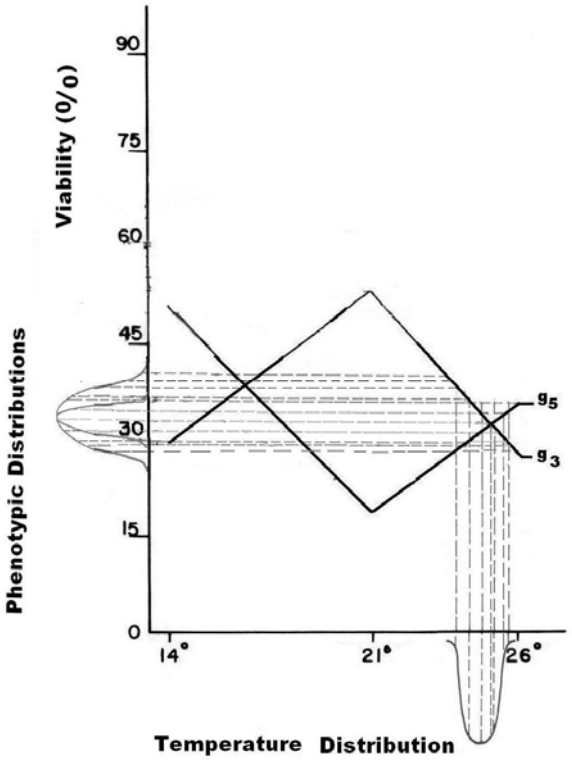

Figure 4. **Rule 2: One phenotypic Distribution**: The viability data, demonstrating the only ***one*** phenotypic distribution, for two genotypes g_3 and g_5 taken from figure 2 when the temperature range varies from 23.6ºC and 25.6ºC provides the evidence of genes **turned off** (mostly non-genetic or environmental stimuli expression effect).

For the present paper, the two genotypes 3 and 5 (g_3 and g_5) are selected to explain not only the gene expression as turned "***on***" or turned "***off***" but also the process of gene transcription and translation towards the development of the viable progeny (viability) as a phenotypic trait.

In Figure 3, when one takes into account raising these two genotypes (g_3 and g_5) at a temperature range between 18.5º and 20.6ºC, one gets two different phenotypic distributions with a mean viability of 24.2 and 49.4 percent for the genotype g_5 and g_3, respectively, indicating the significant impact of the gene action and less impact of the environment (temperature) in determining the viability. It is interpreted in terms of the gene expression effect as genes turned "***on***".

When one cultures these two genotypes at a temperature range of 23.6º to 25.6ºC (Figure 4), one finds that both have the same phenotypic distribution (both genotypes show the same mean viability of 34.2%). There is a little or no gene effect in determining the phenotypic trait (viability). It implies the significant contribution of non-genetic factors (temperature effect) and less impact of gene action. Thus, it is considered as the gene action turned "***off***".

Formulation of Two Rules on Gene Expression

In order to invent the two rules on gene expression effect, the two different temperature ranges are considered: the first temperature range from 18.5º to 20.6ºC to the left of 21ºC where the two genotypes g_3 and g_5 ***do not cross each other***, Figure 3; and the other temperature range from 23.6º to 25.6ºC to the right of 21ºC (Figure 4), where the same two genotypes (g_3 and g_5) ***cross each other***. Based on the experimental data the two rules have been established as detailed below.

Rule 1: ***Two Phenotypic Distributions***:

Here, a temperature range is considered where the two genotypes g3 and g5 ***do not*** cross each other. Thus, considering the temperature range of 18.5º – 20.6ºC to the left of 21ºC (Figure 3), the vertical lines were drawn from x-axis towards each of the two g_3 and g_5 genotypes and a temperature distribution curve was drawn below the x-axis line. From each of the two genotypes, drew horizontal lines towards the y-axis to get the mean viability. Based on these data, two different curves were drawn on facing out the y-axis line, and that outlined two distinct phenotypic distributions with a mean viability of 24.2% for g_5, and 49.4% for g_3 genotypes, respectively. This demonstrates the phenomenon of gene expression effect as turned ***on*** because of the significant difference in the mean viability (25.2%) between the two genotypes. The gene expression effect is mostly genetic and thereby the transcription and translation process of g_3 and g_5 genotypes for viability is ***normal***.

Rule 2: ***One Phenotypic Distribution***:

Now considering the temperature range of 23.6º – 25.6ºC to the right of 21ºC for g_3 and g_5 genotypes where they ***cross*** each other (Figure 3), the vertical lines were drawn from the x-axis towards each of these two genotypes, and a distribution curve was drawn facing out below the x-axis line. In order to get mean viability for each of these two genotypes drew horizontal lines towards the y-axis from genotypes g_3 and g_5. The two curves facing out the y-axis line were formed that overlapped each other. That is, only ***one*** phenotypic distribution curve was obtained, and that produced only ***one*** phenotype with a mean viability of 34.2% for each of two g_3, and g_5 genotypes. This provides the experimental evidence of gene expression effect as turned ***off*** because there was no difference in the viability yielded by each of the two genotypes. The gene expression effect is mostly non-genetic and thus influenced by the temperature stimuli. That identifies the ***disruption*** in the gene expression effect results from the absence or malfunction in the transcription and translation process.

Thus, the data discussed above provide the evidence for the two hypotheses of transcription and translation that lead to: 1) the gene expression effect as turned "***on***", and 2) the gene expression effect that are turned "***off***", for the viability in *Drosophila* when raised under three different temperatures. It is evident that the gene expression effects are not only applicable to prokaryotes but

are also relevant to eukaryotes including humans on one-to-one basis (an extreme human example: an individual who commits suicide under a heavy environmental stress shows the ***suicidal gene*** expression effect as turned "***on***" while the ***normal*** gene expression effect for behavior is turned "***off***". Such suicidal gene effect of an individual human being ranging from an intellectual to a politician is due to the profound stressful environmental stimuli having not been identified yet).

Thus, the experimental approach facilitated in formulating ***two rules*** on gene expression effect. However, both experimental and other hypothetical aspects discussed above provides the evidence that the normal gene expression activity of turning "***on***" (the two genotypes g_3 and g_5 *do not cross* each other) and/or turning "***off***" (two genotypes g_3 and g_5 *cross* each other) for an individual genotype depends upon the surrounding environment and that affects the phenotypic expression. That is the gene expression effect is temperature sensitive and the environment, thus, controls the transcription and translation processes in an individual's daily life.

Acknowledgment: Appreciation is extended to Michelle Orr and Ruth Faye Richards.

References: Gupta, A.P., 1978, Ph.D. Thesis, Harvard University, Cambridge. MA; Gupta, A.P, and R.C. Lewontin 1982, Evol. 36(5): 934-948; Gupta, A.P., 1983, Dros. Inf. Serv. 59: 47-48.

Evolutionary significance of temperature on gene expression.

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The experiment is designed to understand how the environmental stimulus such as the temperature affects the gene expression for a phenotypic trait that involves the process of transcription and translation leading to an evolutionary approach, using *Drosophila pseudoobscura*.

Experimental Procedure

Ten iso-chromosomal lines for the second chromosome of *Drosophila pseudoobscura* from Strawberry Canyon, 200 feet above sea level, California, were used for the experiment (Gupta 1978). These lines were maintained in half-pint milk bottles on Carpenters medium at 24°C before they were used for the experimental work. Heterozygotes (F_1's) between lines were created by mating pairs of iso-chromosomal strains at random (1×2, 3×4, 5×6, 7×8, 9×10) so as to reconstitute the variety of genotypes present in nature (the results for heterozygotes are not discussed in this paper). For our purpose, we have considered the data on absolute viability percent *only* in *parents*. [*Viability* is defined as the percent of fertilized eggs placed in a vial that reached adulthood (eclosion from the pupal case)] to provide the experimental evidence for the occurrence of gene expression phenomenon. The fertile eggs collected varied from 6 to 14 hours of age. For each parental homozygote, eggs were collected and cultured at two densities (40 and 140 eggs/vial) at the specified temperature. Ten replicates were made at two egg densities and at three temperatures (14°, 21°, and 26°C). Each vial contained 10-12 ml of Carpenters medium. The results obtained at 40 eggs/vial are not discussed here as both the egg densities showed the same pattern from gene expression view-point.

Results and Discussion

Figures 1, 2, and 3 show on the x-axis the three different temperatures (14°, 21°, and 26°C) while on y-axis is the viability (%) in strains of *Drosophila pseudoobscura*.

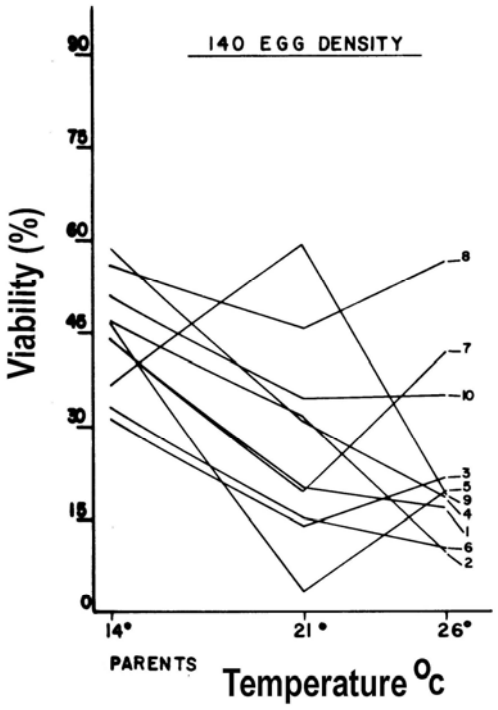

Figure 1. Viability of ten genotypes of *D. pseudoobscura* (numbered from 1 to 10), Strawberry Canyon, California, each raised at three temperatures (14°C, 21°C, and 26°C).

In Figure 1, there are ten different parental genotypes numbered from 1 to 10, each raised at the same time at three different temperatures. The genotypes numbered 1, 2, 4, 6, and 9 produce more progeny at 14°C but less at the other two temperatures (21° and 26°C); genotypes 3 and 7 produce higher viability at 14° and 26° but lowest at the 21°; genotype 5 yields highest viability at 21° but lower at 14° and 26°C; genotype 8 produces more viable progeny at 26°C but lowest at 21°C; genotype 10 yield the highest viability at 14° but lower at 21° and 26°C. The figure leads to the following important evolutionary information: 1) the norm of four genotypes numbered 3, 7, 8, and 9 is of 'V' shaped; 2) while the norm of only one genotype numbered 5 is of inverted 'Λ' shaped; 3) the two genotypes 5 and 7 *cross each other* when nurtured at a temperature range between 14° and 21° C, and between 21° and 26°C; 4) the two genotypes 5 and 8 *cross each other* when nurtured at a temperature range between 14° and 21° C, and between 21° and 26°C; 5) the two genotypes 5 and 9 *cross each other* when nurtured at a temperature range between 14° and 21° C, and between 21° and 26°C. The evolutionary approach of genotypes numbered 5 and 7; 5 and 8; and 5 and 9 demonstrates the phenomenon of genotype × temperature interaction. Based on such facts one cannot predict the outcome of the phenotype developed from a genotype when moved from one temperature to another. Thus, the development of a phenotypic trait of phenotype from a given genotype is temperature sensitive. These data provide the gene expression effect on the development of an individual fruit-fly. This signifies that each genotype is different genetically and the genes differ in their expression from one temperature to another. That is how the genes are expressed differently considering and analyzing the viability data for a genotype in question from one temperature to another. Thus this figure demonstrates the presence of turning "***on***" for *higher* viability at one temperature, and turning "***off***" the genes for *lower* viability when raised at other temperatures for a given genotype. (The selection of a genotype is best where there is no genotype × environmental interaction).

For the present paper, the two genotypes 5 and 7 (g_5 and g_7) from Figure 1 are selected to explain not only the gene expression as turned "***on***" or turned "***off***" but also the process of gene transcription and translation towards the development of the viable progeny (viability) as a phenotypic trait.

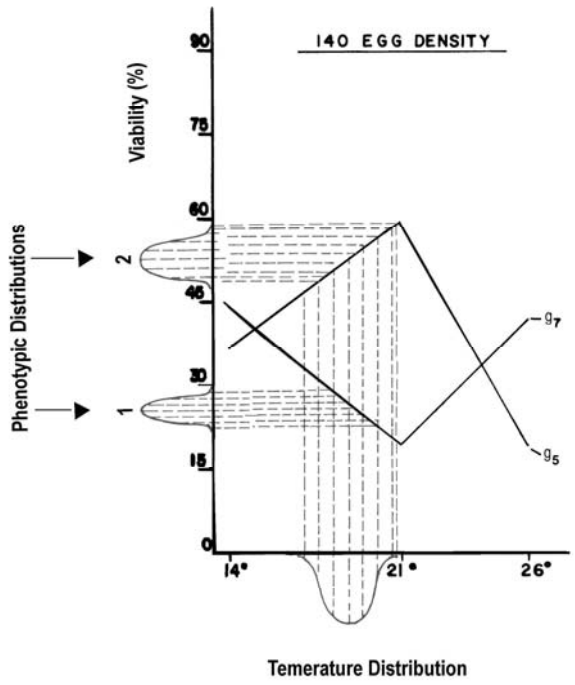

Figure 2. The viability data, illustrating the ***two*** phenotypic distributions numbered 1 and 2, for two genotypes g_5 and g_7 taken from Figure 1 at the temperature range of 17.1°C and 20.6°C.

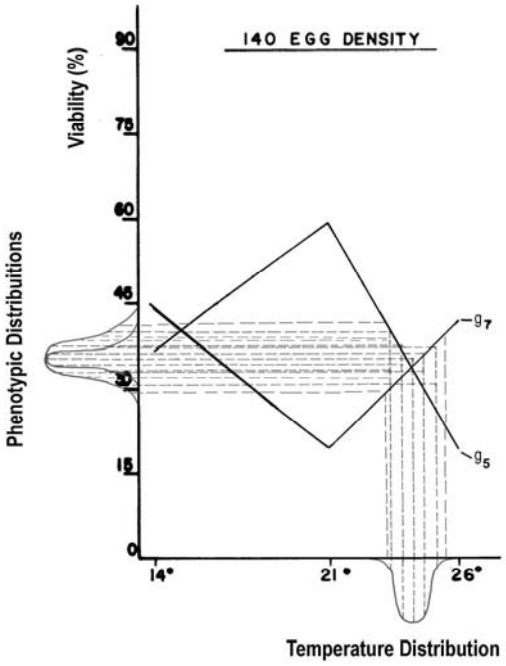

Figure 3. The viability data, demonstrating the only ***one*** phenotypic distribution, for two genotypes g_5 and g_7 taken from Figure 1 when the temperature range varies from 23.3°C and 25.3°C.

In Figure 2, when one takes into account raising these two genotypes (g_5 and g_7) at a temperature range between 17.1° and 20.6°C, one gets *two* different phenotypic distributions numbered 1 and 2. The viability range for phenotypic distribution number 1 varies from 22.1 to 29.1%, while for phenotypic distribution number 2 ranges from 48.5 to 58.2%. However, the mean viability obtained is of 25.6 and 52.1 percent for the genotype g_7 and g_5, respectively. This provides the evidence for a significant impact of the gene action and less impact of the environment (temperature) in determining the viability. It is interpreted in terms of the gene expression effect as genes turned "***on***".

When one cultures these two genotypes at a temperature range of 23.3° to 25.3°C (Figure 3), one finds that both have the same phenotypic distribution (both genotypes show the same mean viability of 36.3%). That is one gets only *one* phenotypic distribution from two genotypes. There is a little or no gene effect in determining the phenotypic trait (viability). It implies the significant contribution of non-genetic factors (temperature effect) and less impact of gene action. Thus, it is considered as the gene action turned "***off***".

It is evident that the gene expression effects are not only applicable to prokaryotes but are also relevant to eukaryotes including humans and plants. It is interesting to *note* that all human individuals in the world share a common gene expression effect and that is *stealing*. It is clear that each of us in our lives have performed the act of stealing. For example: cheating (stealing the response for the question) on a test by student; or a child takes away the food (act of stealing) from brother or sister; or an academic made a photocopy (an act of stealing) for personal use not permitted

by the Institution's policy and so on. However, the extent of stealing behavior will differ from one individual to another and thereby causing a difference in gene expression for such individuals. I name such a gene responsible for it as the "*Stealing gene"* [It is not yet known which gene (s) and/or the chromosome is responsible for it].

The present subsection deals with the evolutionary inter-relationship of transcription and translation process for respiration required by an individual organism and an individual human including plant photosynthesis. The scientific knowledge of gene expression plays an important role in an individual's life ranging from bacteria to human. For example, every living-being including aerobic bacterium needs oxygen to respire and in turn liberates the carbon dioxide into the atmosphere. It is the plant that uses the liberated carbon dioxide from the atmosphere for the process of photosynthesis and in turn releases oxygen as a waste product and sugar (energy) to be consumed by a living individual. It becomes clear that the processes of photosynthesis and respiration are interrelated. However, nothing has been elucidated so far how the transcription and translation process (a process for protein/enzyme synthesis) plays an important evolutionary role to photosynthesis and respiration required by an individual organism or an individual human. Figure 4 demonstrates the relationship of transcription, translation, respiration and photosynthesis to an individual organism and an individual human. Here, the transcription process is from DNA (gene) to mRNA while translation is from RNA to amino acid chain that leads to protein/enzyme synthesis. For example, a normal functioning of the transcription and translation process maintains the homeostatic temperature of a human-being as constant under any environmental condition. However, any interruption at any stage causes fever (from low to extreme high) and could be fatal depending upon the degree of interference. The process of photosynthesis releases the oxygen to be inhaled by a living organism and individual human for day-to-day activity in life. In order to inhale oxygen and exhale carbon dioxide during respiration process, individual needs energy and that comes through the use of covalent bonds during the translation and transcription process. The carbon dioxide thus released through the respiration process is absorbed by the plants for photosynthesis. This completes the cycle of relationship between the transcriptions, translation, photosynthesis, and respiration, thereby fulfilling the process of gene expression effect required by an individual organism including a human for survival.

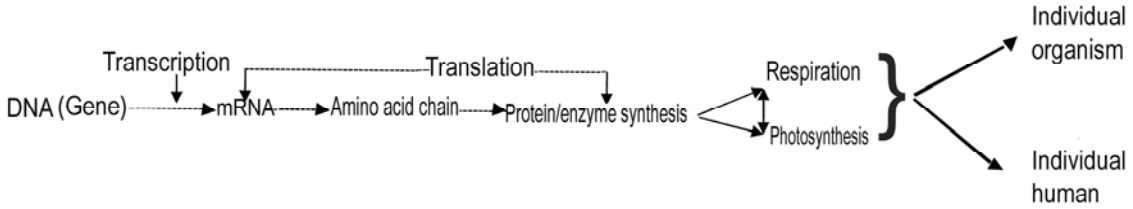

Figure 4. A model on gene expression effect demonstrating the relationship of the transcription, translation, respiration, photosynthesis to an individual organism and individual human.

Also, the Figures 2 and 3 obtained from *D. pseudoobscura*, Strawberry canyon genotypes (200 feet above sea level) confirm the findings of *two* rules published by Gupta (2009) for *D. pseudoobscura*, Santa Cruz Island genotypes (at sea level). Furthermore, the genotypes from the two populations differ in their gene expression effect. For example, 40% genotypes (Figure 1) from Strawberry Canyon population show the gene expression effect as of "V' shaped, while only 28%

genotypes from Santa Cruz Island show such an effect (see Figure 2, Gupta 2009). This is due to the difference in existing environmental conditions encountered by these two populations even though both the populations were maintained in laboratory for several years.

Acknowledgment: Appreciation is extended to Ruth Faye Richards and Michelle Orr.

References: Gupta, Anand P., 2009, Dros. Inf. Serv. 92: 32-37; Gupta, A.P., 1978, Ph.D. Thesis, Harvard University, Cambridge. MA.

Toxic effects of Patulin to some development stages of *Drosophila melanogaster*.

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Introduction

Mycotoxins are secondary metabolites of moulds, and their compounds have toxic effects on the living organisms. The toxic effect of mycotoxins on animal and human health is referred to as mycotoxicosis (Peraica *et al*., 1999). Mycotoxins may cause a range of toxic responses from acute toxicity to long term or chronic health problems, including immunosuppression and even carcinogenesis. Many mycotoxins may be produced by one mould; in particular, many *Aspergillus* and *Penicillium* species can produce several mycotoxins simultaneously, depending on the environmental and substrate conditions (Stec *et al*., 2009).

Patulin (PAT), which gets its name from *Penicillium patulinum*, is one of the most well-known mycotoxins. 50 lg/l of PAT in apple juice was considered a safe level by the World Health Organization (WHO) (van Egmond, 1989).

PAT is a potent genotoxic compound (Saxena *et al*., 2009). In addition to this, it has been reported that patulin has carcinogenic features in mice (Wogan, 1965).

The adverse effects of patulin were studied in many animals. There are some studies even in *Drosophila melanogaster*. Reiss (1975) showed that patulin has strong insecticidal activities in *Drosophila melanogaster.*

But according to understanding the likely adverse effects of patulin, there are no studies of development stages of *Drosophila* according to the best of our knowledge. *Drosophila melanogaster* provides a powerful system in which to use genetic and molecular approaches to investigate human genetic diseases (Chien *et al*., 2002). It also has many advantage for a model organism, because it is known as fruit fly *Drosophila melanogaster* has several features such as different ecological adaptation to the environment, transport giant chromosomes, simple food requirements, and having a lot of genetic variation.

Materials and methods

In this study we used the Oregon-R strain of *Drosophila melanogaster*. All experiments were carried out 25 ± 1°C and approximately 60% of relative humidity. The flies in stock and this study

were kept on Standard Drosophila Medium (SDM) composed of maize-flour, agar, sucrose, dried yeast and propionic acid (Çakır and Bozcuk, 2000).

Patulin was dissolved in 10% DMSO (Dimethyl sulfoxide). 1, 5, 10, and 15 µM test solutions were prepared and added in 50 ml bottles of SDM. For experiments average same aged individuals were obtained from stock culture, and for each bottle 5 males and 5 females were crossed.

After the pupal stage, parents were removed from the media. Parents living up to the pupal stage and offspring obtained throughout days 1-9 by taking into consideration the total number of individuals was calculated. The experiments were repeated two times.

Table 1. Maternally toxic effects of Patulin on *Drosophila melanogaster.*

Concentration (µM/50 mL)	Total number of parental individuals	Mortality
0 µM Control	10	10
Control+DMSO (10 %)	10	10
1 µM Patulin	10	10
5 µM Patulin	10	9
10 µM Patulin	10	8
15 µM Patulin	10	8

Table 2. The effect of Patulin on the number of offspring of *Drosophila melanogaster.*

Concentration (µM/50 mL)	Female (♀)	Male (♂)	Total no. of Individuals
0 µM Control	576	480	1056
Control+DMSO (10%)	655	449	1104
1 µM Patulin	595	388	983
5 µM Patulin	524	365	889
10 µM Patulin	379	314	693
15 µM Patulin	308	290	598

Results

The possible effect of Patulin (PAT), as a mycotoxin, has been examined on maternal mortality, development stages, and the number of offspring of Oregon-R strain of *Drosophila melanogaster* in this study. According to developmental stages of *Drosophila melanogaster*, any effect has not been found (Data not shown). The data obtained between C (control) and C + DMSO is not a serious difference. This shows that DMSO is a solvent that can be used.

In Table 2, C + DMSO compared to Control showed an increase in terms of total number of individuals. This increase is important compared to $p < 0.05$. 1, 5, 10, 15 µM concentrations of Patulin showed a decrease in seriousness according to C + DMSO (10%). The results in Table 2 were evaluated from the statistical angle in Figure 1, and standard errors were calculated according Student t-test.

Conclusion

Patulin previously is admitted as an antibiotic and used. Later, it was concluded patulin may have toxic effects.

Patulin did not show any lethal effects on maternal individuals (Table 1). However, a regular decrease in the number of offspring was showed starting from 1 µM to 15 µM concentrations (Table 2). Earlier studies have been parallel with our work. Indeed, Dickens and Jones (1961) showed that patulin causes tumors in rats. Mutagenic and teratogenic effects were also observed in some other studies (Roll *et al*., 1990; Korte, 1980; Thust *et al*., 1982). However, The International Agency for Research on Cancer (1986) has emphasized that for patulin there was not found sufficient evidence for carcinogenic effects on humans. Patulin was studied using the Ames test, but results were not significant (Würgler *et al*., 1991).

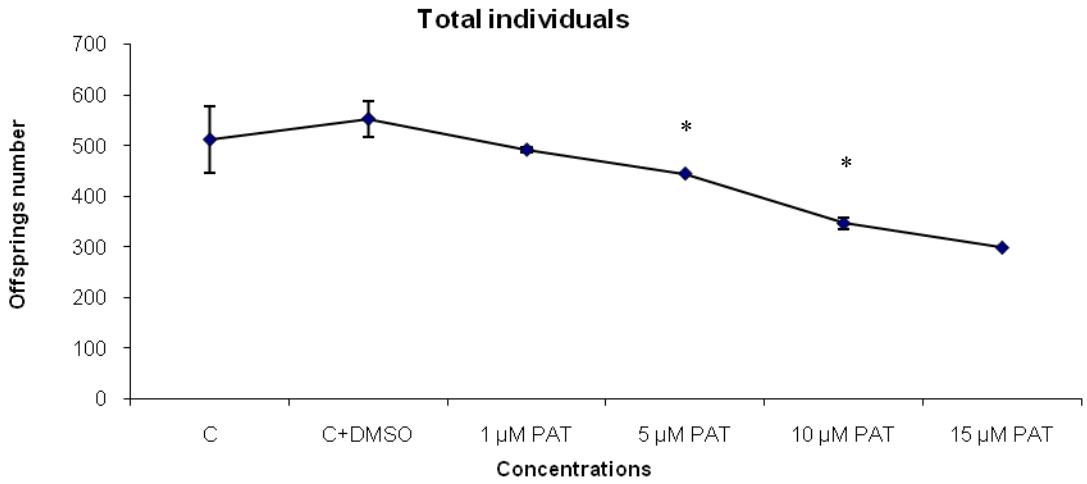

Figure 1. The levels number of total offspring individuals for patulin solutions as compared with C and C+DMSO (1 µM, 5 µM, 10 µM and 15 µM). According to Student ttest. * shows the differences between value $p < 0.05$. C: Control, DMSO: Dimethyl sulfoxide, PAT: patulin.

Possible effects of patulin certainly should be examined, because patulin is a natural contaminant such as other mycotoxins. But mutagenic and teratogenic effects of patulin have not been exactly clarified.

References: Dickens, F., and H.E.H. Jones 1961, Br. J. Cancer 15: 85-100; Çakır, Ş., and A. Bozcuk 2000, Turk. J. Biol. 24: 321–329; IARC 1986, IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. IARC: Lyon, France, Vol. 40, pp. 83-98; Korte, A., 1980, Mutat. Res. 78: 41–49; Peraica, M., B. Radic, A. Lucic, and M. Pavlovic 1999, Bulletin of the World Health Organization. 1999, 77 (9); Reiss, J., 1975, Chem. Biol. Interact. 10: 339-342; Roll, R., G. Matthiaschk, and A. Korte 1990, J. Environ. Pathol. Toxicol. Oncol. 10: 1–7; Saxena, N., K.M. Ansari, R. Kumar, A. Dhawan, P.D. Dwivedi, and M. Das 2009, Toxicology and Applied Pharmacology 234: 192-201; Stec, J., J. Żmudzki, J. Rachubik, and M. Szczotka 2009, Bull. Vet. Inst. Pulawy. 53: 129-134; Thust, R., S. Kneist, and J. Mendel 1982, Mutat. Res. 103: 91–97; Würgler, F.E., U. Friederich, and J. Schlatter 1991, Mutat. Res. 261: 209–216; van Egmond, H.P., 1989, Food Addit. Contam. 6: 139-188; Wogan, G.N., ed., 1965, *Mycotoxins in Foodstuffs*. The M.I.T. Press, Cambridge, Mass.; Chien, S., L.T. Reiter, E. Brier, and M. Gribskov 2002, Nucleic Acids Research 30: 149-151.

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Esterase-5 gene sequences from the *Drosophila buzzatii* cluster species.

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Introduction

The autosomal gene Esterase-5 (Est-5) was described as a possible member of the carboxyl/cholinesterases gene family (Cygler *et al.,* 1992; Hemilä *et al.,* 1994; Oakeshott *et al.,* 1999, 2005). The members of this gene family have high evolutionary rates and low amino acid identity probably due to their diversification in DNA sequence and biochemical function, relative to the ecological and behavioral changes of the hosts species (Oakeshott *et al.,* 1999, 2005). In *Drosophila spp t*wo groups of carboxyl/cholinesterases were described: α-esterases and β-esterases, associated to the hydrolyse of α and β-naftil-acetate, respectively. The α-esterase group was characterized from *Drosophila melanogaster* (Robin *et al.,* 2000). In *D. buzzatii* species the Esterase-5 gene encodes possibly the Esterase-2 protein that is homologous to Esterase-9 from *D. melanogaster* (Oakeshott *et al.,* 1993; East *et al.,* 1990). That protein is expressed in digestive trait and Malpighi tubes in *D. buzzatii*, indicating a possible relationship with alimentary behavior (East *et al.,* 1982); however, the true function is still unknown (East *et al.,* 1990).

The *D. buzzatii* cluster (*D. repleta* group: *D.buzzatii* complex) is a monophyletic group (Ruiz *et al.,* 1993; Manfrin *et al.,* 2001), composed by seven species: *D. buzzatii, D. serido, D. antonietae, D. seriema, D. gouveai, D. borborema,* and *D. koepferae*, and the main diagnostic characteristic is the aedagus morphology (Silva and Sene, 1991). They are endemic of the South American continent, with the exception of *D. buzzatii* that was introduced in other continents (Barker *et al.,* 1985). The species of this cluster are cryptic and cactophilic, whose larvae feed exclusively on decayed tissues of cacti (Pereira *et al.,* 1983). The distribution of the *D. buzzatii* cluster species ranges from Northeast region of Brazil to Chaco Domain in Argentina and is associated with different species of cacti (Manfrin and Sene, 2006). The cladogenetic events in this cluster are associated to pre-Quaternary vicariant events (Manfrin *et al.,* 2001; De Brito *et al.,* 2002), and the population structure and diversification are associated to the retraction and expansion events of the dry vegetation (caatinga and savanna like vegetations) and moist vegetation (rainforest like vegetations) of the South America, during the paleoclimatic changes, as the Quaternary glaciations (Sene *et al.,* 1988).

Historical events, ecological interactions, and genetic characteristics of the species are fundamental to understanding the current patterns of distribution, species diversification, and population variation. For the *Drosophila buzzatii* cluster a lot of historical information is available; however, little information exists regarding the genes associated with ecological interactions, such as the adaptation to the use of decaying tissues of cactus to feed their larvae. Thus, this study aimed to evaluate information in sequences of the Esterase-5 gene in species of the *D. buzzatii* cluster for future works.

Material and Methods

We extracted DNA from 18 individuals of natural populations of the species of the *D. buzzatii* cluster: *D. buzzatii* (2 individuals), *D. koepferae* (3 individuals), *D. borborema* (1 individual), *D.*

serido (3 individuals), *D. seriema* (3 individuals), *D. antonietae* (3 individuals), and *D. gouveae* (3 individuals). For DNA extraction, we used the Puregene kit (GENTRA Systems). We performed the PCR reaction using three sets of primers (Table 1), designed from *D. borborema* sequence from GeneBank (DQ453800) (Figure 1). The PCR reaction conditions were: 1 µl DNA template, 5 µl Buffer, Magnesium Chloride free, 3 µl Magnesium Chloride (2mM/ µl), 4 µl dNTP (4 pmol/µl), 2 µl of each primer (5 pmol/ µl), 0.2 µl Taq polymerase (5 U/ µl) and 32.8 µl of water in a final volume of 50 µl per reaction. The thermocycler program used was one cycle of 94°C, 4 minutes; 35 cycles of 94°C, 40 seconds, 53°C, 40 seconds, 72°C, one minute, and 72°C 3 minutes, and a final extension of 4°C without limit of time. The sequencing reaction was performed directly from the PCR fragments following the protocol in Piccinali *et al.* (2007). The sequences were checked by eye using the program Chromas lite v 2.0 (Free Software from: http://www.technelysium.com.au/chromas_lite.html, access: 18/12/2009) and aligned with Piccinali *et al.* (2007) sequences and *D. virilis* (CH940652) and *D. mojavensis* (CH933806) from Gene Bank using Bioedit program v. 7.0.9.0 (Hall, 1999). To check for possible phylogenetic information, we performed a Maximum Parsimony test (10000 replications) in PAUP v 4.0 program (Swofford, 2001).

Table 1. Characteristics of the primers used to amplify the fragments of Esterase-5 gene.

Name of the primer	Sequence	Size (bp)	Molecular Weight	Annealing temperature °C
Ester-2F	TGG ACT GAA GGA CCA GGT TT	20	6197.1	57.3
Ester-2R	CTT GTC GTA CTC GGG CAG AT	20	6124.1	59.4
Ester-CH1 F	GGG CAC CTT GCA CAA CTA TT	20	6058.0	57.3
Ester-CH1R	AGC CAT GCC AGA AGA TCC TA	20	6076.0	57.3
Ester-CH2F	ATA TG TGT GCA CGG AGC AG	20	6187.0	57.3
Ester-CH3R	CTT CAT CTT CTT GCT GGA CT	20	6006.0	55.3

Results and Discussion

We tested all sets of primers (Table 1) and the combinations of these primers to produce DNA sequences that cover at least the exon 4 and 5 from the Esterase-5 gene. In the first test we used the set of primers Ester-2F and Ester-2R (Figure 1) that amplified a DNA fragment with approximately 1158 bp in all seven species. The set Ester-2F and Ester-2R cover a big portion of the exon 4 and 5 from the Esterase-5 gene. However, the sequences from the species *D. antonietae* and *D. gouveae* showed low quality of the sequences and some samples of the species *D. seriema, D. antonietae, D. gouveae, D. koepferae,* and *D. buzzatii* do not show PCR products. A second test was performed to produce an internal fragment with a better quality of the sequences. We used the set Ester-2F and Ester-CH1R that showed amplified products of 781 bp (Figure 1). However, these products showed low concentration of the PCR products in the species *D. antonietae, D. gouveae,* and *D. buzzatii,* and we made a re-amplification to produce PCR products in high concentration to perform the DNA sequence reaction. The re-amplified products do not show ghost bands, and the concentration was enough to perform sequencing reactions in all species. In a third test we used the set Ester-CH2F and Ester-2R to cover the final portion of the Esterase-5 gene and the exon 5. This set of primers produced fragments with 1039 bp, but can only be obtained from the species *D. borborema, D. seriema, D. koepferae,* and *D. buzzatii.* Even performing re-amplification and changing the PCR conditions, DNA sequences from the species *D. antonietae* and *D. gouveae* could not be obtained. The other combinations of the primers (Ester-2F and Ester-CH3R, Ester-CH2F and Ester-CH1R,

Ester-CH2F and Ester-CH3R, Ester-CH1F and Ester-2R, Ester-CH1F and Ester-CH1R, Ester-CH1F and Ester-CH3R) did not produce DNA fragments even with modifications in the PCR conditions. The consensus sequence sizes varied from 614 bp in the *D. antonietae,* 740 bp from *D. gouveae,* 888 bp from *D. borborema,* 1032 bp from *D. serido*, 1062 bp from *D. kopferae,* 1085 bp from *D. seriema,* and 1091 from *D. buzzatii* (see Appendix 1).

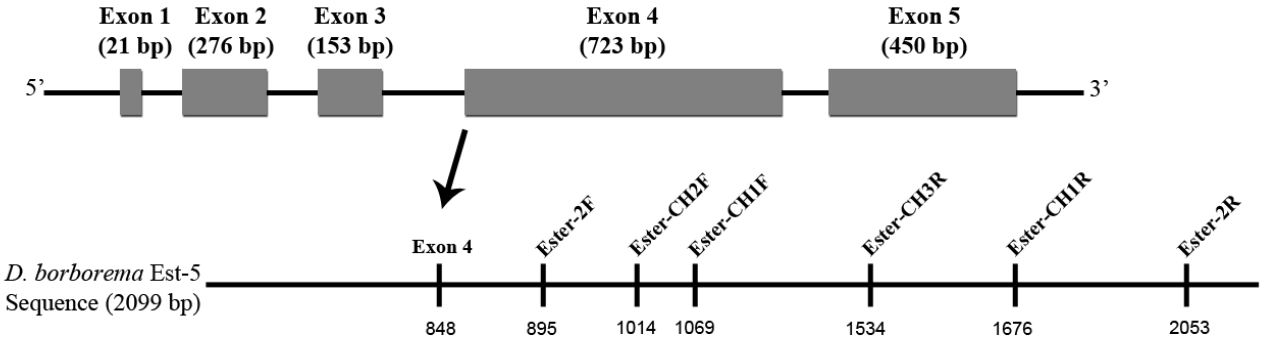

Figure 1. Location of the primers, based on the *D. borborema* sequence from GeneBank, used in this work. In the top of the figure, representation of Esterase-5 gene and exons positions. In the low position of the figure, primer location, the numbers represent the position of the primers in base pairs, related of the *D. borborema* from GeneBank.

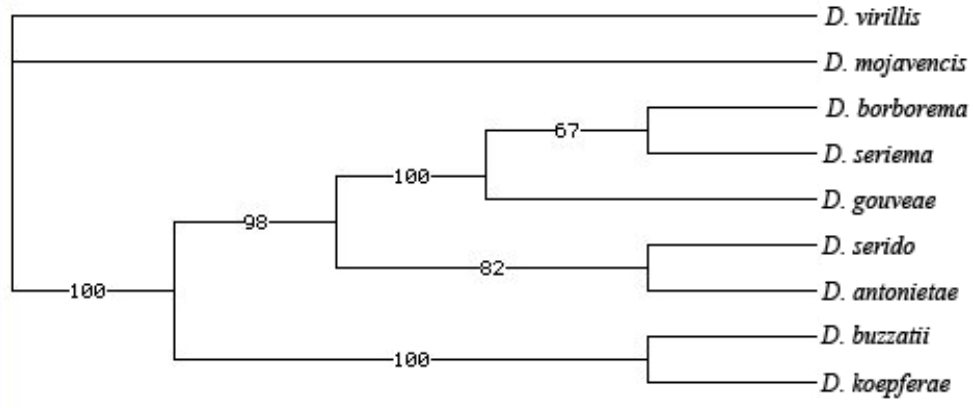

Figure 2. Maximum Parsimony tree based on the consensus sequence of the species used in this work. *D. virilis* and *D. mojavencis* was used as a outgroup. Values of bootstrap calculated based on 10000 replications.

The Maximum Parsimony analyses showed that the species of the *D. buzzatii* form a clade (booststrap values of 100%), separated from the external species *D. virillis* and *D. mojavencis* (Figure 2). The species in the cluster formed two clades. One encompassed *D. buzzatii* and *D. koepferae* (bootstrap values of 100%). The second one encompassed the other five species with bootstrap values of 98%. This second clade is divided in two groups: one with *D. serido* and *D. antonietae* species (bootstrap values of 82%) and the second group with the species *D. borborema*, *D. seriema,* and *D. gouveae* (bootstrap values of 100%). This phylogenetic hypothesis using the Esterase-5 gene

information is very similar with the ones elaborated using mitochondrial DNA (Manfrin *et al.,* 2001) and chromosomal inversions (Ruiz and Wasserman, 1993; Ruiz *et al.,* 1996). The similarity between the phylogenies obtained with the Esterase-5 gene and the other phylogenies (Manfrin *et al.,* 2001; Ruiz and Wasserman, 1993; Ruiz *et al.,* 1996) seems to be related to the historical relationship with the species of the *D. buzzatii* cluster; even the Esterase-5 showed many indications that it is a gene related to adaptative functions (East *et al.,* 1982). In this work we generated sequences from the Esterase-5 gene from the seven *D. buzzatii* cluster species, and it is a first step to perform future work. The genetic sequences are the base to develop new specific primer sets to be used in molecular systematics, phylogeography, adaptation, and ecological works and more.

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References: Barker, J.S.F., F.M. Sene, P.D. East, and M.A.Q.R. Pereira 1985, Genética 67: 161-170; Cygler, M., J. Schrag, *et al*. 1992, Biotechnology & Genetic Engineering Reviews 10: 143-184; De Brito, R.A., M.H. Manfrin, and F.M. Sene 2002, Genetics and Molecular Biology 25(2): 161-171; East, P., 1982, Ecological Genetics and Evolution: 323–338; East, P., A. Graham, and G. Whitington 1990, Ecological and Evolutionary Genetics of *Drosophila*: 389–406; Hall, T., 1999, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT; Hemilä, H., T.T. Koivula, and L. Palva 1994, Biochimica et Biophysica Acta 1210(2): 249; Manfrin, M.H., R.A. De Brito, and F.M. Sene 2001, Annals of the Entomological Society of America 94(3): 333-346; Manfrin, M.H., and F.M. Sene 2006, Genetica 126(1-2): 57-75; Oakeshott, J.G., C. Claudianos, P.M. Campbell, R.D. Newcomb, and R.J. Russell 2005, Comprehensive Molecular Insect Science 5: 309–381; Oakeshott, J.G., C. Claudianos, R.J. Russell, and G.C. Robin 1999, Bioessays 21(12): 1031-1042; Oakeshott, J.G., E.A. Van Papenrecht, T.M. Boyce, M.J. Healy, and R.J. Russell 1993, Genética 90(2): 239-268; Pereira, M.A.Q.R., C.R. Vilela, and F.M. Sene 1983, Ciência e Cultura 35: 1313-1319; Piccinali, R.V., L.J. Mascord, J.S.F. Barker, J.G. Oakeshott, and E. Hasson 2007, Journal of Molecular Evolution 64(2): 158-170; Robin, G.C.Q., C. Claudianos, R.J. Russell, and J.G. Oakeshott 2000, Journal of Molecular Evolution 51(2): 149-160; Ruiz, A., A.M. Cansian, M.R. Alvez, and F.M. Sene 1996, Revista Brasileira de Genética 19: 269; Ruiz, A., and M. Wasserman 1993, Heredity 70: 582-596; Sene, F.M., M.A.Q.R. Pereira, and C.R. Vilela 1988, Pacific Science 42(1-2): 81-88; Silva, A.F.G., and F.M. Sene 1991, Revista Brasileira de Entomologia 35: 455-468; Swofford, D., 2001, PAUP* 4.0 beta 5: Phylogenetic Analysis Using Parsimony (and other methods), Sinauer Associates Sunderland, MA.

Appendix 1. Sequences aligned by the program Bioedit v 7.0.9.0 (Hall, 1999) using Blossun 62. * DNA Sequence of *D. borborema* from GeneBank (DQ453800). ** DNA Sequence from Robin (2000).

```
                    10        20        30        40        50
                ....|....|....|....|....|....|....|....|....|....|
D. bor *        ATGTCAGAAAGGTGTTTGTTTTCAATAAAAACATACATTTAAATTCCATT
D. bor          --------------------------------------------------
D. buzz **      --------------------------------------------------
D. buzz         --------------------------------------------------
D. koep         --------------------------------------------------
D. anto         --------------------------------------------------
D. gouv         --------------------------------------------------
D. serido       --------------------------------------------------
D. serie        --------------------------------------------------
```

```
                  60        70        80        90       100
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    ATCATAAATTAATGTGAATTTAATAATAATTTCATTTCATTTAGTTTGGA
D. bor      ---------------------------------------------------
D. buzz **  ---------------------------------------------------
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------

                 110       120       130       140       150
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GAGCTGTGAAATATCATTGCCCGTGGGCCAAATCCGGGGCGTTAAGCGCC
D. bor      ---------------------------------------------------
D. buzz **  ---------------------------------------------------
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------

                 160       170       180       190       200
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GCAGCCTTTACGATGATGATTACTATAGCTTCGAGCGTTTGCCCTTTGGC
D. bor      ---------------------------------------------------
D. buzz **  ---------------------------------------------------
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------

                 210       220       230       240       250
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    AAGCCGCCAGTGGGTGAACTACGCTTTAAGGCACCTGTGCCAGTCGAGCC
D. bor      ---------------------------------------------------
D. buzz **  -------------TACACTGCGCTTTAAGGCACCTGTGCCGGTTGAGCC
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------

                 260       270       280       290       300
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    CTGGTCCGGTGTGCTGGACTGCACCCACTTTGCCGAAAAGCCCGTGCAGA
D. bor      ---------------------------------------------------
D. buzz **  CTGGTCCGGTGTGCTGGACTGCACCCACTTTGCAAATAAGCCGGTGCAGA
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------
```

```
                 310       320       330       340       350
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    AGGGCTTGTTAACAGGCATCATAGAGGGCAGCGAGGACTGCCTTTATTTG
D. bor      ---------------------------------------------------
D. buzz **  AGGGCTTGCTAACGGGCATCATAGAAGGCAGCGAGGACTGCCTTTATCTG
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------

                 360       370       380       390       400
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    AATGTATACGCCAAACAGGTGACTTGCTAAAAAATAATATATCACTTTGA
D. bor      ---------------------------------------------------
D. buzz **  AATGTATACGCCAAACAGGTGACTTGCTAAAAAATAATATATCACTTTGA
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------

                 410       420       430       440       450
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    TGGATTGTTCAAATGTTCAAGTGATCTCACTGATAACAGCTGAAGAGCGC
D. bor      ---------------------------------------------------
D. buzz **  TGGATTGTTCAAATTTCCAAGTGATTTCACTGATAACAGCTGAAGAGCGC
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------

                 460       470       480       490       500
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    AAAACCCCTACCTGTTATGGTCTATATATACGGAGGCGCTTTCTCAATAG
D. bor      ---------------------------------------------------
D. buzz **  GAAACCATTACCTGTTATGGTCTATATATACGGAGGCGCTTTCTCCATAG
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------

                 510       520       530       540       550
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GCGAGGCCACTCGGGATATCTACTCGCCCGATTATTTTATGGCTAAAGAT
D. bor      ---------------------------------------------------
D. buzz **  GCGAGGCCACTCGGGAGATCTACTCGCCCGATTACTTTATGGCTAAAGAT
D. buzz     ---------------------------------------------------
D. koep     ---------------------------------------------------
D. anto     ---------------------------------------------------
D. gouv     ---------------------------------------------------
D. serido   ---------------------------------------------------
D. serie    ---------------------------------------------------
```

```
                  560       570       580       590       600
              ....|....|....|....|....|....|....|....|....|....|
D. bor *      GTGGTTCTAGTCACCCTCAACTATCGCGTTGATTGTCTAGGTAAGTGATT
D. bor        ---------------------------------------------------
D. buzz **    GTGGTTCTAGTCACCCTCAATTATCGCGTTGATTGCCTAGGTAAGTGATT
D. buzz       ---------------------------------------------------
D. koep       ---------------------------------------------------
D. anto       ---------------------------------------------------
D. gouv       ---------------------------------------------------
D. serido     ---------------------------------------------------
D. serie      ---------------------------------------------------

                  610       620       630       640       650
              ....|....|....|....|....|....|....|....|....|....|
D. bor *      GTAAACTTATCGTTTGATTATCTCTTAAATAAGTTAGTTATATTAGTTGT
D. bor        ---------------------------------------------------
D. buzz **    GTAAACTTATCGTTTGATTATCTCTTAAAGAAGTTTGTTATATTAGTTGT
D. buzz       ---------------------------------------------------
D. koep       ---------------------------------------------------
D. anto       ---------------------------------------------------
D. gouv       ---------------------------------------------------
D. serido     ---------------------------------------------------
D. serie      ---------------------------------------------------

                  660       670       680       690       700
              ....|....|....|....|....|....|....|....|....|....|
D. bor *      TCAGCTAGTTTCTCTTATCAATTTGGGTATAAAGTTCGGCCTAGCTTTAA
D. bor        ---------------------------------------------------
D. buzz **    TTAGCTAGTTTCTCTTATCAATGTGGGTATAAAGTTCCGGCTAGCTTTAA
D. buzz       ---------------------------------------------------
D. koep       ---------------------------------------------------
D. anto       ---------------------------------------------------
D. gouv       ---------------------------------------------------
D. serido     ---------------------------------------------------
D. serie      ---------------------------------------------------

                  710       720       730       740       750
              ....|....|....|....|....|....|....|....|....|....|
D. bor *      ATTATTTTTT-TGGTAAAAGCTTTTTCTCTTGCTCCCCTAGCTAAGCTTT
D. bor        ---------------------------------------------------
D. buzz **    GTTATTTTTTATGGCAAAAGCTTTTTCTCTTGCTCCCCTAGCTAAGCTTT
D. buzz       ---------------------------------------------------
D. koep       ---------------------------------------------------
D. anto       ---------------------------------------------------
D. gouv       ---------------------------------------------------
D. serido     ---------------------------------------------------
D. serie      ---------------------------------------------------

                  760       770       780       790       800
              ....|....|....|....|....|....|....|....|....|....|
D. bor *      CGCACTCAGCGTACATTCGAACTTGCTATTTTCACTCGTTTTCTCTCTCT
D. bor        ---------------------------------------------------
D. buzz **    CGCACTCAGCGTACATTCGGACTTGCTGTTTTCACTCTCTCTCTCTCTCT
D. buzz       ---------------------------------------------------
D. koep       ---------------------------------------------------
D. anto       ---------------------------------------------------
D. gouv       ---------------------------------------------------
D. serido     ---------------------------------------------------
D. serie      ---------------------------------------------------
```

```
                 810       820       830       840       850
               ....|....|....|....|....|....|....|....|....|....|
D. bor *       CTCTCTTTCTCTTTTCTCTTTTCTTATTTCCTTTTTCTT-----TTTCTT
D. bor         ---------------------------------------------------
D. buzz **     CTCTTTCACCGTTTCCCCCTCTTTCTTTCTCCTTTTATTCCATTTCTCTT
D. buzz        ---------------------------------------------------
D. koep        ---------------------------------------------------
D. anto        ---------------------------------------------------
D. gouv        ---------------------------------------------------
D. serido      ---------------------------------------------------
D. serie       ---------------------------------------------------

                 860       870       880       890       900
               ....|....|....|....|....|....|....|....|....|....|
D. bor *       TAAGGTTTCCTTAGTCTAAAAGACCCCAGTTTGGAGGTGCCCGGAAATGC
D. bor         ---------------------------------------------------
D. buzz **     TAAGGTTTCCTTAGTCTAAAAGACCCCAGCTGTGAGGTGCCGGGAAATGC
D. buzz        ---------------------------------------------------
D. koep        ---------------------------------------------------
D. anto        ---------------------------------------------------
D. gouv        ---------------------------------------------------
D. serido      ---------------------------------------------------
D. serie       ---------------------------------------------------

                 910       920       930       940       950
               ....|....|....|....|....|....|....|....|....|....|
D. bor *       TGGACTGAAGGACCAGGTTTTAGCTATCAAATGGGTAAATCAATATATTT
D. bor         ---------------------------------------------------
D. buzz **     TGGGCTAAAGGATCAGGTGTTAGCTATCAAATGGGTAAATCAATATATTT
D. buzz        ------------CAGGTTTTAGCTATCAAATGGGTAAATCAATATATTT
D. koep        ----------GACCAGGTTTTAGCTATCAAATGGGTAAATCAATATATTT
D. anto        ---------------------------------------------------
D. gouv        ---------------------------------------------------
D. serido      ---------------------------------------------------
D. serie       -----------------TTTTAGCTATCAAATGGGTAAATCAATATATTT

                 960       970       980       990       1000
               ....|....|....|....|....|....|....|....|....|....|
D. bor *       CCTATTTCAATGGTGATGTGAACAACATAACGGTGTTTGGTGAAAGCGCC
D. bor         ---------------------------------------------------
D. buzz **     CCTATTTCAATGGTGCTGTGAACAACATAACGGTGTTCGGTGAAAGCGCC
D. buzz        CCTATTTCAATGGTGATGTGAACAACATAACGGTGTTCGGTGAAAGCGCC
D. koep        CCTATTTCAATGGTGATGTGAACAACATAACGGTATTCGGTGAAAGCGCC
D. anto        ---------------------------------------------------
D. gouv        ---------------------------------GTTTGGTGAAAGCGCC
D. serido      -----------GGTGATGTGAAGAACATAACGGTGTTTGGTGAAAGCGCC
D. serie       CCTATTTCAATGGTGATGTGAACAACATAACGGTGTTTGGTGAAAGCGCC

                 1010      1020      1030      1040      1050
               ....|....|....|....|....|....|....|....|....|....|
D. bor *       GGCGGCTGCTCCACCCACTATATGATGTGCACGGAGCAGACGCGCGGACT
D. bor         ----------------------------ACGGAGCAGACACGTGGACT
D. buzz **     GGCGGCTGCTCCACGCACTATATGATGTGCACGGAGCAGACGCGTGGACT
D. buzz        GGCGGCTGCTCCACGCACTATATGATGTGCACGGAGCAGACGCGTGGACT
D. koep        GGCGGCTGCTCCACGCACTATATGATGTGCACGGAGCAGACGCGTGGACT
D. anto        ----------------------------ACGGAGCAGACGCGTGGACT
D. gouv        GGCGGCTGCTCCACCCACTATATGATGTGCACGGAGCAGACACGTGGACT
D. serido      GGCGGCTGTTCCACCCACTATATGATGTGCACAGAGCAGACGCGTGGACT
D. serie       GGCGGCTGCTCCACCCACTATATGATGTGCACGGAGCAGACACGTGGACT
```

```
                1060      1070      1080      1090      1100
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    CTTCCACAAGGCCATTCCCATGTCGGGCACCTTGCACAACTATTGGTCCA
D. bor      CTTCCACAAGGCCATTCCCATGTCGGGCACCTTGCACAACTATTGGTCCA
D. buzz **  CTTCCACAAGGCCATTCCTATGTCGGGCACCTTGCACAACTATTGGTCTA
D. buzz     CTTCCACAAGGCCATTCCTATGTCGGGCACCTTGCACAACTATTGGTCTA
D. koep     CTTCCACAAGGCCATTCCTATGTCGGGCACCTTGCACAACTATTGGTCTA
D. anto     CTTCCACAAGGCCATTCCCATGTCGGGCACCTTGCACAACTATTGGTCCA
D. gouv     CTTCCACAAGGCCATTCCCATGTCGGGCACCTTGCACAACTATTGGTCCA
D. serido   CTTCCACAAGGCCATTCCCATGTCGGGCACCTTGCACAATTATTGGTCCA
D. serie    CTTCCACAAGGCCATTCCCATGTCGGGCACCTTGCACAACTATTGGTCCA

                1110      1120      1130      1140      1150
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    ATACC-CCTCCCGCAGACTTTGCCTATCGACTGGCAAA-GGGGAATGGGT
D. bor      ATACG-TCGCCCGCAGACTTTGCCTATCGTCTGGCCAA-GTTGAATGGGT
D. buzz **  ATACA-CCGCCCGCAGACTTTGCCTATCGCCTGGCCAA-GTTGAATGGGT
D. buzz     ATACA-CCGCCCGCAGACTTTGCCTATCGCCTGGCCAA-GTTGAATGGGT
D. koep     ATACA-CCGCCCGCAGACTTTGCCTATCGCCTGGCCAA-GTTGAATGGGT
D. anto     ACACGGCCNNCCGCAGACTTTGCCTATCGTCTGGCCAAAGGTGAATGGGT
D. gouv     ATACC-CCGCCCGCAGACTTTGCCTATCGACTGGCCAA-GGTGAATGGGT
D. serido   ACACGGC-GCCCGCAGACTTTGCCTATCGTCTGGCCAA-GGTGAATGGGT
D. serie    ATACC-CCTCCCGCAGACTTTGCCTATCGTCTGGCCAA-GGTGAATGGGT

                1160      1170      1180      1190      1200
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    ATGAGGGC-GAAAACAATGATCGCCAGGTGCTGGACTATTTGCGCACTGT
D. bor      ATGAGGGC-GAAAACAATGATCGCCAGGTGCTGGACTATTTGCGCACTGT
D. buzz **  TTGAAGGC-GAAAACAATGATCGCCAGGTGCTGGACTATTTGCGTACTGT
D. buzz     TTGAAGGC-GAAAACAATGATCGCCAGGTGCTGGACTATTTGCGTACTGT
D. koep     TTGAGGGC-GAAAACAATGATCGCCAGGTGCTGGACTATTTGCGTACTGT
D. anto     ATNAGGGCTGAAAACAATGATCGCCAGGTGCTGNACTATTTGCGCACTGT
D. gouv     ATGAGGGC-GAAAACAATGATCGCCAGGTGCTGGACTATTTGCGCACTGT
D. serido   ATGAGGGC-GAAAACAATGATCGCCAGGTGCTGGACTATTTGCGCACTGT
D. serie    ATGAGGGC-GAAAACAATGATCGCCAGGTGCTGGACTATTTGCGCACTGT

                1210      1220      1230      1240      1250
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    CCCAGCAGAGCAGTTGGTTAACCACAGCCTGCTCACTCCT-GAGGATCGG
D. bor      GCCGGCAGAGCAGTTGGTTAACCACAGCCTGCTCACTCCTTGAGGATCGG
D. buzz **  GCCGGCAGAACAGCTGGTTAACCACAGTCTGCTCACCCCT-GAAGATCGG
D. buzz     GCCGGCAGAACAGCTGGTTAACCACAGTCTGCTCACCCCT-GAGGATCGG
D. koep     GCCGGCAGAACAGCTGGTTAACCACAGTCTGCTCACCCCT-GAGGATCGG
D. anto     GCCGGCAGAGCAGCTGGTTAGCCACACCCTGCTCACCCCT-GAGGATCGG
D. gouv     ACCAGCAGAGCAGCTGGTTAACCACAGCCTGCTCACACCT-GAGGATCGG
D. serido   GCCGGCAGAGCAGCTGGTTAGCCACAGCCTGCTCACCCCT-GAGGATCGG
D. serie    GCCGGCAGAGCAGTTGGTTAACCACAGCCTGCTCACCCCT-GAGGATCGG

                1260      1270      1280      1290      1300
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    CGCACCGGACTTATTTATGCCTTTGGCCCCACCGGGGAGCCATACGTGAT
D. bor      CGCAACGGACTTATCTATGCCTTTGGCCCCACCGTGGAGCCATACGTGAT
D. buzz **  CGCAACGGACTCATCTATGCCTTTGGCCCCACCGTGGAACCATACGTGAT
D. buzz     CGCAACGGACTCATCTATGCCTTTGGCCCCACCGTGGAGCCATACGTGAT
D. koep     CGCAACGGACTCATCTATGCCTTTGGCCCCACCGTGGAGCCATACGTGAT
D. anto     CGNAACGGACTCATCTATGCNTTTGGCCCCACCGTGGAGCCATACGTGAT
D. gouv     CGCAACGGACTTATCTATGCCTTTGGCCCCACCGTGGAGCCATACGTGAT
D. serido   CGCAACGGACTCATCTATGCCTTTGGCCCCACCGTGGAGCCATACGTGAT
D. serie    CGCAACGGACTTATCTATGCCTTTGGCCCCACCGTGGAGCCATACGTGAT
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                1310      1320      1330      1340      1350
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GGTAGACTGTGTGGCTCCAAAGCCACAGTTGGAAATGGTGCGCAATGCTT
D. bor      GGTAGACTGTGTGGCTCCAAAGCCACAGCTGGAGATGGTGCGCGATGCTT
D. buzz **  GACAGACTGTGTTACTCCAAAGCCACAGCTGGAGATGGTGCGCAAAGCTT
D. buzz     GGCAGACTGTGTAGCTCCAAAGCCACAGCTGGAGATGGTGCGCGAAGCTT
D. koep     GGCAGACTGTGTAGCTCCAAAGCCACAGCTGGAGATGGTGCGCGAAGCTT
D. anto     GGTAGACTGTGTGGCTCCAAAGCCACAGCTGGAGATGGTGCGCGATGCTT
D. gouv     GGTAGACTGTGTGGCTCCAAAACCACAGCTGGAGATGGTGCGCGATGCTT
D. serido   GGTAGACTGTGTGGCTCCAAAGCCACAGCTAGAGATGGTGCGCGATGCTT
D. serie    GATAGACTGTGTGGCTCCAAAGCCACAGCTGGAGATGGTGCGCGATGCTT

                1360      1370      1380      1390      1400
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GGAGCAATAAGTTACCAGCCATGCTGGGCGGCACCTCTTTCGAAGGTCTC
D. bor      GGAGCAATAAGTTACCAGTCATGCTGGGCGGCACCTCTTTCGAAGGTCTC
D. buzz **  GGAGCAATAAGTTACCAGTCATGCTGGGCGGTACTTCTTTCGAAGGTCTC
D. buzz     GGAGCAATAAGTTACCAGTCATGCTGGGCGGTACCTCTTTCGAAGGTCTC
D. koep     GGAGCAATAAGTTACCAGTCATGCTGGGCGGTACCTCTTTCGAAGGTCTC
D. anto     GGAGCAATAAGTTACCAGTCATGCTGGGCGGTACCTCTTTCGAAGGTCTC
D. gouv     GGAGCAATAAGTTACCAGCCATGCTGGGCGGCACCTCTTTCGAAGGTCTC
D. serido   GGAGCAATAAGTTACCAGTCATGCTGGGCGGTACCTCTTTCGAAGGTCTC
D. serie    GGAGCAATAAGTTACCAGTCATGCTGGGCGGCACCTCTTTCGAAGGTCTC

                1410      1420      1430      1440      1450
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    TTCATGTATCCCGCCTTGAAGGCTAATCCTAAGGGCATGGATAGCCTGCC
D. bor      TTCATGTATCCCGCCTTAAAGGCCAATCCTAAGGGCATGGATAGCCTGCC
D. buzz **  TTCATGTATCCCGCCTTGAAGGCCAATCCTAAGGGCATGGATAGCCTGCC
D. buzz     TTCATGTATCCCGCCTTGAAGGCCAATCCTAAGAGCATGGATAGCCTGCC
D. koep     TTCATGTATCCCGCCTTGAAGGCCAATCCTAAGGGCATGGATAGCCTGCC
D. anto     TTCATGTATCCCGCCTTGAAGGCCAATCCTAAGGGCATGGATAGCCTGCC
D. gouv     TTCATGTATCCCGCCTTAAAGGCCAATCCTAAGGGCATGGATAGCCTGCC
D. serido   TTCATGTATCCCGCCTTGAAGGCCAATGCTAAGGGCATGGATAGCCTGCC
D. serie    TTCATGTATCCCGCCTTAAAGGCCAATCCTAAGGGCATGGATAGCCTGCC

                1460      1470      1480      1490      1500
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GCAGGATCTGCTGCGATTGACGCCTTATGAAGTGCGTGTGCTCAATACGG
D. bor      GCAGGATCTGTTGCGATTGACGCCTTATGAAGTGCGTGTGCTCAATACGG
D. buzz **  GCAGGATCTGTTGCGATTGACGCCCCATGAAGTGCGTGTGCTCAATACGG
D. buzz     GCAGGATCTCTTGCGATTGACGCCCCATGAAGTGCGTGTGCTCAATACGG
D. koep     GCAGGATCTGTTGCGATTGACGCCCCATGAAGTGCGTGTGCTCAATACGG
D. anto     GCAGGATCTGTTGCGATTAACGCCCTATGAAGTGCGTGTGCTCAATAAGG
D. gouv     GCAGGATCTGTTGCGATTGACGCCTTATGAAGTGCGTGTGCTCAATACGG
D. serido   GCAGGATCTGTTGCGATTGACGCCCCATGAAGTGCGTGTGCTCAATACGG
D. serie    GCAGGATCTGTTGCGATTGACGCCTTATGAAGTGCGTGTGCTCAATACGG

                1510      1520      1530      1540      1550
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    AGCAACAGAATTTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
D. bor      AGCAACAGAATCTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
D. buzz **  AGCAACAGAATTTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
D. buzz     AGCAACAGAATTTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
D. koep     AGCAACAGAATTTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
D. anto     AACAACAGAATCTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
D. gouv     AGCAACAGAATCTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
D. serido   AACAACAGAATCTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
D. serie    AGCAACAGAATCTAGAGTCCAGCAAGAAGATGAAGCAATTGTACTTTGGC
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                1560      1570      1580      1590      1600
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GATGACACGCCCAGCTCCAAGCTCATCATGAACTTCATGGACGTGAGTT-
D. bor      GATGCCACGCCCAGCTCCAAGTTCATCATGAACTTCATGGACGTGAGCTG
D. buzz **  GATGCTACACCCAGCTCCAAGCTCATCATGAACTTCATGGACGTGAGCT-
D. buzz     GATGCTACACCCAGCTCCAAGCTCATCATGAACTTCATGGACGTGAGCT-
D. koep     GATGCTACACCCAGCTCCAAGCTCATCATGAACTTCATGGACGTGAGCT-
D. anto     GATGCCACGCCCAGCTCCAAGCTCATCATGAACTTTATGGACGNNNNTN-
D. gouv     GATGACACGCCCAGCTCCAAGCTCATCATGAACTTCATGGACGTGAGCT-
D. serido   GATGCCACGCCCAGCTCCAAGCTCATCACGAACTTCATGGACGTGAGTT-
D. serie    GATGCCACCCCCAGCTCCAAGTTCATCATGAACTTCATGGACGTGAGTT-

                1610      1620      1630      1640      1650
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    CGAATCACTTAAGTTTAACTATATAAC--TACTCTA-ATTCTTTAT----
D. bor      CGAATCACTTAAGTTTAACTATATAACTGTACTCTA-ATTCTTTAT----
D. buzz **  CGAATCACTTAAGTTTAACTATATAAC--TACTCTA-ATTCTTTGT----
D. buzz     CGAATCACTTAAGTTTAACTATATAAC--TACTCTA-ATTCTTTGT----
D. koep     CGAATCACTTAAGTTTAACTATATAAC--TACTCTA-ATTCTTTGT----
D. anto     NNNATCACTTAAGTTTAATTATATACT-GTACTCTATATACTGTACT---
D. gouv     CGAATCACTTAAGTTTAACTATATAACTGTACTCTA-ATTCTTTAT----
D. serido   CGAATCACTTAAGTTTAACTATATATT-GTACTCTATATACTGTACTCTA
D. serie    CGAATCACT----TTTAACTATATAACTGTACTCTA-ATTCTTTAT----

                1660      1670      1680      1690      1700
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    ---------ATTTCTACTTTTAGTACTATTCCTATAGGATCTTCTGGCAT
D. bor      ---------ATTTCTACTTTTAGTACTATTCTTATAGGATCTTCTGGCAT
D. buzz **  ---------ATTTCTACTTTTAGTACTATTCCTATAGGATCTTCTGGCAT
D. buzz     ---------ATTTCTACTTTTAGTACTATTCCTATAGGATCTTCTGGCAT
D. koep     ---------ATTTCTACTTTTAGTACTATTCCTATAGGATCTTCTGGCAT
D. anto     --------------------------------------------------
D. gouv     ---------ATTTCTACTTTTAGTACTATTCTTATAGGATCTTCTGGCAT
D. serido   ATTCTTTATATTTCTACTTTTAGTACTATTCCTATAGGATTTTCTGGCAT
D. serie    ---------ATTTCTACTTTTAGTACTATTCTTATAGGATCTTCTGGCAT

                1710      1720      1730      1740      1750
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GGCTTCCATCGCACTTTGCAAGCTCGCCTGGCATACGCCACGGCCCCCAC
D. bor      GGCTTCCATCGCACTTTGCAAGCTCGCCTGGCATACGCCACGGCCCCCAC
D. buzz **  GGCTTCCATCGCACTTTGCAAGCTCGCTTGGCATACGCCACGGCTCCCAC
D. buzz     GGCTTCCATCGCACTTTGCAAGCTCGCTTGGCATACGCCACGGCTCCCAC
D. koep     GGCTTCCATCGCACTTTGCAAGCTCGCTTGGCATACGCCACGGCTCCCAC
D. anto     --------------------------------------------------
D. gouv     GGCTTCCATCGCACTTTGCAAGCTCGCCTGGCATACGCCACGG-------
D. serido   GGCTTCCATCGTACTTTGCAAGCTCGCCTGGCATACGCCACGGCTCCCAC
D. serie    GGCTTCCATCGCACTTTGCAAGCTCGCCTGGCATACGCCACGGCTCCCAC

                1760      1770      1780      1790      1800
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    TTACTTCTATCGCTTTGATTTTGATTCACCTGGTTTCAATTTCTACCGTA
D. bor      TTACTTCTATCGCTTTGATTTTGATTCACCTGGTTTCAATTTCTACCGTA
D. buzz **  ATACTTCTATCGCTTTGATTTTGATTCACCTGATTTCAATTTCTACCGTA
D. buzz     ATACTTCTATCGCTTTGATTTTGATTCACCTGATTTCAATTTCTACCGTA
D. koep     ATACTTCTATCGCTTTGATTTTGATTCACCTGATTTCAATTTCTACCGTA
D. anto     --------------------------------------------------
D. gouv     --------------------------------------------------
D. serido   TTACTTCTATCGCTTTGATTTTGATTCACCTGATTTCAATTTCTTCCGTA
D. serie    TTACTTCTATCGCTTTGATTTTGATTCACCTGATTTCAATTTCTACCGTA
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                1810      1820      1830      1840      1850
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    AGAAATTCTG-TGGGGATGACATCAAGCAGGGCGTTGCCCATGCCGATGA
D. bor      AGAAATTCTG-TGGGGATGACATCAAGCAGGGCGTTGCCCATGCCGATGA
D. buzz **  AAAAATTCTG-TGGCGATGACATCAAGCAGGGCGTGGCCCATGCCGATGA
D. buzz     AAAAATTCTG-TGGCGATGACATCAAGCAGGGCGTTGCCCATGCCGATGA
D. koep     AAAAATTCTG-TGGCGATGACATCAAGCAGGGCGTTGCCCATGCCGATGA
D. anto     --------------------------------------------------
D. gouv     --------------------------------------------------
D. serido   AGAAATTCTGCTGGCGATGACATTAAGCAGGGCGTTGCCCATGCCGATGA
D. serie    AGAAATTCTG-TGGGGATGACATCAAGCAGGGCGTTGCCCATGCCGATGA

                1860      1870      1880      1890      1900
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GTTGAGCTATTTGTTCCGCAACTCGGAGTCGTGGAAACTGGACAAAGCAT
D. bor      GTTGAGCTATTTGTTCCGCAACTCGGAGTCGTGGAAACTGGACAAAGCAT
D. buzz **  GTTGAGCTATTTGTTCCGTAACTCGGAGTCGTGGAAACTGGACAAAGCCT
D. buzz     GTTGAGCTATTTGTTCCGTAACTCGGAGTCGTGGAAACTGGACAAAGCCT
D. koep     GTTGAGCTATTTGTTCCGTAACTCGGAGTCGTGGAAACTGGACAAAGCCT
D. anto     --------------------------------------------------
D. gouv     --------------------------------------------------
D. serido   GTTGAGCTATTTGTTCCGCAACTCGGAGTCGTGGAAACTAGACAAAGCCT
D. serie    GTTGAGCTATTTGTTCCGCAACTCGGAGTCGTGGAAACTGGACAAAGCCT

                1910      1920      1930      1940      1950
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    CCGCTGAATATCGCACCATTCAACGGCTGGTCGACATTTGGACATCCTTT
D. bor      CCGCTGAATATCGCACCATTCAACGGCTGGTCGACA--------------
D. buzz **  CTGCTGAATATCGCACCATTCAACGGCTGGTCGACATTTGGACATCCTTT
D. buzz     CTGCTGAATATCGCACCATTCAACGGCTGGTCGACATTTGGACATCCTTT
D. koep     CTGCTGAATATCGCACCATTCAACGGCTGGTCGACATTTGGACATCCTTT
D. anto     --------------------------------------------------
D. gouv     --------------------------------------------------
D. serido   CCGCTGAATATCGCACCATTCAACGGCTGGTCGACATTTGGACATCCTTT
D. serie    CCGATGAATATCGCACCATTCAACGTCTGGTCGACATTTGGACATCCTTT

                1960      1970      1980      1990      2000
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GCAGCTACTTCCAATCCCAACTGCGCTGAGACAGCGCATCTCGATTGGCA
D. bor      --------------------------------------------------
D. buzz **  GCAGCTACTTCCAATCCCAACTGCGCT-----------------------
D. buzz     GCAGCTACTTCCAATCCCAACTGCGCTGAGACTGCGCATCTCGATTGGCA
D. koep     GCAGCTACTTCCAATCCCAACTGCGCTGAGACTGCGCATCTCGA------
D. anto     --------------------------------------------------
D. gouv     --------------------------------------------------
D. serido   GCAGCTACTTCCAATCCCAACTGCGCTGAGACAGCGCATCTCGATTGGC-
D. serie    GCAGCTACTTCCAATCCCAACTGCGCTGAGACAGCGCATCTCGATTGGCA

                2010      2020      2030      2040      2050
            ....|....|....|....|....|....|....|....|....|....|
D. bor *    GCCAGCTAAGCAGGAGCAGCCTCAGCGTGTGCTCAACATTGGCAAAGAGG
D. bor      --------------------------------------------------
D. buzz **  --------------------------------------------------
D. buzz     GCCAGCGAAGCAGGAGCAGCCTCAGC-----------------------
D. koep     --------------------------------------------------
D. anto     --------------------------------------------------
D. gouv     --------------------------------------------------
D. serido   --------------------------------------------------
D. serie    GCCAGCTAAGCAGGAGCAGCCTCAGC-----------------------
```

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                2060      2070      2080      2090      2100
           ....|....|....|....|....|....|....|....|....|....|
D. bor *   TGGAGCTCATCGATCTGCCCGAGTACGACAAGCTGCTCGTCTGGGATAGT
D. bor     --------------------------------------------------
D. buzz ** --------------------------------------------------
D. buzz    --------------------------------------------------
D. koep    --------------------------------------------------
D. anto    --------------------------------------------------
D. gouv    --------------------------------------------------
D. serido  --------------------------------------------------
D. serie   --------------------------------------------------

                2110      2120
           ....|....|....|....|....|..
D. bor *   CTATATAAGAAGGAACACTTATTTTAG
D. bor     ---------------------------
D. buzz ** ---------------------------
D. buzz    ---------------------------
D. koep    ---------------------------
D. anto    ---------------------------
D. gouv    ---------------------------
D. serido  ---------------------------
D. serie   ---------------------------
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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)-325-4821; 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. 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.

Toxic effect of sevin on *Drosophila melanogaster*.

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Abstract

The toxicity of Sevin, a systematic carbamate pesticide, has been evaluated in the *Drosophila melanogaster* by larval feeding method. Eggs of same age (± 4 hours) were cultured on the Sevin containing media for their rest of life. Toxicity of Sevin was analyzed using parameters like rate of development, viability, morphology, and fecundity.

Key words: Sevin, Rate of development, Viability, Morphology, Fecundity

Introduction

The man-made chemicals are deliberately added to the environment as they are being widely used to manage several categories of pests. Unfortunately, most of these are not strictly selective and are also toxic to non-target species, which includes man himself (Davies *et al.*, 1975; McEwen and Stephenson, 1979). The impact in non-targets may lead to toxic effect and mutations that ultimately influence the progeny by heredity. Carbaryl, a carbamate pesticide, causes behavioral and neurological (Sideroff and Santolucito, 1972; Dsi, 1974; Anger and Setzer, 1979; Branch and Jacqz, 1986), reproductive, and carcinogenic problems (Cantor, 1992; and Davis, 1993) in exposed systems. Doull (1975) stated that "It is essential that the formulated forms as well as their subsequent changed formulations be tested. Hence, the present work was done on *Drosophila melanogaster* using carbamate pesticide so as to assess toxicity results.

Materials and Method

Most widely used Carbaryl, that is Sevin (CAS No.63-25-2), was tested. The standard Oregon-K (O.K.) strain of *Drosophila melanogaster* has been used in the present studies. All the experiments were maintained at 25 ± 1°C and RH 60%. The same aged eggs (± 4 hours) were obtained by Delcour (1969) method after crossing males with the virgin females. The same eggs at a density of 50 eggs/vial were exposed to different doses (2.5, 5.0, 7.5, 10.0, 12.5 and 15 mg/100ml of medium) of Sevin separately for their rest of their life as most of the insects are exposed to pesticides during their larval stages in which stage extensive feeding is seen (Vogel, 1977). Five hundred eggs were allocated to each dose of Sevin, and toxicity on fruit fly was analyzed using parameters like rate of development, viability, morphology, and fecundity. The results of rate of development and morphological traits were expressed by Mean ± S.E. A simple t-test was used to know the significance between control and treated groups. Similarly, ANOVA was used to calculate the statistical significance of treated series to control with respect to morphological characters and fecundity.

Results

The concentrations above 17 mg of Sevin were found to be too toxic. Most of the eggs did not hatch and larvae die soon after hatching. Hence, concentrations below 17 mg ranging from 2.5 mg to 15.0 mg were selected to know their toxic influence on *Drosophila*.

The mean developmental time of *Drosophila* in different concentrations of Sevin is shown in Table 1. It varied from 10.06 ± 0.23 days of control to 25.33 ± 0.27 days in 15.0 mg of Sevin food media. As the amount of chemical in food is increased, there is a delay in the developmental time. However, the concentrations 2.5 mg and 5.0 mg are ineffective to bring significant changes. But, the remaining concentrations (7.5, 10.0, 12.5 and 15.0 mg) produced a delay, which is statistically significant to control.

Table 1. Effect of Sevin on rate of development of *Drosophila melanogaster*.

Concentration (mg/100ml)	Rate of development (Days)
Control	10.06 ± 0.23
2.5	10.43 ± 0.37
5.0	11.65 ± 0.61
7.5	12.31 ± 0.25*
10.0	16.23 ± 0.44*
12.5	20.14 ± 0.53*
15.0	25.33 ± 0.27*

Note: All values are Mean ± S.E
*Control versus treated significant at 5% level by simple t-test.

Table 2. Effect of Sevin on Viability of *Drosophila melanogaster.*

Concentration (mg/100ml)	Viability (Percentage)
Control	93.01
2.5	90.54
5.0	76.23*
7.5	70.19*
10.0	67.45*
12.5	45.32*
15.0	20.54*

*Significant to control ($p<0.05$) by ANOVA.

Survival value as a measure of lethality is expressed in Table 2. The viability has been reduced from 93.01% (control) to lowest of 20.54% (15.0 mg). Except for 2.5 mg of Sevin, other concentrations significantly affected the viability of *Drosophila*.

Table 3. Effect of Sevin on the morphology of *Drosophila melanogaster*.

Concentration (mg/100ml)	Pupa length (mm)	Body length of fly (mm)	Wing length (mm)
Control	2.96 ± 0.71	2.83 ± 0.85	2.09 ± 0.55
2.5	2.88 ± 0.84	2.74 ± 0.54	2.04 ± 0.64
5.0	2.45 ± 0.66*	2.31 ± 0.60*	2.02 ± 0.66
7.5	2.34 ± 0.49*	2.12 ± 0.31*	1.92 ± 0.45*
10.0	2.22 ± 0.41*	1.98 ± 0.47*	1.88 ± 0.57*
12.5	2.16 ± 0.39*	1.88 ± 0.50*	1.78 ± 0.46*
15.0	2.05 ± 0.82*	1.67 ± 0.39*	1.66 ± 0.89*

Note: All values are Mean ± S.E
*Control versus treated significant at 5% level by simple t-test.

As represented in Table 3, the morphological traits, namely pupa length, body length of fly, and wing length are directly influenced by the nature of the food. The food with 5.0 mg and above concentration of Sevin significantly altered the pupa length and body length compared to control. However, 7.5 mg and higher are effective to make significant alterations in the wing length when compared to their respective control group. From this it is clear that the effect of pesticide on morphological traits is directly dependent on the amount itself, and there is a relation between dosage and effect produced.

Table 4 depicts the result on the fecundity. Control group laid a total of 6934 eggs with a value of 45.66 eggs by single female per day. The egg laying potency has been reduced to 3861 with a value of 19.92, which is lowest and is due to the high amount of 15.0 mg Sevin. However, the 2.5 mg of Sevin is not able to produce significant changes. while all the remaining have effectively brought significant fecundity changes. Furthermore, there is also dose dependent effect on fecundity by Sevin.

Table 4. Effect of Sevin on fecundity of *Drosophila melanogaster.*

Concentration (mg/100ml)	Total fecundity	Daily egg production / female
Control	6934	45.66
2.5	6899	43.86
5.0	6245	40.12*
7.5	6093	34.68*
10.0	5120	25.43*
12.5	4665	22.59*
15.0	3861	19.92*

*Significant to control ($p<0.05$) by ANOVA.

Discussion

Toxic effect of the Sevin was analyzed by using parameter namely –rate of development, viability, morphological traits and fecundity in *Drosophila*. The present results confirmed that there is a difference between treated and control groups. The prolonged rate of development is due to chemical's effect. This is in support of Luning's (1966) view, where the lengthening of developmental time is a meaningful and best indication of a chemical's effect on somatic cells of test system. Findings of Luning (1966), Sorsa and Pfeifer (1973b), Laamanen *et al.* (1976) with different chemicals are similar. In other systems, Huckabee and Griffith (1974) and Dial (1978) are of the same opinion. Sevin has effect on the survivality of *Drosophila*. There is a linear relationship between lethality and the amount of Sevin present in the food. Same type of dose response toxic effect has also been shown by Marton (1974), Laamanen *et al.* (1976), Sorsa and Pfeifer (1773a). Morphological changes are due to the differential response of genotype with varying environmental condition like food, temperature, and density. The reduced morphological characters are the result of chemical present in food. The effects on such traits are fair indicators of somatic variation, because of chemical. Same results are noticed by Robertson (1959), Chinnici *et al.* (1976), and Lalor *et al.* (1976). Due to toxic food there is lowering of egg laying by *Drosophila*. According to Lints (1971), fecundity is extremely sensitive to environmental factors. Similar observations are made by Georghiou (1965) and Lints and Lints (1971).

From the above results and discussion, we are of the opinion that Sevin is toxic and has potency to influence the somatic changes in *Drosophila*.

References: Anger, K.W., and J.V. Setzer 1979, J. Toxicol. Environ. Health 5: 793-808; Branch, R.A., and E. Jacqz 1986, Amer. J. Med. 80: 659-664; Cantor, K.P., 1992, Cancer Res. 52: 2447-2455; Chinnici, J.P., M.A. Booker, and G.C. Llewellyn 1976, J. Invertebrate pathology 27: 255-258; Davies, J.E., S.A. Poznanski, R.F. Smith, and V.H. Freed 1975, International dynamics of pesticide poisoning. In: *Environmental Dynamics of Pesticides*. (Haque, R., and V.H. Freed, eds.). Plenum press, New York. 275-287; Davis, J.R., 1993, Arch. Environ. Contam. Toxicol. 24: 87-92; Delcour, J., 1969, Dros. Inf. Serv. 44: 133-134; Dial, A.N., 1978, Terotology 17: 83-92; Doull, J.,

1975, Factors influencing toxicology. In: *Toxicology - the Basic Science of Poisons*. (Casarett, L.J., and J. Doull, eds.). McMilan Publ. Co., New York. 133-147; Dsi, I., 1974, Toxiclol. Appl. Pharm. 27: 465-476; Georghiou, G.P., 1965, In: *Carbamate Insecticides: Chemistry, Biochemistry and Toxicology*. (Kuhr, R.J., and H.W. Dorough, eds.). CRC Press, USA.; Huckabee, J.W., and N.A. Griffith 1974, Trans. Amer. Fish. Soc. 103: 822-825; Laamanen, I., M. Sorsa, D. Bamford, V. Gripenberg, and T. Meretoja 1976, Mutation Res. 40: 185-190; Lalor, J.H., J.P. Chinnici, and G.C. Llewellyn 1976, Develop. Industrial Microbiol. 17: 442-449; Lints, F.A., 1971, Gerontologia 17: 33-51; Lints, F.A., and C.V. Lints 1971, Expl. Gerontol. 6: 417-426; Luning, K.G., 1966, Nature 209: 84-86; Marton, A., 1974, Boil. Abst. 57: 34078; McEwen, F.L., and R. Stephenson 1979, John Wiley and Sons, New York. 1-20; Robertson, F.W., 1959, Univ. Texas. Publ. 5914: 89-98; Sideroff, S.I., and J.A. Santolucito 1972, Physiol. Behav. 9(3): 459-462; Sorsa, M., and S. Pfeifer 1973a, Hereditas 74: 89-102; Sorsa, M., and S. Pfeifer 1973b, Hereditas 75: 273-277; Vogel, E.W., 1977, Identification of carcinogens by mutagen testing in *Drosophila*. In: *Origins of Human Cancer*. (Heatt, H.H, J.D. Watson, and J.A. Winsten, eds.). Book C., Cold Spring Harbor Laboratory, 1483-1497.

The effects of exogenous estrogen and progesterone on developmental stages of *Drosophila melanogaster.*

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Abstract

In this article, Exogenous Estrogen and Progesteron's developmental stages of *Drosophila melanogaster*, offspring gender and number of individuals impacts were examined. Only Standard *Drosophila* Medium (SDM) containing bottles were used as control group in the study. 1% methanol used as solvents were taken into consideration. For application groups, 3 ml solution of different concentrations was added in each bottle (200 ml SDM). 7 male and 7 female individuals were crossed in any bottle. In this study, for both steroid hormones any negative effect was not observed on sex ratio and developmental stages of the *Drosophila melanogaster*. In terms of numbers of offspring individuals estrogen and progesterone groups have seen an increasing trend than the control group. This study applied Exogenous Estrogen and Progesterone does not have an exchange on sex ratio and developmental stages of *Drosophila melanogaster* has been concluded. In addition, according to control group both steroid hormones cause an increase in the number of offspring individuals were removed as a result.

Keywords: Estrogen, Progesterone, Drosophila

Introduction

Mammalian sex hormones such as estrogens, androgens, and progesterone belong to steroids, a group of compounds, which have a basic sterane carbon skeleton (1). It has been reported the steroid hormones play an important role within the oocyte during meiotic maturation of mammalian oocytes *in vivo* and *in vitro* (2).

The steroid hormone estrogen is shaped in ovarium. This hormone plays a key role on the formation of secondary sex characters, development motor activity of uterus, on fat distribution and bone.

Progesterone are essential for reproduction is also a steroid hormone, which is familiar clinically and physiologically as regulators of physiological processes. The physiological effects of progesterone include differentiation of the endometrium, control of implantation. Progesterone also plays an important role in oocyte release from the ovary (3).

Exogenous estrogen and progesterone cause a very adverse effect on the organism (4, 5). In this study, we tried to observe the possible effects of exogenous estrogen and progesterone. To our best knowledge, effects of these hormones have not been worked on development stages of *Drosophila melanogaster*. It provides a powerful system in which to use genetic and molecular approaches to investigate human genetic diseases (6). It also has the advantages of a model organism; because, known as fruit fly, *Drosophila melanogaster* has several features, such as different ecological adaptation to the environment, transport giant chromosomes, simple food requirements, and having a lot of genetic variation.

Materials and Methods

In this work Oregon R wild type strain of *Drosophila melanogaster* was used. All experiments were carried out 25 ± 1ºC and at approximately 60% of relative humidity. The fly stocks and experimental groups were kept on Standard *Drosophila* Medium containing maize-flour, agar, sucrose, dried yeast and propionic acid (7). Progesterone and estradiol solutions of 1, 2, 5, and 10 μM were prepared in 1% methanol and they were added in 50 ml of SDM in bottle. No hormone was added to SDM for the preparation of control groups. 1% methanol was not taken into consideration.

The experiments were carried out with the average same aged individuals, which were obtained from stock culture. 7 males and 7 females were crossed for each bottle. Parents after the pupa stage were removed from the media. Offspring obtained throughout days 1-9 by taking into consideration the total number of individuals and gender was calculated. The experiments were performed in triplicates.

Table 1. The effect of Estradiol and Progesterone on the number of offspring of *D. melanogaster*.

Hormone concentration (mM/50 mL)	Female (%)	Male (%)	Total no of individuals (%)
0 mM Control	345 (53; -)	305 (47; -)	650
1 mM Estradiol	409 (49; +)	422 (51;+)	831 (28)
2 mM Estradiol	493 (49; +)	519 (51; +)	1012 (56)
5mM Estradiol	608 (51; +)	573 (49; +)	1181 (82)
10 mM Estradiol	657 (51; +)	632 (49; +)	1289 (98)
1 mM Progesterone	333 (52; +)	313 (48;+)	646 (i)
2 mM Progesterone	376 (52; +)	352 (48; +)	728 (12)
5 mM Progesterone	370 (53; -)	325 (47; +)	695 (i)
10mM Progesterone	422 (50; +)	422 (50; +)	844 (30)

i = inclusive

Results

These hormones on developmental stages of *Drosophila melanogaster* any effect has been found. In the case of the control groups, the hormone treated-groups reached to pupa stage day 7 and completed their developmental stages day 9 without any problems. Besides, in the case of the control groups no noteworthy change was observed in sex ratio of offspring of the hormone treated-groups.

The differences between sex-ratio is statistically important ($p < 0.05$); the increasing of total number of offsprings according to control group is computed on a percentage basis; i = inclusive.

Conversely, the number of total offspring of the hormone-treated groups were increased by both hormones at all the concentrations tested from 1 to 10 μM. On the other hand, it was found that Estradiol gave rise to more increase in the number of total offspring than Progesterone. For example, only 30% increase rate could be achieved with Progesterone at 10 μM concentration, where 98% increase was obtained with Estradiol. Increase rate was defined as percentage (%) = (OC − OH)/OC × 100, where OC is the number of the total offspring; OH is the number of the total offspring at any hormone concentration (Table 1). These results elucidated that Estradiol was better hormone than Progesterone in terms of the promotion of offspring.

The results in Table 1 were evaluated from the statistical angle in Figure 1 and Figure 2, and standard errors were calculated according Student ttest.

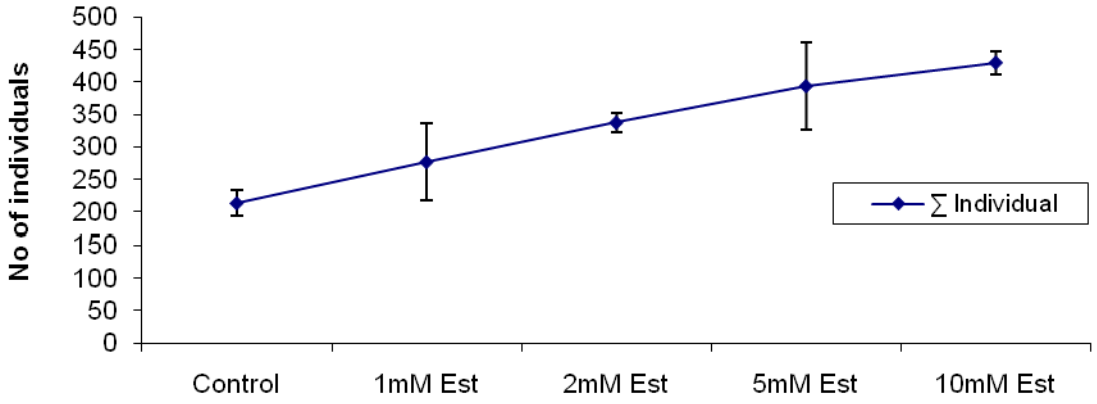

Figure 1. The levels number of offspring individuals for Estrogen groups(1 μM, 2 μM, 5 μM and 10 μM). According to Student ttest. *; **; shows the differences between value $p < 0.05$; $p < 0.01$, respectively.

Discussion

In this study, the role of exogenous estrogen and progesterone on developmental stages of *Drosophila melanogaster*, the number and sex ratio of offspring were investigated.

Estrogene and progesterone are substances that are naturally occurring in both human and animals in identical molecular forms (8). It has been reported steroid hormones have very adverse effects on the human health (4, 5). In addition to this, Androgen therapy leads to hirsutism, acne, coarsening of the voice, hypertrophy of the clitoris and male-pattern baldness (9).

In this study we examined the possible effect of exogenous estrogen and progesterone. Exogenous Estrogen and Progesterone do not have an exchange on sex ratio and developmental stages of *Drosophila melanogaster* have been concluded. In addition, according to control group

both steroid hormones are to cause increases in the number of offspring individuals were removed as a result.

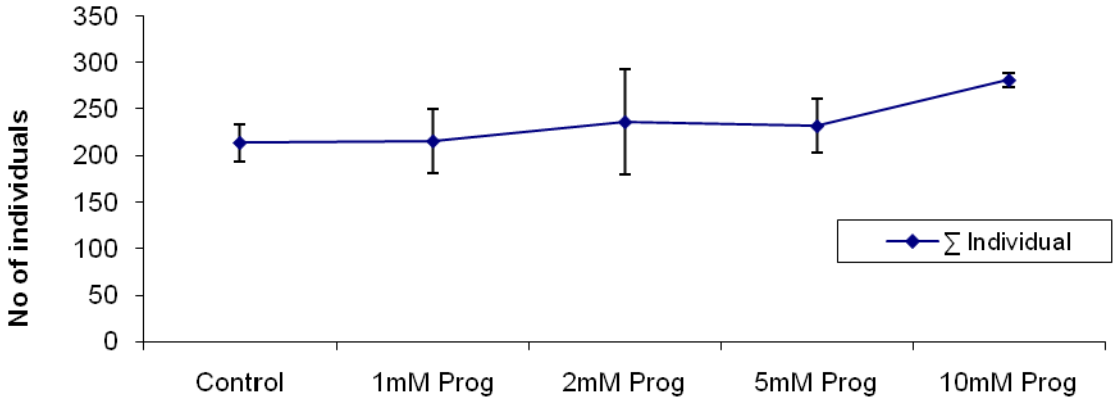

Figure 2. The levels number of offspring individuals for Progesterone groups(1 μM, 2 μM, 5 μM and 10 μM). According to Student ttest. *shows the differences between value $p < 0.05$, respectively.

References: (1) Janeczko, A., and A. Skoczowski 2005, Folia Histochemica et Cytobiologica 43: 71-79; (2) Shimada M., N. Kawano, and T. Terada 2002, Reproduction 124: 557–564; (3) Buffet, N.C., G. Meduri, P. Bouchard, I. 2005, Human Reproduction Update 11: 293–307; (4) Bern, H.A., and F.J. Talamantes 1981, In Herbst Al. 11: 20-4; (5) Crews, D., and M.C. Moore 1986, Science 231: 121-125; (6) Chien, S., L.T. Reiter, E. Brier, and M. Gribskov 2002, Nucleic acids Research 30: 149-151; (7) Çakır Ş., and A. Bozcuk 2000, Turk. J. Biol. 24: 321-329; (8) Andersson, A.M., and N.E. Skakkebæk 1999, European Journal of Endocrinology 140: 477–485; (9) Griffin, J.E., and J.D. Wilson 1992, In: *Textbook of Endocrinology.* (Wilson, JD., and D.W. Foster, eds.). 8th ed, pp799-852, Philadelphia: WB Saunders.

A genetic analysis of the cytological region 46F-47B containing the *Drosophila melanogaster* homolog of the *ELAC2* PCA susceptibility gene.

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Introduction

Human ELAC2 belongs to the metallo-β-lactamase superfamily, a large group of prokaryotic and eukaryotic metalloproteins that perform a variety of diverse functions. *ELAC2* was originally described as a putative Prostate Cancer (PCA) susceptibility gene located on chromosome 17p (Tavtigian *et al*., 2001). Mutation screening of high-risk families revealed four single-nucleotide

substitutions that alter the predicted amino acid sequence of the ELAC2 protein. However, the role of sequence variants of ELAC2 in the genesis of PCA remains unclear.

In vitro biochemical studies showed that ELAC2 is a tRNA processing endonuclease, the RNase Z enzyme (Schiffer *et al*., 2002; Takaku *et al*., 2003). It generates the mature 3'-end of tRNA molecules by removal of the 3'-trailer elements of pre-tRNAs. The *Drosophila* genome contains a single ortholog of human *ELAC2* in the 47A1 region on the 2R chromosome (FlyBase ID FBgn0028426; annotation symbols are Dmel\JhI-1 and CG3298). We previously identified this gene as *Juvenile hormone-inducible protein 1*, *JhI-1* (Dubrovsky *et al*., 2000). Silencing of *JhI-1 in vivo* by RNAi in *Drosophila* S2 cells disrupts tRNA maturation and triggers accumulation of pre-tRNA molecules with 3' extensions (Dubrovsky *et al*., 2004). Since our data confirm that *JhI-1* encodes the *Drosophila* tRNA 3'-processing endonuclease, we renamed this gene *dRNase Z*.

In order to understand *in vivo* function of dRNase Z, we have begun to generate a library of diverse mutant alleles at this locus. Ethyl methanesulfonate (EMS) has a low-target specificity and primarily causes single base-pair changes. However, the small size of the *dRNase Z* gene may potentially reduce the frequency of mutation. Here, we have determined the efficiency of the EMS-mediated approach to induce mutations within the *dRNase Z* locus and present a cytogenetic map of the 46F-47B region using available deletion and lethal lines.

Materials and Methods

Drosophila Stocks:

Descriptions for balancer chromosomes and mutations used in this study can be found in FlyBase (http://flybase.bio.indiana.edu). The following stocks were obtained from the Bloomington Stock Center: *w;L*^{2}*,Pin*^{1}*/CyO,Kr-GFP* (BL5194), *w;Df(2R)12/CyO* (BL5425), *w;Df(2R)BSC*^{281}*/CyO* (BL23666), *w;Df(2R)BSC*^{595}*/CyO* (BL25428), *w;Df(2R)ED*^{2076}*/SM6a* (BL8909), *w;Df(2R)ED*^{2098}*/SM6a* (BL9277), *w;Df(2R)X1/CyO* (BL1702), *w;Df(2R)27/CyO* (BL8109), *Syb*$^{21\text{-}15}$*/CyO* (BL9873), *Hr46*$^{12\text{-}6}$*/CyO* (BL9862), *lola*^{00642}*/CyO* (BL10946), *yw;JhI-1*^{DG04402}*/SM6a* (BL20379). All fly strains were maintained on a standard cornmeal agar medium in a humidified incubator at 25°C. During collection of virgin females, flies were alternated between a 25°C incubator during the day and an 18°C incubator at night in order to facilitate collection.

EMS Mutagenesis and Genetic Analysis:

Newly eclosed *w*^{1118} males were aged for 2-3 days, starved for about 5 hours in an empty bottle, and then fed with 25 mM ethyl methane sulfonate (EMS) in 1% sucrose solution for about 12 hours as described previously (Grigliatti, 1998). Males were left to recover overnight in fresh bottles with regular food before being crossed with virgin females of genotype *w;Drl/CyO*. The F_0 crosses were set up *en masse*. Flies from each F_0 bottle were transferred to fresh bottles every day for 4 days. Individual F_1 *Cy* males were collected and crossed to 2-3 *w;Df(2R)12/CyO,Kr-GFP* virgin females. Crosses were scored for balanced versus unbalanced flies (*Cy:Cy*$^{+}$), and those which contained only balanced progeny indicated the presence of a new lethal mutation over the deficiency chromosome. Balanced males and females carrying the EMS-mutated chromosome were identified by the *w*$^{+}$ marker present on the *CyO,Kr-GFP* balancer chromosome (Figure 1). Each F_2 stock carrying a single mutagenized second chromosome were given a unique number and retested for lethality with *w;Df(2R)12/CyO,Kr-GFP* flies.

Complementation groups were identified among the recessive lethal mutations by *inter se* crosses performed at 25°C. Cytogenetic mapping of complementation groups was achieved through additional crosses with lines containing known mutations and deletions.

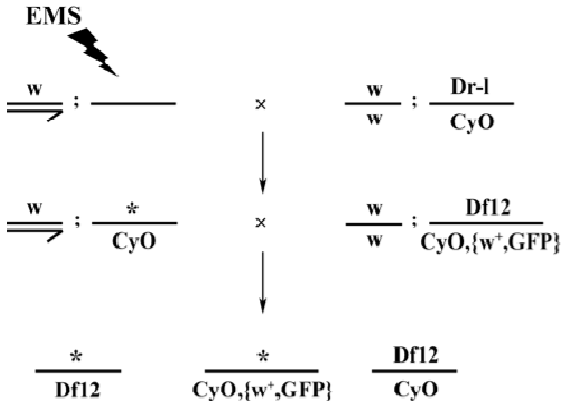

Figure 1. Genetic scheme for the EMS mutagenesis designed to isolate lethal mutations in the 46F1-47B1 cytological region that includes *dRNase Z*. Mutagenized second chromosomes were screened by non-complementation with *Df(2R)12*. Flies carrying lethal alleles were identified in the F_2 cross by the w^+ marker present on the balancer chromosome.

Results and Discussion

The frequency of mutations affecting protein function depends on the size of the coding region, the number of amino acids crucial for its activity, and the treatment conditions during mutagenesis. In order to estimate the mutation frequency at the *dRNase Z* locus, we used a standard mutagenesis protocol with 25 mM EMS. Following the mating scheme diagrammed in Figure 1, mutant strains were selected through non-complementation with chromosomal deficiency *Df(2R)12*. This deficiency spans the genomic region 46F-47B of the second chromosome and was chosen due to its relatively small size. F_0 *w* males were treated with EMS, allowed to recover, and crossed to *w;Drl/CyO* females. From the resulting progeny, F_1 males with the *CyO* balancer chromosome were selected and individually crossed with *w;Df(2R)12/CyO,{w+,GFP}* females. In three independent experiments we screened 3,850 second chromosomes from fertile F_1 male progeny and recovered thirty recessive lethal mutations that did not complement *Df(2R)12* when scored for the presence of flies with straight wings (Cy^+). The stocks were established by crossing w^+ siblings from the F_2 cross.

Inter se testing of the thirty strains was conducted in order to organize allelic mutations into discrete complementation groups (Supplementary Table 1). Most recessive lethal lines fell into seven groups showing a simple pattern of allelism, but one group exhibited interallelic complementation (Figure 2). This group is comprised of two long alleles, BM157 and TH9, that overlap three separate groups of alleles that complement with each other but not with long alleles. Overall, analyses defined eight groups with the allele number ranging from one to ten per group.

The complementation groups were next assigned to relative positions within the 46F-47B cytological region uncovered by *Df(2R)12*. The region was subdivided into small intervals using six deletions acquired from the Bloomington Center. As shown in Figure 2, the breakpoints of *Df(2R)BSC*^{281}, *Df(2R)BSC*^{595}, *Df(2R)X1*, *Df(2R)27*, *Df(2R)ED*^{2076}, and *Df(2R)ED*^{2098} produce 5 intervals: 46F1-47A1, 47A1-47A3, 47A7-47A9, 47A9-47A10, and 47A10-47B1. Flies from each complementation group were crossed with each deletion to test their ability to complement lethality (Supplementary Table 2). Individual complementation groups were mapped to both the 46F1-47A1 and 47A9-47A10 intervals. Pairs of complementation groups were mapped to the intervals 47A1-47A3, 47A7-47A9, 47A10-47B1. As no additional deletion breakpoints within these three intervals are available, the relative positioning of the complementation groups in each interval was not possible.

Our next goal was to associate the eight complementation groups with genes in the 46F-47B region. Annotation of the *Drosophila* genome suggests that this cytological region contains as many as forty known and predicted protein coding genes. For approximately half of them no mutant alleles

are available, and the other half is mostly represented by insertional alleles with no available phenotypic data. There are only four genes in this cytological region – *dRNase Z* (formerly *JhI-1*), *Hormone receptor-like in 46* (*Hr46*), *Synaptobrevin* (*Syb*), and *Longitudinals lacking* (*lola*) – which are uncovered by *Df(2R)12* and for which recessive lethal alleles are available. Flies from each complementation group were crossed with mutant lines Syb^{21}, $Hr46^{12}$, $lola^{642}$, and $JhI\text{-}1^{DG}$. The results of the test for allelism are shown in Supplementary Table 2 and summarized in Figure 2.

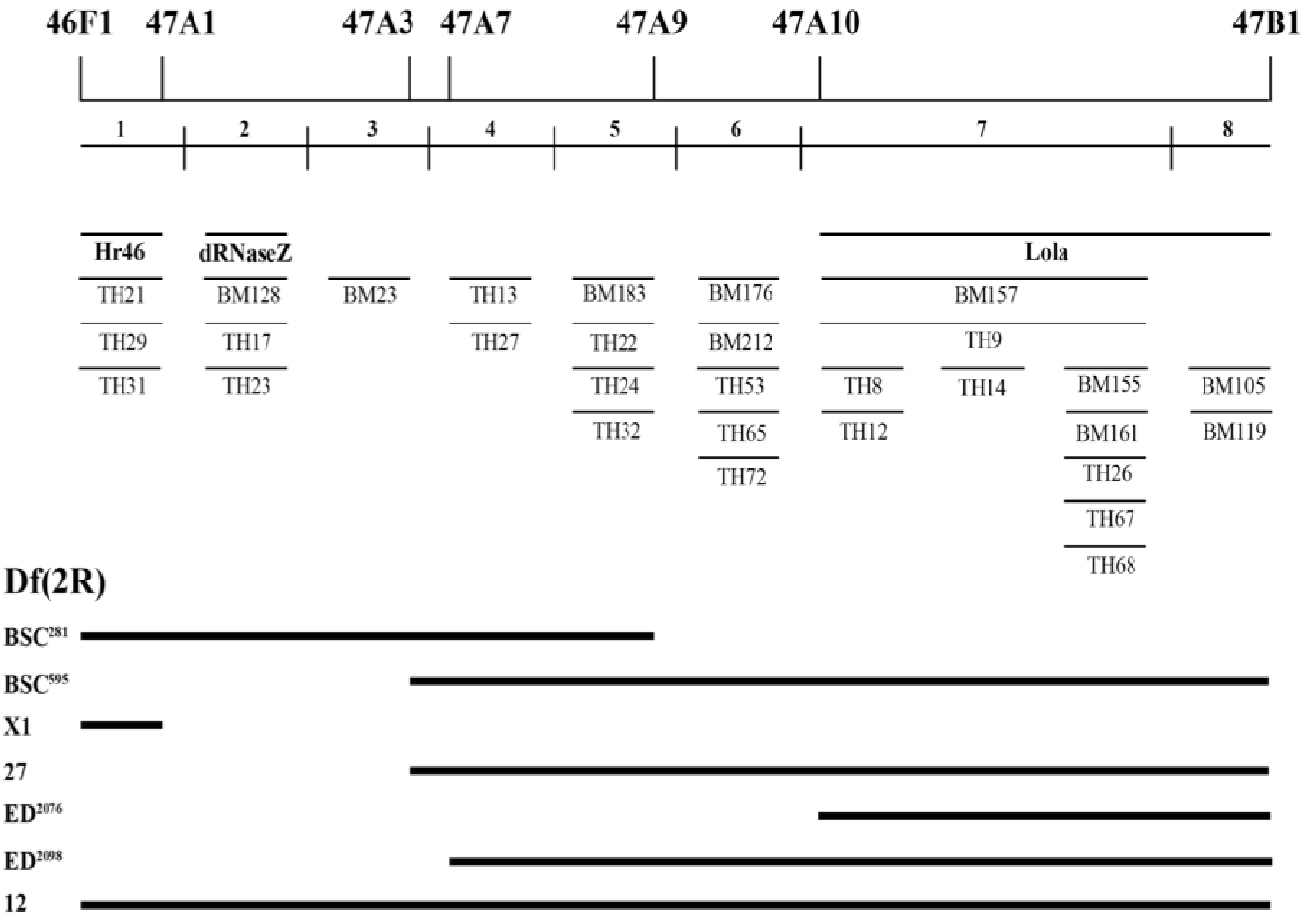

Figure 2. Cytogenetic map of complementation groups within the 46F1-47B1 region on the 2R chromosome. Bold lines at the bottom of the figure represent the deficiencies that were used in this study. The upper line shows the cytological endpoints of these deficiencies as they are reported in FlyBase. Below is the numerical designation of the eight complementation groups. Listed under each number are the alleles belonging to complementation groups assigned to a specific cytological interval. Three known genes are also indicated in bold in relation to the complementation groups and cytological intervals.

There are four complementation groups that could not be associated with known genes: one in the 47A1-47A3 interval, two in the 47A7-47A9 interval, and one in the 47A9-47A10 interval. These are mutations that apparently represent predicted but as yet uncharacterized genes in the region. Among our lethal lines we did not find any alleles for the *Syb* locus. However, we identified three new alleles for *Hr46* mapped to the 46F1-47A1 interval, and twelve for *lola*. Interestingly, $lola^{642}$ did not

Table 1. Complementation among EMS-induced alleles recovered within the 46F1-47B1 region. All recessive lethal lines were tested for complementation in pairwise combinations. Each test can be described as: *lethal(1)/CyO* X *lethal(2)/CyO*. Crosses were scored for *Cy* versus Cy^{+} flies.

	TH9	TH12	TH13	TH14	TH17	TH21	TH22	TH23	TH24	TH26	TH27	TH29	TH31	TH32	TH53	TH65	TH67	TH68	TH72	BM23	BM105	BM119	BM128	BM155	BM157	BM161	BM176	BM183	BM212
TH8	**119:9**	**100:0**	77:51	108:36	89:43	69:33	84:44	116:57	68:41	94:63	113:48	97:32	118:69	91:45	87:40	97:51	62:47	84:39	78:48	107:57	95:44	72:55	94:86	95:40	**90:8**	102:64	95:55	104:61	73:43
TH9		**110:8**	78:28	**123:0**	84:25	92:57	83:29	107:37	83:26	**93:10**	77:40	114:53	89:46	96:26	77:24	81:33	**96:1**	**102:7**	99:45	105:60	94:42	73:45	64:52	**103:0**	**107:1**	**157:1**	103:64	98:46	87:21
TH12			76:28	82:43	78:37	75:29	78:39	87:36	97:27	107:34	71:35	80:30	73:33	83:28	74:37	106:44	86:45	91:42	110:77	93:61	114:70	73:51	69:47	78:44	**61:2**	84:61	76:43	87:58	66:29
TH13				80:23	108:40	84:37	74:48	93:33	100:58	63:28	**106:0**	94:52	89:57	N/D	52:30	67:39	113:70	92:58	84:49	111:43	87:33	98:72	83:60	84:38	104:37	108:50	104:62	98:62	95:47
TH14					106:57	98:54	76:40	113:47	82:39	118:29	107:35	91:24	84:59	110:33	72:32	94:48	82:33	96:63	106:62	104:45	76:25	77:36	105:47	98:70	**113:3**	113:67	84:72	121:74	96:69
TH17						60:40	97:35	**102:0**	101:35	114:51	112:58	108:68	82:42	94:43	94:43	101:56	78:55	106:71	77:73	101:41	84:39	98:60	**101:0**	88:68	119:56	67:21	84:47	91:65	63:31
TH21							78:43	78:38	85:37	73:39	85:41	**117:0**	**105:0**	104:47	93:38	82:41	96:44	65:46	68:53	95:37	97:85	74:52	87:70	93:73	84:39	66:20	82:32	98:46	73:26
TH22								91:53	**107:0**	71:58	69:33	137:55	102:55	**104:0**	83:26	104:68	79:45	102:70	87:67	108:31	87:56	69:35	76:71	98:54	78:63	73:45	99:47	**104:0**	76:35
TH23									108:47	N/D	75:29	87:58	95:27	114:43	80:36	98:41	103:61	97:49	67:52	109:68	82:32	83:50	**81:0**	99:35	76:67	131:72	97:49	126:51	105:58
TH24										80:41	118:81	59:43	90:30	**101:0**	76:28	86:44	72:66	97:41	76:33	117:48	74:32	89:42	79:25	83:39	86:35	117:61	106:59	**121:0**	84:52
TH26											74:51	87:52	124:62	108:71	85:43	71:33	**96:0**	**112:0**	98:55	100:76	62:36	70:35	124:81	**97:0**	**109:0**	**155:0**	118:54	106:67	92:56
TH27												71:39	101:27	101:55	94:25	71:42	89:49	81:46	86:27	96:53	83:29	97:54	103:56	67:46	70:31	87:51	66:28	74:39	103:71
TH29													**118:0**	116:52	91:42	64:43	62:54	98:47	82:31	120:47	89:61	91:29	90:32	63:42	99:67	116:74	112:52	78:46	93:54
TH31														79:55	88:21	74:40	103:35	103:60	111:53	88:38	78:38	101:59	101:50	70:50	76:35	118:65	69:35	82:38	71:39
TH32															94:42	68:33	74:37	71:47	92:49	82:35	85:51	92:55	88:50	72:39	71:31	76:36	95:62	**122:0**	74:44
TH53																**62:0**	84:50	85:50	**68:0**	107:59	104:59	79:51	82:54	63:46	103:40	110:62	**117:0**	108:56	**111:0**
TH65																	82:45	68:38	**106:0**	83:53	79:37	70:39	75:39	87:56	62:35	N/D	**80:0**	73:47	N/D
TH67																		**103:0**	73:39	66:36	61:27	88:56	73:38	**61:7**	**88:0**	**107:0**	76:35	76:34	75:49
TH68																			108:48	73:33	67:39	61:44	89:38	**53:17**	**101:0**	**72:0**	76:52	65:52	84:42
TH72																				68:44	91:47	62:32	71:41	83:36	77:45	71:45	**87:0**	64:39	**72:0**
BM23																					138:59	184:102	134:71	74:61	83:68	77:50	77:41	91:37	69:47
BM105																						**128:0**	122:54	89:38	69:32	71:32	66:36	79:32	69:43
BM119																							141:67	78:34	83:52	73:62	61:39	85:45	84:59
BM128																								62:39	75:34	69:56	72:45	91:66	79:43
BM155																									**78:6**	**83:0**	90:46	67:41	67:37
BM157																										**97:0**	87:36	71:44	88:41
BM161																											72:45	85:68	73:37
BM176																												89:42	**88:0**
BM183																													95:60

Table 2. Cytogenetic mapping of complementation groups. Representative alleles from each group were tested for complementation with deletion and lethal lines from the 46F1-47B1 cytological region. Crosses were scored for *Cy* versus Cy^+ flies.

	$Hr46^{12}$	Syb^{21}	$JhI\text{-}1^{DG}$	$lola^{642}$	BSC^{281}	BSC^{595}	DfX1	Df27	ED^{2076}	ED^{2098}
TH8	82:45	75:38	93:51	**116:0**	109:53	**94:0**	85:49	**80:0**	**88:0**	**108:0**
TH14	95:55	100:40	91:44	**102:0**	97:48	**113:0**	75:41	**84:0**	**68:0**	**91:0**
TH27	80:56	83:57	110:48	75:38	**97:0**	**137:0**	76:41	**106:0**	65:44	**89:0**
TH29	**147:0**	74:32	72:30	80:44	**111:0**	97:74	**71:0**	89:43	109:63	72:48
BM23	72:45	74:57	74:42	94:73	**102:0**	79:48	23:7	71:44	73:48	85:41
BM105	60:38	95:39	81:41	**107:0**	87:48	**93:0**	43:36	**112:0**	**105:0**	**102:0**
BM128	95:60	98:56	**118:0**	76:38	**128:0**	83:46	84:33	97:50	94:69	83:60
BM155	84:40	75:46	68:38	**131:0**	75:31	**97:0**	81:47	**117:0**	**98:0**	**104:0**
BM157	73:28	70:41	73:47	**94:0**	79:69	**106:0**	89:36	**132:0**	**84:0**	**127:0**
BM176	86:36	95:55	84:44	95:50	89:47	**88:0**	56:38	**97:0**	55:39	**86:0**
BM183	79:42	84:36	87:48	103:67	**127:0**	**118:0**	42:22	**98:0**	80:43	**111:0**

complement both groups in the most distal interval, 47A10-47B1. *Lola* is a large and complex protein-coding gene. It occupies over 60 kb of genomic DNA and consists of thirty-two exons producing at least eighty splicing variants (Ohsako *et al*., 2003). The interallelic complementation between different *lola* mutations arises from alternative trans-splicing of common and variable exons (Horiuchi *et al*., 2003). This would account for the complex complementation pattern observed in our results. Most importantly, we recovered three alleles for *dRNase Z* mapped to the 47A1-47A3 interval. BM128, TH17, and TH23 do not complement each other and the $JhI\text{-}1^{DG}$ recessive lethal mutation, an established allele for *dRNase Z*. As it required a screen of 3,850 chromosomes to find these three alleles, we estimate that the rate of mutation for this gene is approximately one per 1,200 mutagenized chromosomes. Because the three alleles are recessive lethal, we predict that they knock out the *dRNase Z* gene. Therefore, these strains will enable future studies to elucidate the *in vivo* function of this gene.

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References: Dubrovsky, E.B., V.A. Dubrovskaya, A.L. Bilderback, and E.M. Berger 2000, Dev. Biol. 224: 486-495; Dubrovsky, E.B., V.A. Dubrovskaya, L. Levinger, S. Schiffer, and A. Marchfelder 2004, Nucleic Acids Res. 32: 255-262; Grigliatti, T.A., 1998, Mutagenesis, In: Drosophila melanogaster: *A Practical Approach*. (Roberts, D.B., ed.). IRL Press, Oxford, pp. 55-83; Horiuchi, T., E. Giniger, and T. Aigaki 2003, Genes Dev. 17: 2496-2501; Ohsako, T., T. Horiuchi, T. Matsuo, S. Komaya, T. Aigaki 2003, Gene 311: 59-69; Schiffer, S., S. Rosch, and A. Marchfelder 2002, EMBO J. 21: 2769-2777; Takaku, H., A. Minagawa, M. Takagi, and M. Nashimoto 2003, Nucleic Acids Res. 31: 2272-2278; Tavtigian, S.V., J. Simard, D.H. Teng, V.

Abtin, M. Baumgard, A. Beck, N.J. Camp, A.R. Carillo, Y. Chen, P. Dayananth *et al*. 2001, Nature Genet. 27: 172-180.

Male remounting in three species of *Drosophila montium* subgroup.

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Species of *Drosophila* exhibit elaborate courtship followed by mating. Although genus *Drosophila* consists of more than 2250 species, the sexual behavior of about 250 species has been studied so far. The mean copulation duration in these species varies from just 60 seconds to several hours. During this time the male transfers the sperm to the female. Male mating another female or male remating is a common feature in mass cultures of many *Drosophila*. Female remating is not as frequent as that of male remating, but yet it is reported in a few species. When copulation is complete, generally the male will not mount the same female again. Here we report the males of three species of *montium* subgroup remounting the same female. Four species of *montium* subgroup viz, *D. agumbensis, D. gangotrii, D. jambulina* and *D. nagarholensis* collected from University Garden, Manasagangotri, Mysore, India were used. Virgin females and bachelor males were collected from progeny of wild-caught females. One male and one female were aspirated into an Elens-Wattiaux mating chamber (observation chamber), and observed for two hours. If there was no mating within two hours, then the pairs were discarded. Among flies which paired, the copulation duration was recorded. The pairs were allowed to stay together, and the remounting if any was also noted along with the copulation duration of a second mount and subsequent mounts.

Table 1. Showing courtship latency, mating latency and copulation duration of the different species of *Drosophila* (values are in seconds).

Species	No. of pairs observed	Courtship latency	Mating latency	Copulation duration (seconds) (No. of pairs mated is given in parenthesis)			
				1st mount	2nd mount	3rd mount	4th mount
D. agumbensis	20	120 ± 12.2	2866 ± 42.4	127 ± 23.2 (00)			
D. gangotrii	20	149 ± 34.3	76 ± 18.7	231 ± 120.6 (19)	23 ± 8.0 (9)		
D. jambulina	20	165 ± 20.4	198 ± 23.2	187 ± 32.9 (18)	73 ± 0.2 (11)	27 ± 0.6 (7)	
D. nagarholensis	20	70 ± 10.7	83 ± 12.9	607 ± 60.1 (18)	19 ± 3.0 (5)	17 ± 0.7 (5)	10 ± 2.1 (3)

The duration of mounting (copulation) as well as remounting is shown in Table 1. The courtship latency and mating latency is also shown in the table. It was interesting to note that *D. agumbensis* did not show remounting. The males of the remaining three species *viz*, *D. gangotrii, D. jambulina* and *D. nagarholensis* remounted the same female immediately after first mount. There was no relation between courtship latency or mating latency or with copulation duration (first mount).

However, the duration of second and subsequent mounts gradually shortened. The incomplete sperm transfer in the first mount must have been the cause for second and subsequent mounts.

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Esterase loci differences, specificities, and body expression patterns in species of the *Drosophila guarani* group (Diptera; Drosophilidae).

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Abstract

The level of genetic differentiation within populations has received considerable attention as it can indicate the vitality and the potentiality of the population to respond to environmental changes. Several works have combined morphological, isoenzymes, and DNA sequencing to produce better and more complete results about the evolutionary history and genetic structure of populations and species. Thus, the aim of this work was to evaluate the esterase loci differences, specificities, and body expression patterns in two species of *Drosophila guarani* group, in order to obtain a tool for future studies using a combined analysis of isoenzymes, DNA, and morphology. Our esterase loci analysis showed that there are genetic composition differences between species and that these markers could be used in studies of natural populations' genetic variability. However, to obtain better results for *D. maculifrons*, the individual sample should not have the body cut in parts, such as head, thorax and abdomen, because there is head specific locus.

Introduction

Isoenzyme electrophoresis has been used in population and evolutionary researches since 1966 as a way to evaluate populational genetic variability through the direct product of gene expression (Mateus *et al*., 2005). Esterases in *Drosophila* form a polymorphic group of isoenzymes (Johnson, 1974) and can be related with several body functions, such as juvenile hormone levels regulation, digestive processes, reproductive behavior, and insecticide degradation. These enzymes have been detected in all life phases and in many tissues of this group of organisms, which demonstrates the importance of this class of enzymes in the insect normal development (Karotam *et al*., 1993; Gu and Zera, 1994; Feyereisen *et al*., 1995).

The *D. guarani* group belongs to the quinaria-tripuntacta section of the *Drosophila* subgenus and has 16 neotropical species (Bächli, 2009). According to Gottschalk *et al*. (2008), six of these species have been recorded in the Brazilian territory: *D. alexandrei*, *D. guaru*, *D. ornatifrons*, *D. griseolineata*, *D. guaraja*, and *D. maculifrons*. Like other Brazilian species, the *D. guarani* group has been poorly studied, and the data on ecology, systematics, genetics, and evolution its species are scarse. Therefore, much more work related to these aspects should be done.

In this context, this study evaluated for the first time the esterase loci genetic differences and body expression patterns using two species of the *D. guarani* group: *D. ornatifrons* (*D. guarani* subgroup), and *D. maculifrons* (*D. guaramunu* subgroup). For the first species, esterase pattern of the whole body was investigated, whereas for the second, body expression pattern (head, thorax and abdomen) and also esterase specificity to α- and β-naphthyl acetates were analyzed in order to provide a tool for future combined works using isozyme, DNA, and morphology data.

Material and Methods

The *Drosophila* species were collected in 2008 in a conservation area in Guarapuava, Parana, Brazil (25°23'36" N, 51°27'19" W), named Parque Municipal das Araucárias, which covers approximately 100 ha, and 42.75% of the total area is composed by Araucaria forest. Flies collected were identified, and individuals of the species *D. maculifrons* and *D. ornatifrons* were stored individually at -20°C for later electrophoretic analysis.

The analysis of esterase activity was performed through electrophoresis on 10% polyacrylamide gel, according to the method described by Ceron (1988). The identification of esterases in the gel was performed using α- and β-naphthyl acetates as substrates, and the products of the reactions were stained by Fast Blue-RR salt. For *D. ornatifrons*, the esterase pattern of the whole body was analyzed. For *D. maculifrons*, the pattern of body expression (head, thorax and abdomen) and also esterase specificity for α- and β-naphthyl acetates were investigated. The enzyme loci were numbered in ascending order of anodal mobility.

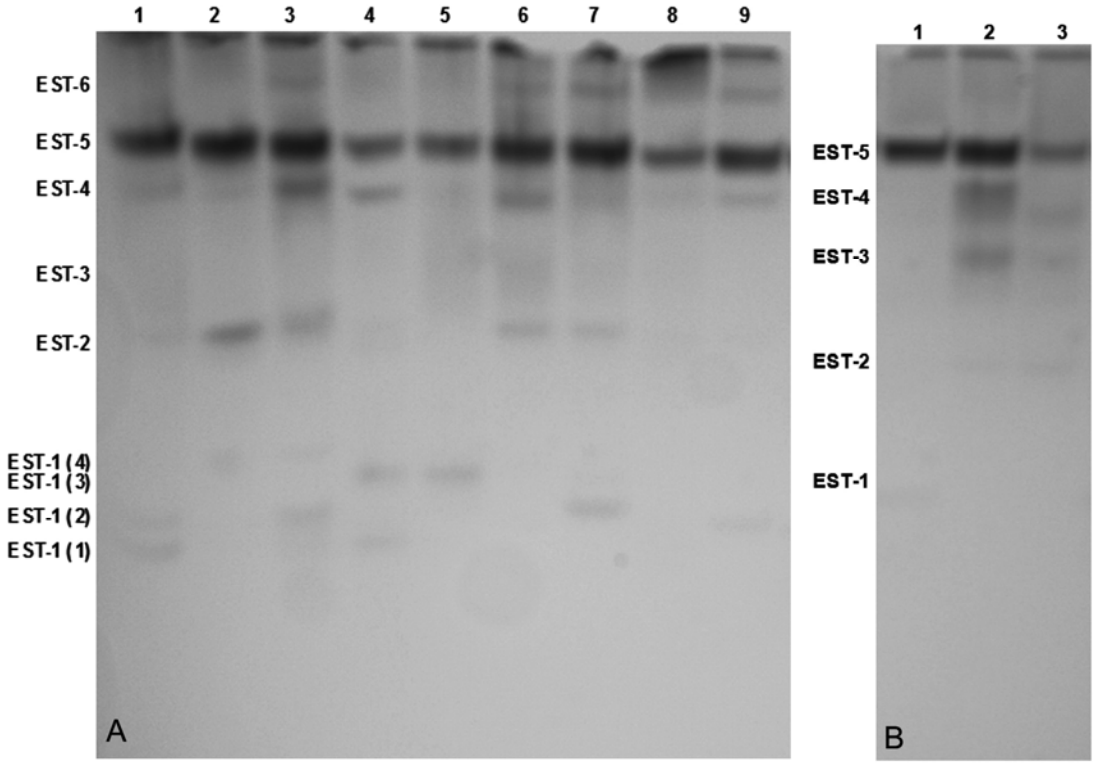

Figure 1. Esterase profile of (A) *Drosophila ornatifrons* and (B) *Drosophila maculifrons* head (1), thorax (2) and abdomen (3).

Results and Discussion

Esterase profile analysis in *D. ornatrifrons* species revealed the presence of six loci, four α-esterases (EST-1, EST-2, EST-5 and EST-6) and two β-esterases (EST-3 and EST -4). All these loci were monomorphic, except by EST-1, which presented four alleles (Figure 1-A). *Drosophila maculifrons* presented five monomorphic loci (Figure 1-B). EST-1, EST-2 and EST-5 were α-esterase exclusive as they were not stained in the presence of only β-naphthyl acetate. EST-3 and EST-4 loci were preferentially β-esterases as they were visualized when in contact with one or the other substrate independently, but in the presence of both substrates at the same time they preferentialy reacted with β-naphthyl acetate. The analysis of the esterase profile of both species revealed differences in the genetic composition, which is consistent with the patterns found for other groups of insects (Bueno *et al*., 2003; Reyes *et al*., 2004, as examples).

The esterase body expression pattern in *D. maculifrons* revealed that the EST-1 locus is exclusive of the head. EST-2, EST-3 and EST-4 were detected in the chest and abdomen. EST-5 had no specific pattern of body expression as it was observed in all body part samples. The differences in body expression pattern of esterase loci in *D. maculifrons* species suggests that individuals should not be cut in parts (head, thorax and abdomen) in studies where the aim is to access the genetic variability of these enzymes, as suggested for other *Drosophila* species (Mateus *et al*., 2005; Cavasini *et al*., 2008), because it will generate loss of information as there is head specific locus.

However, new studies about physiology, enzyme activity and gene expression should be performed in different tissues in order to investigate the functional and differential expression observed for these loci on this species. The results of such work could be related to the differences in body/tissue expression pattern during development and also under the effects of contaminants, which can affect the specificity of these enzymes.

Our results showed that there is genetic differentiation among the species studied, indicating the necessity for further studies using natural populations of *D. guarani* species group, which may contribute to clarify the phylogenetic relationships among the still controversial *D. guarani* and *D. guaramunu* subgroups (Robe *et al*., 2005). Furthermore, in the *D. maculifrons* genetic variability analysis, regarding the methodology used here, individuals should not be cut in body parts because there is a head specific locus.

References: Bächli, G., 2009, Taxodros <http://www.taxodros.unizh.ch/>, accessed in October 2009; Bueno, C.E.M.S., C.E.M. Otoboni, J.M. Santos, and F.J. Cividanes 2003, Rev. Cient. Eletr. Agron. 4: 1-5; Cavasini, R., E.C. Gustani, P.T. Rodrigues, D.P. Simão, L.P.B. Machado, and R.P. Mateus 2008, Dros. Inf. Serv. 91: 90-91; Ceron, C.R., 1988, PhD Thesis, Departamento de Biologia, IB-USP, São Paulo, SP, Brasil; Feyereisen, R., 1995, Toxicol. Lett. 82/83: 83-90; Gu, X., and A.J. Zera 1994, Mol. Biol. 107: 553-560; Gottschalk, M.S., P.R.P. Hofmann, and V.L.S. Valente 2008, Check List 4: 485–518; Johnson, G.B., 1974, Science 184: 28-37; Karotam, J., A.C. Delves, and J.G. Oakeshott 1993, Genetica 88: 11-28; Mateus, R.P., L.P.B. Machado, and F.M. Sene 2005, Dros. Inf. Serv. 88: 46-48; Reyes, M., J.C. Bouvier, T. Boivin, E. Fuentes-Contreras, B. Sauphanor 2004, Agric. Técn. (Chile) 64: 229-237; Robe, L.J., V.L.S. Valente, M. Budnik, and E.L.S. Loreto 2005, Mol. Phylogenet. Evol. 36: 623-640.

Occurrence of *P* element in natural populations of *Drosophila melanogaster* in Ukraine.

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Abstract

Using PCR and sequencing we have demonstrated the presence of *P* elements in genomes of *Drosophila melanogaster* from natural populations of Ukraine. The degree of gonadal reduction revealed indirectly indicates low or, in some cases, zero activity of the mobile element in the inspected natural populations of *Drosophila*. All studied populations have been found to represent the M' cytotype. In this study, we have shown for the first time that *P* element, absent two decades ago, has invaded Ukrainian populations of *Drosophila melanogaster*. This invasion is part of the worldwide expansion of *P* element in *Drosophila melanogaster*. In Ukraine, an initial stage of the invasion has been suggested to occur, and further evolution of the M' cytotype into P cytotype is expected.

Keywords: *P* element, *Drosophila melanogaster*

Introduction

Recent data suggest that according to different estimates up to 22 percent of the *Drosophila melanogaster* genome is represented by mobile elements (ME) pertaining to diverse families (Kapitonov and Jurka, 2003). The *P* element family (autonomous ME, 2.9kb long) includes MEs capabale of promoting so-called P-M hybrid disgenesis under a certain crossing scheme (Kidwell, 1985). Hybrid disgenesis is usually accompanied by such effects as high gonadal reduction frequency, elevated rates of mutation, and recombination in males (Broadhead *et al*., 1977). Based on the presence of *P* element and the hybrid disgenesis manifestation all known fruit fly lineages are divided into three major types: a) no *P* element (M cytotype), b) *P* element present (P and Q cytotypes) (Kidwell, 1985). Beside these three, there are also two variants: M' (the M type yet containing *P* element) and P', the P strain unable to repress the effects of hybrid dysgenesis. Hybrid dysgenesis usually manifests itself in reduction of the size and stage of development of gonads in *Drosophila* of the F1 generation from crosses between *P*-element-containing males and females lacking *P* element, and is the result of *P* element movement in the fly germ cells. Although the exact nature of the process of *P* element movement suppression (as a defensive mechanism against GD) is still poorly understood, the two current hypotheses (that are not mutually exclusive) link it either to the activity of suppressor proteins produced by incomplete copies of *P* elements (Rio, 1990) or the activity of Piwi-interacting (pi) RNAs (Simmons *et al*., 2007; Jensen *et al*., 2008). Whatever the mechanism, flies inherit maternally a means to suppress movement of *P* element, and this is the basis of their division into so-called cytotypes. Present day cytotypes are distinguished depending on the ability of flies to induce (tested on males) or repress (tested on females) active movement of *P* elements. In this way, P cytotype lineages have both inducing and repressing abilities, P' cytotype strains have only inducing ability, Q strains have only repressing ability, and M cytotype flies have

neither (see in Itoh *et al*., 2007) and are devoid of *P* elements. M' cytotype defines flies that lack both inducing and repressing abilities, but still contain *P* elements (Bingman *et al*., 1982).

In laboratory lineages and natural populations of *Drosophila* collected before 1950, *P* element has not been detected (Kidwell, 1983). Its invasion of the *Drosophila melanogaster* genome is believed to have started somewhere in the middle of the twentieth century in the Caribbean or southeastern North America, probably originating through a horizontal transfer from *D. willistoni* (Daniels *et al*., 1990; Clarck and Kidwell, 1997; Itoh *et al*., 2007). By the 1980s, the invasion had spread all over the world including Europe, but it hadn't been found to occur in populations from the former Soviet Union and Australia (Kidwell, 1983; Zakharov, 1984). In the 1990s, it was detected in Japanese populations (Gamo *et al*., 1990). Concerning Ukraine, a work published in 2006 demonstrated the presence of incomlete copies of this element in a natural population from Uman collected in 1983 (Kovalenko *et al*., 2006). However, no detailed research on ME in natural populations of *Drosophila* in Ukraine has been carried out so far.

The aim of the present study was to investigate the occurrence of *P* element in natural populations of *Drosophila melanogaster* in Ukraine.

Figure 1. Fly collection sites.

Materials and Methods

We studied lineages of *Drosophila melanogaster* originating from different natural populations collected at several locations in Ukraine in 2008 and 2009 (Figure 1), as well as the

laboratory line *Canton S*, a wild type lineage that may sometimes not contain the mobile *P* element (Roberts, 1986), being part of the collection of General and Molecular Genetics Department of Taras Shevchenko National University of Kyiv and courteously granted by Genetics Department of Lomonosov Moscow State University in 1992.

Cytotype determination was based on the gonadal dysgenesis (GD) assay and included two kinds of crosses (Kidwell *et al*., 1977; Engels and Preston, 1980) – female *Canton-S* (M cytotype) were crossed with wild caught males to test the GD induction potential in wild flies (cross A), and wild females were crossed with Harwich (P cytotype) males to test the repression potential of the tested wild flies (cross B). Cytotype was determined as described in Itoh *et al*. (2001). Gonad biotomy and visual inspection of their developmental status was used to measure GD. Only unilateral and bilateral ovary and seminal gland reduction were counted. For each population, 50 individuals of each sex were analyzed. Percent ratio of GD was calculated using the formula %GD = ½%GD(1) + %GD(2), where %GD(1) stands for the proportion of individuals with unilateral reduction of the ovary/seminal gland taken as a percent of the whole sample; %GD(2) means the proportion of individuals with bilateral gonadal reduction taken as a percent of the whole sample.

We used a 437 bp region of *P* element DNA sequence to look for possible base substitutions and confirm the results of *P* element detection by PCR. This region is believed to be part of all known types of incomplete *P* element copies and the complete one. Total DNA was extracted from adult individuals of each population using QIAamp DNA Micro Kit (Qiagen, USA). We employed PCR to amplify the 437 bp *P* element fragment using primers 5'-ACGTTTGCTTGTTGAGAGGA-3' and 5'-AACAGGACCTAACGCACAGT-3' specific to the abovementioned fragment ranging from the 41th to the 477th base of the gene coding for the *P* element transposase.

Sequence alignment was performed using the Vector NTI software.

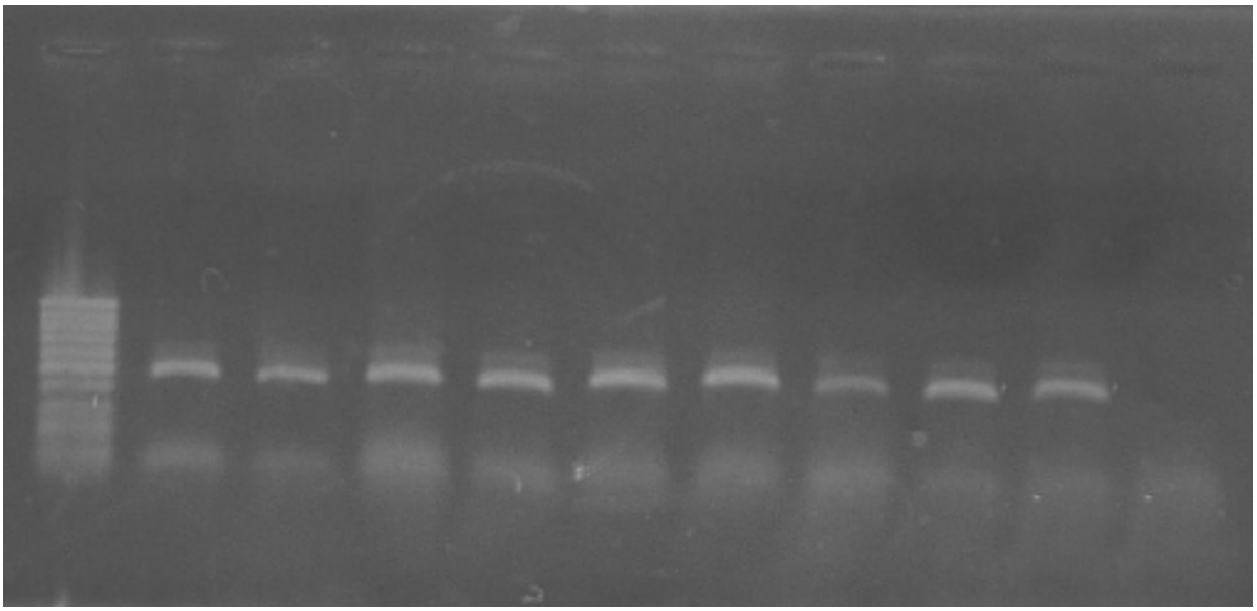

Figure 2. PCR analysis of fly lineages. M – molecular weight marker, 1-9 – *P* element transposase fragments in DNA of flies from the studied natural populations, 10 – DNA of the *Canton-S* line (no fragments).

Results and Discussion

Individuals of *Drosophila melanogaster* were collected in different regions of Ukraine planned so as to form a latitudinal cross section of the country from the north to the south, as up to the 1980s not all natural populations of this species contained *P* elements, and specifically populations from the territory of the former Soviet Union had not been found to contain MEs of this type at all (Kidwell, 1985).

As it can be seen from Figure 2, DNA of all lineages, except for *Canton S*, produced products of the expected length. To further ascertain that these bands represented *P* elements, we extracted and sequences the products. Figure 3 demonstrates the region of *P* element (reference: GenBank accession #AB331393) we amplified. The amplified sequences were identical to the reference, and we found no base substitutions in any of the studied populations. Therfore, we can state that all of the studied natural populations contain copies of *P* element. However, insomuch as the primers were specific to a small fragment of the transposase gene, we were unable to distinguish which copies were full-size and what types of incomplete copies occur. Size distribution of the detected copies requires further investigation.

```
(41)    ACGTTTGCTT GTTGAGAGGA AAGGTTGTGT GCGGACGAAT TTTTTTTTGA  (90)
(91)    AAACATTAAC CCTTACGTGG AATAAAAAAA AATGAAATAT TGCAAATTTT  (140)
(141)   GCTGCAAAGC TGTGACTGGA GTAAAATTAA TTCACGTGCC GAAGTGTGCT  (190)
(191)   ATTAAGAGAA AATTGTGGGA GCAGAGCCTT GGGTGCAGCC TTGGTGAAAA  (240)
(241)   CTCCCAAATT TGTGATACCC ACTTTAATGA TTCGCAGTGG AAGGCTGCAC  (290)
(291)   CTGCAAAAGG TCAGACATTT AAAAGGAGGC GACTCAACGC AGATGCCGTA  (340)
(341)   CCTAGTAAAG TGATAGAGCC TGAACCAGAA AAGATAAAAG AAGGCTATAC  (390)
(391)   CAGTGGGAGT ACACAAACAG AGTAAGTTTG AATAGTAAAA AAAATCATTT  (440)
(441)   ATGTAAACAA TAACGTGACT GTGCGTTAGG TCCTGTT               (477)
```

Figure 3. The 437 bp region of the *P* element of *Drosophila melanogaster* we amplified.

Occurrence of *P* element in all studied populations suggests that this transposon may have spread all over the territory of Ukraine since the 1980s.

Gonadal dysgenesis and cytotype determination results are summarized in Table 1. As seen from Table 1, both the GD induction (cross A) and GD repression (cross B) potentials of all studied populations are low. This indicates that all populations represent the M type cytotype. The presence of *P* elements demonstrated by PCR indicates that they all have specifically M' cytotype, *i.e*. they have yet acquired *P* element but still lack the accompanying molecular machinery which evolves during further stages of *P* element invasions. The results of GD induction assessment crosses (cross A) suggest that the *P* elements are still not actively transposing in these populations. Although we didn't study the exact size distribution of different *P* element copies, the absence of notable *P* element transposition in the populations studied may also indirectly suggest that they lack complete copies. The logic behind this inference is that complete copies are the only ones capable of active transposition, and other types of *P* elements are able to move only in the presence of complete copies which serve as a transposase donor. However, this requires further investigation.

It is known from literature that natural populations of *Drosophila melanogaster* are characterized with a phenomenon called "mutation outburst" (Golubovski, 1985) detected as an enhanced rate of mutation compared to the background level. There are suggestions that the activity of mobile elements may account for such outbursts (Golubovski, 1985). Our previous research didn't

find any signs of such events in natural populations of *Drosophila* in 2005-2006 (Protsenko and Kozeretska, 2006, 2007). Therefore, the genomes of the flies from the populations inspected do bear *P* element, but its active movement was not registered at the time of the study.

Table 1. GD and cytotype determination results.

Fly lineage	GD% in F1 offspring						Cytotype
	unilateral GD		bilateral GD		Total GD		
	cross A	cross B	cross A	cross B	cross A	cross B	
Conton-S	0	9	0	60	0	69	M
Odesa	1	14	0	68	1	82	M'
Cooling Pond	0	18	0	48	0	66	M'
Magarach	0	3	0	88	0	91	M'
Uman	1	10	0	62.5	1	72.5	M'
Kyiv	0	4	2	2	2	26	M'
Lubny	0	7	0	18	0	25	M'
Pyryatyn	0	2	0	60	0	62	M'
Varva	0	9	0	76	0	85	M'
Chornobyl	0	11	0	62	0	73	M'
Harwich		0		0		0	

Therefore, the presence of *P* element copies has been registered in all studied populations of *Drosophila melanogaster* in Ukraine. All populations in the study represented M' cytotype, which suggests a comparatively recent invasion of Ukrainian populations of *Drosophila melanogaster* by this element. Ukrainian populations, thus, are experiencing a transitional state of a *P* element invasion, and evolution of P cytotype is expected in future. Search for possible occurrence of P cytotype in other regions of Ukraine not investigated in this study would potentially bring insights into the timing of this transitional period, as in case P cytotype is found somewhere, its sooner spread over the territory of Ukraine may be expected. The low level of gonadal reduction in A crosses suggests the absence of active transposition of mobile elements in natural populations of *Drosophila* in Ukraine. The results obtained are interesting not only on a local scale. They contribute to the worldwide pattern of *P* element invasion of *Drosophila melanogaster* populations.

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References: Bingman, P.M., M.G. Kidwell, and G.M. Rubin 1982, Cell 29: 995-1004; Broadhead, R.S., J.F. Kidwell, and M.G. Kidwell 1977, J. Hered. 68: 323-6; Clarck, J.B., and M.G. Kidwell 1997, Proc. Natl. Acad. Sci. USA 94: 11428-11433; Daniels, S.B., K.P. Preston, L.D. Strausbaugh, M.G. Kidwell, and A. Chovnik 1990, Genetics 124: 339-355; Engels, W.R., and C.R. Preston 1980, Genetics 95: 111-128; Gamo, S., M. Sakajo, and K. Ikeda 1990, Jpn. J. Genet. 65: 277-285; Golubovski, M.D., and E.S. Belyaeva 1985, Genetika 21: 1662-1670 (in Russian)**;** Itoh, M., N. Sasai, Y. Inoue, and M. Watada 2001, Heredity 86: 206-212; Itoh, M., N. Takeuchi, M. Yamaguchi, M.-T. Yamamoto, and I.A. Boussy 2007, Genetica 131: 21-28; Jensen, P.A., J.R. Stuart, M.P. Goodpaster, J.W. Goodman, and M.J. Simmons 2008, Genetics 179: 1785-1793; Kapitonov, V.V., and J. Jurka 2003, Proc. Natl. Acad. Sci. USA 100: 6569-6574; Kidwell, M.G., 1983, Proc. Natl. Acad. Sci. USA 80: 1655-1659; Kidwell, M.G., 1985, Genetics 111: 337-350; Kidwell, M.G., J.F. Kidwell, and J.A. Sved 1977, Genetics 86: 813-833; Kovalenko, L.V., L.V. Zahkarenko, M.A. Voloshyna, T.V. Karamysheva, N.B. Rubtsov, and I.K. Zakharov 2006, Genetika 42: 748-756 (in Russian); Protsenko, O.V., and I.A. Kozeretska 2006, Research series. Factors of

experimental evolution of organisms 3: 49-53 (in Ukrainian); Protsenko, O.V., and I.A. Kozeretska 2007, Research series. Advances and problems in genetics, selection and biotechnology 1: 288-292 (in Russian); Rio, D.C., 1990, Annu. Rev. Genet. 24: 543-578; Roberts, D.B., 1986, Drosophila: *A Practical Approach*. Oxford. 295 pp; Simmons, M.J., D.-F. Ryzek, C. Lamour, J.W. Goodman, N.E. Kummer, and P.J. Merriman 2007, Genetics 176: 1945-1955; Zakharov, I.K., 1984, Genetika 20: 42-50 (in Russian).

Effects of fumonisin B_1 to developmental stages of F2 offspring of *Drosophila melanogaster.*

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Introduction

Mycotoxins are secondary metabolites of moulds, and their compounds have toxic effects on the living organisms. The toxic effect of mycotoxins on animal and human health is referred to as mycotoxicosis (Peraica *et al*., 1999). Fumonisin B_1 is a mycotoxin produced by mainly in *Fusarium moniliforme*. Fumonisins (FBs) family includes fumonisin A_l, A_2, B_1 and B_2. Fumonisin B_1 is the best known and worked on (Gelderblom *et al*., 1988; Bezuidenhout *et al*., 1988). Fumonisins occur infrequently in foodstuffs, such as sorghum, asparagus, rice, beer, and mung beans (Creppy, 2002).

It has been shown that Fumonisin B_1 causes a number of heavy damage effects in animals, including equine leukoencephalomalacia (Gelderblom *et al*., 1988; Marasas *et al*., 1988; Kellerman *et al*., 1990), porcine pulmonary edema (Harrison *et al*., 1990), hepatotoxicity (Gelderblom *et al*., 1996), nephrotoxicity, and genotoxity (Knasmuller *et al*., 1997).

Since Fumonisin B_1 has a toxic effect on many organisms, we have investigated any lasting impact on development stages of *Drosophila melanogaster*. *Drosophila melanogaster* provides a powerful system in which to use genetic and molecular approaches to investigate human genetic diseases (Chien *et al*., 2002). It also has many advantages for a model organism, because, known as fruit fly, *Drosophila melanogaster* has severel features, such as different ecological adaptation to the environment, transport giant chromosomes, simple food requirements, and having a lot of genetic variation.

Materials and Methods

Oregon R wild type strain of *Drosophila melanogaster* was used in this work. All experiments were carried out 25 ± 1°C and at approximately 60% of relative humidity. The fly stocks and experimental groups were kept on Standard Drosophila Medium (SDM) containing maize-flour, agar, sucrose, dried yeast and propionic acid (Çakır and Bozcuk, 2000). Fumonisin B_1 was dissolved 10% DMSO (Dimethyl sulfoxide). 1, 3, 5 and 10 µM test solutions were prepared and added in 50 ml bottles of SDM. For experiments average same aged individuals obtained from stock culture and for

each bottle 5 male and 5 female were crossed. Individuals were fed SDM with test compounds chronically. Parents after the pupa stage were removed from the media. Obtained F1 offspring were crossed among themselves as 5 males and 5 females in bottles containing only the SDM. F2 offspring obtained throughout days 1-9 by taking into consideration the total number of individuals and gender was calculated. The experiments were repeated two times.

Results

In this study, the effects of fumonisin B_1 were evaluated in *Drosophila melanogaster*. Parents were crossed in bottles containing SDM with test compounds of fumonisin B_1. Obtained F1 offsprings were crossed in bottles containing only the SDM.

Table 1. The effect of Fumonisin B_1 on the number of F2 offspring of *Drosophila melanogaster.*

Concentration (µM/50 mL)	Female (♀)	Male (♂)	Total no of Individuals
0 µM Control	424	392	816
Control+DMSO (10 %)	404	357	761
1 µM Fumonisin B_1	392	342	734
3 µM Fumonisin B_1	305	307	612
5 µM Fumonisin B_1	255	246	501
10 µM Fumonisin B_1	249	204	453

The possible effect of Fumonisin B_1, a mycotoxin, have been examined on development stages of F1 and F2 offspring and the number of F2 offspring of R Oregon Strain of *Drosophila melanogaster* in this study. According to developmental stages of *Drosophila melanogaster*, any effect has not been found in F1 and F2 offspring (Data not shown).

The data obtained between C (Control) and C + DMSO is not a serious difference. This shows that DMSO is a solvent can be used. 1, 3, 5, 10 µM concentrations of Fumonisin B_1 showed a decrease in seriousness according to Control+DMSO (10%).

The results in Table 1 were evaluated from the statistical angle in Figure 1 and standard errors were calculated according Student ttest.

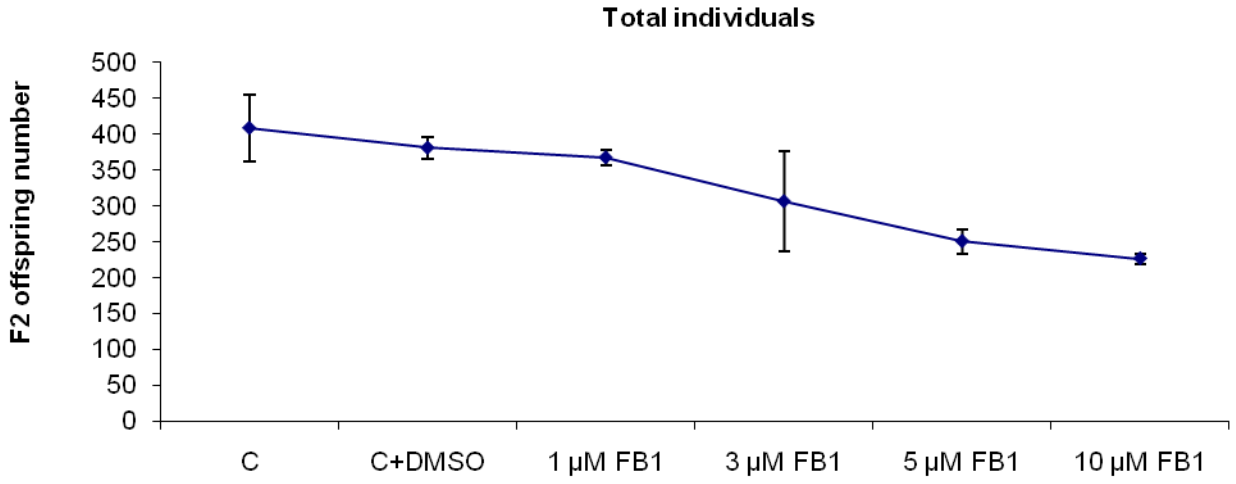

Figure 1. The levels number of total F2 offspring individuals for Fumonisin B_1 solutions as compared with C and C+DMSO (1 µM, 3 µM, 5 µM and 10 µM). According to Student ttest. C: Control, DMSO: Dimethyl sulfoxide, FB_1: Fumonisin B_1.

Discussion

We have investigated any permanent impact on development stages of *Drosophila melanogaster* in this study. Thus F1 and F2 generations of *Drosophila melanogaster*, obtained from parents that were fed in bottles containing SDM with test compounds of Fumonisin B_1 chronically, were checked in terms of developmental stages of *Drosophila melanogaster*. But any effect has not been found. In addition to this, the number of F2 offspring was calculated in respect of gender and total number. A significant decrease was observed in all concentrations according to Control+DMSO (10%) (Table 1).

These results have concluded that Fumonisin B_1 has lasting effect on *Drosophila melanogaster*. There are very few reports available on the adverse effects of *Fusarium moniliforme* (Gelderblom *et al*., 1988). This fungus occurs worldwide on a variety of plant hosts (Nelson *et al*., 1991). Fumonisins are toxic metabolites of the fungus *Fusarium moniliforme*, which is a common contaminant of corn everywhere in the world. The fumonisins are carcinogenic in laboratory rats, and cause acute toxicity of domestic animals that mimics field cases of disease attributed to contamination of feed by *Fusarium moniliforme* (Norred, 1993). Fumonisin B_1 is the major fumonisin present both in cultures and in naturally contaminated samples (Nelson *et al*., 1991).

References: Bezuidenhout, S.C., W.C.A. Gelderbloom, C.P. Gorst-Allman *et al*., 1988, J. Chem. Soc., Chem. Commun. 743-745; Chien, S., L.T. Reiter, E. Brier, and M. Gribskov 2002, Nucleic acids Research. 30: 149-151; Creppy, E.E., 2002, Toxicology Letters 127: 19–28; Çakır Ş., and A. Bozcuk 2000, Turk J Biol. 24: 321–329; Gelderblom, W.C., K. Jaskiewicz, W.F.O. Marasas, P.G. Thiel, R.M. Horak, R. Vleggaar, and N.P.J. Kriek 1988, Appl. Environ. Microbiol. 54: 1806-1811; Gelderblom, W.C.A., S.D. Snyman, S. Abel, S. Lebepe-Mazur, C.M. Smuts, L. Van der Westhuizen, and W.F.O. Marasas 1996, Adv. Exp. Med. Biol. 392: 279-296; Harrison, L.R., B.M. Colvin, J.T. Greene, L.E. Newman, and J.R. Cole 1990, J. Vet. Diag. Invest. 2: 217-221; Kellerman, T.S., W.F.O. Marasas, P.G. Thiel, *et al*., 1990, Onderstepoort J. Vet. Res. 57: 269-275; Knasmuller, S., N. Bresgen, F. Kassie, V. Mersch-Sundermann, W. Gelderblom, E. Zohere, and P.M. Eckl 1997, Mutat. Res. 13: 39-48; Marasas, W.F.O., T.S. Kellerman, W.C.A. Gelderblom, J.A.W. Coetzer, P.G. Thiel, and J.J. van der Lugt 1988, Onderstepoort J. Vet. Res. 55: 197-203; Nelson, P.E., R.D. Plattner, D.D. Shackelford, and A.E. Desjardins 1991, Applied and Environ. Microbial. 57: 2410-2412; Norred, W.P., 1993, J. Toxicol. Environ. Health. 38: 309-28; Peraica, M., B. Radic, A. Lucic, and M. Pavlovic 1999, Bulletin of the World Health Organization. 77 (9).

Spatial and temporal variation in Drosophilidae (Diptera) abundance in three environments with different vegetal cover levels in a park in Porto Alegre, southern Brazil.

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Introduction

The Drosophilidae family encompasses 3,952 species distributed in 73 genera and two subfamilies (Steganinae and Drosophilinae). The subfamily Drosophilinae is the most diversified one, and includes 3,240 species distributed across 44 genera, of which *Drosophila* is the most speciose, with 1,159 species recorded (Bächli, 2009). Some of these species are endemic, while others are cosmopolitan and may often disperse in association with human activities (Tidon-Sklorz and Sene, 1999).

The *Drosophila* species are observed in essentially any environment, from the sea level to considerable altitudes, and in temperate as well as in equatorial zones (Throckmorton, 1975). However, Lachaise (1979) suggests that these species are subject to restrictions as regards the habitats they live in. Other authors have also stressed the trend exhibited by certain species towards occupying inner forests or else some sort of open environment (Dobzhansky and Pavan, 1950; Sene *et al.*, 1980). Studies that have evaluated drosophilid assemblages in forest fragments demonstrate the clear segregation between the faunistic composition of the inner forest and the disturbed areas. These same studies have revealed that species diversity increases gradually with forest fragment size (Martins, 1987, 1989). Climatic variables such as humidity, rainfall, temperature, and incidence of sunlight, among others, are determining factors in the occurrence of drosophilid species (Pavan, 1959). Similarly, biotic factors like the kind of vegetation that form natural gradients and changes associated to latitude, for example, are also important (Powell, 1997). Therefore, the composition and structure of a drosophilid assemblage depends on the habitat in which it was established. Studies on drosophilid assemblages aim at offering a simplified explanation of the complex systems and circumstances that are repeatedly observed in different habitats. The recognition of patterns at these organizational levels affords to propose hypotheses about the ecology and evolution of the groups studied (Begon *et al.,* 1990). Due to increased global habitat destruction, studies on the diversity of insects in various environments are highly important regarding the comprehension of biological assemblages and the devising of conservation strategies (Purvis and Hector, 2000).

The present study provides information on the spatial and temporal structure of the Drosophilidae family in three environments (open field, forest edge and inner forest) along the four seasons during one year in Gabriel Knijnik Park, in the city of Porto Alegre, southern Brazil.

Materials and Methods

Adult drosophilids were collected in 2004, in Gabriel Knijnik Park (30º06'12.6"S, 51º12'10.5"W). The park covers an area of 119,545 m² and is located in the city of Porto Alegre, southern Brazil (Figure 1). Collections were made in the four seasons of the year: summer (February), autumn (April), winter (July) and spring (October). Three environments in the park were evaluated and classified according to the vegetal cover: (i) field, (ii) forest edge, and (iii) inner forest (Figure 2). The collection sites in the inner forest were located at least 200 m away from the forest edge.

To attract Drosophilidae flies 5 kg of banana and 5 kg of orange placed on the ground and covered with yeast were used. Baits were distributed as identical amounts in the three studied

environments. After three days, the insects were captured with entomological nets and taken to the laboratory where they were identified by their external morphology. Sibling species identification was carried out by dissection and analysis of male genitalia. The species belonging to the *willistoni* subgroup of *Drosophila* (*D. willistoni* and *D. paulistorum*) were identified by electrophoresis of the Acid Phosphatase-1 enzyme (Acph-1), according to Garcia *et al.* (2006).

Drosophilid populations were compared using the absolute numbers and frequencies of the species recorded. The χ^2 test was used to assess the variations in these species' frequencies across the different environments and seasons of the year.

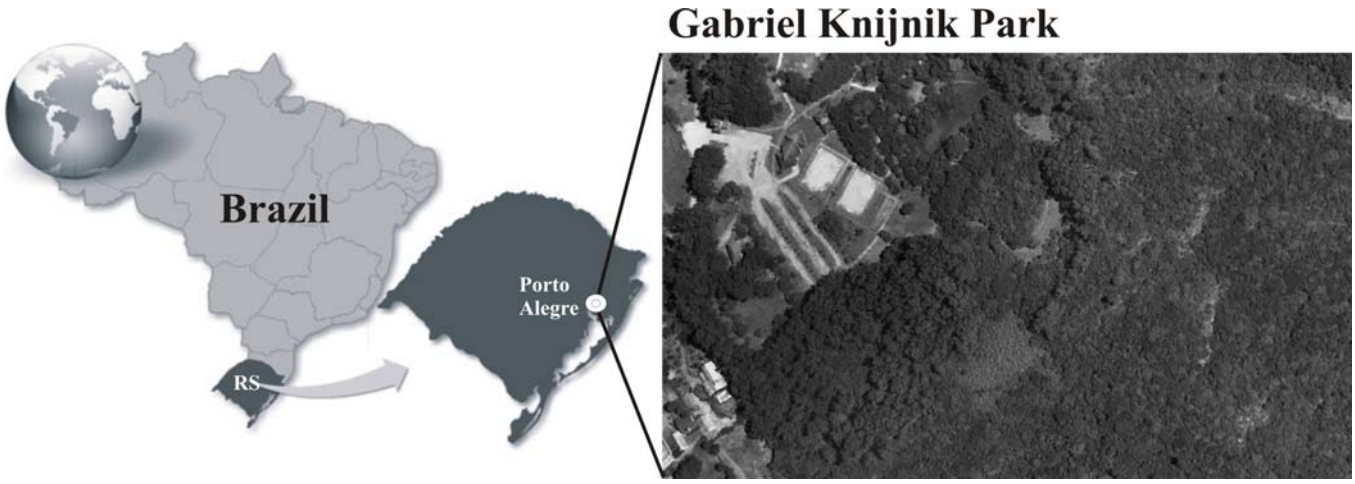

Figure 1. Map of Brazil showing the state of Rio Grande do Sul (RS), where the city of Porto Alegre is located (white circle). The zoomed out image shows an aerial photograph of Gabriel Knijnik Park.

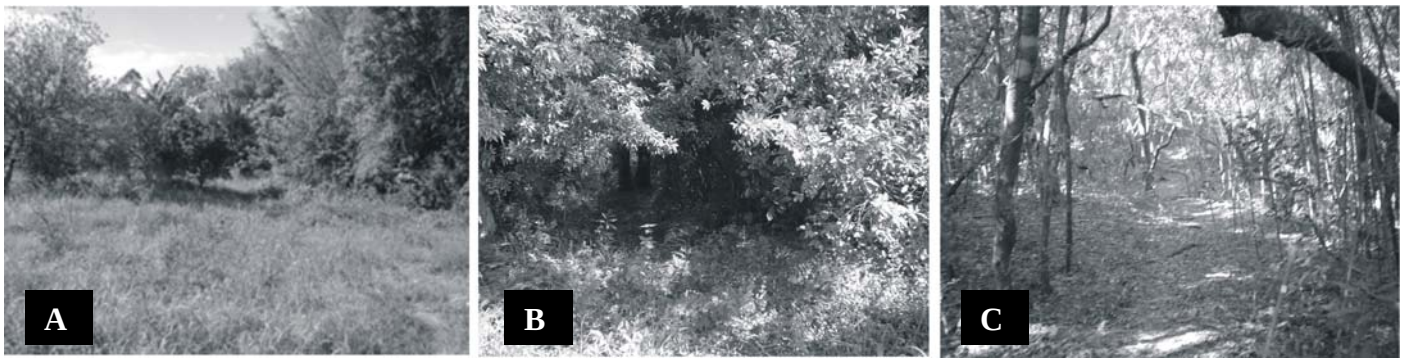

Figure 2. Areas sampled in Gabriel Knijnik Park, Porto Alegre, Brazil. A = Field; B = Forest edge; C = Inner forest.

Results and Discussion

The collections conducted throughout 2004, in the four seasons, in the three environments of Gabriel Knijnik Park afforded to gather 1,377 individuals that belonged to 25 species of the Drosophilidae family. From these, five species were exotic (*Zaprionus indianus*, *Drosophila simulans*, *D. melanogaster*, *D. ananassae*, and *D. immigrans*) (Table 1).

Table 1. Absolute number of drosophilids collected in the four seasons in the year 2004, in the three environments investigated in Gabriel Knijnik Park, Porto Alegre, Brazil. F = Field; E = Forest edge; I= Inner forest.

Species	Author, year	Seasons												TOTAL (%)
		Summer			Autumn			Winter			Spring			
		F	E	I	F	E	I	F	E	I	F	E	I	
Drosophila willistoni	Sturtevant, 1916	12	154	62	37	40	143	4	2	6	4	4	5	473 (34.35)
D. simulans†	Sturtevant, 1919	165	48	2	47	18	33	28	4	0	4	1	0	350 (25.42)
D. mediopunctata	Dobzhansky and Pavan, 1943	0	0	1	0	0	1	11	33	187	12	25	25	295 (21.42)
Zaprionus indianus†	Gupta, 1970	16	7	0	51	18	3	0	0	0	0	0	0	95 (6.90)
D. mediosignata	Dobzhansky and Pavan, 1943	0	0	0	0	0	0	1	2	17	0	0	0	20 (1.45)
D. griseolineata	Duda, 1927	0	4	1	0	0	6	0	1	3	3	0	2	20 (1.45)
D. maculifrons	Duda, 1927	0	0	0	0	0	0	2	2	11	0	2	0	17 (1.23)
D. capricorni	Dobzhansky and Pavan, 1943	0	10	1	0	0	0	0	0	4	0	0	0	15 (1.09)
D. polymorpha	Dobzhansky and Pavan, 1943	0	5	0	0	1	0	4	0	3	0	0	0	13 (0.94)
D. melanogaster†	Meigen, 1830	0	12	0	0	0	0	0	0	0	0	0	0	12 (0.87)
D. nappae	Vilela *et al*., 2004	0	0	0	0	0	0	0	0	12	0	0	0	12 (0.87)
D. paulistorum	Dobzhansky and Pavan, 1949	0	3	2	0	0	4	0	0	0	2	0	0	11 (0.80)
D. nebulosa	Sturtevant, 1916	0	2	0	4	3	1	0	0	0	0	0	0	10 (0.73)
D. bocainensis	Pavan and Da Cunha, 1947	0	0	0	0	0	0	0	1	2	0	3	1	7 (0.51)
D. bandeirantorum	Dobzhansky and Pavan, 1943	0	0	0	0	0	0	0	0	2	0	0	4	6 (0.44)
D. immigrans†	Sturtevant, 1921	0	1	0	0	0	0	0	1	0	3	0	1	6 (0.44)
D. mercatorum	Patterson and Wheller, 1942	0	0	0	1	0	0	2	0	1	0	0	0	4 (0,29)
D. sturtevanti	Duda, 1927	0	1	1	0	0	1	0	0	0	0	0	0	3 (0.22)
D. angustibucca	Duda, 1925	0	0	0	0	0	0	0	0	0	1	0	1	2 (0.14)
D. neocardini	Streisinger, 1946	0	1	0	0	0	0	0	0	0	0	0	0	1 (*)
D. pallidipennis	Dobzhansky and Pavan, 1943	0	0	0	0	0	0	1	0	0	0	0	0	1 (*)
D. ornatifrons	Duda, 1927	0	0	0	0	0	0	1	0	0	0	0	0	1 (*)
D. annulimana	Duda, 1927	0	0	0	0	0	0	0	0	1	0	0	0	1 (*)
D. ananassae†	Doleschall, 1858	0	0	0	0	0	0	0	0	1	0	0	0	1 (*)
D. parabocainensis	Carson, 1954	0	0	0	0	0	0	0	0	0	0	0	1	1 (*)
TOTAL		193	248	70	140	80	192	54	46	250	29	35	40	1377
(%)		(14.02)	(18.01)	(5.08)	(10.17)	(5.81)	(13.94)	(3.92)	(3.34)	(18.16)	(2.11)	(2.54)	(2.91)	

(*) Frequency under 0.1%. † Exotic species.

The highest absolute number of individuals was collected in summer (511 specimens), after which came autumn (412), winter (350) and spring (104). The highest species diversity was observed in winter (17 species), followed by summer (13), spring (11), and autumn (10) (Table 1).

When the total number of individuals collected throughout the year is considered, the most abundant species were *D. willistoni* (34.35%), *D. simulans* (25.42%), *D. mediopunctata* (21.42%), and *Z. indianus* (6.90%). Species representativeness varied across the sampling periods, for the species listed above. *Drosophila willistoni*, *D. simulans,* and *Z. indianus* occurred at significantly higher frequencies in summer ($\chi^2 = 378.73$; df = 3; $P < 0.0001$) and autumn ($\chi^2 = 255.43$; df = 3; $P < 0.0001$). In opposition, *D. mediopunctata* was significantly more frequent in winter ($\chi^2 = 518.15$; df = 3; $P < 0.0001$) and spring ($\chi^2 = 121.90$; df = 3; $P < 0.0001$) (Figure 3).

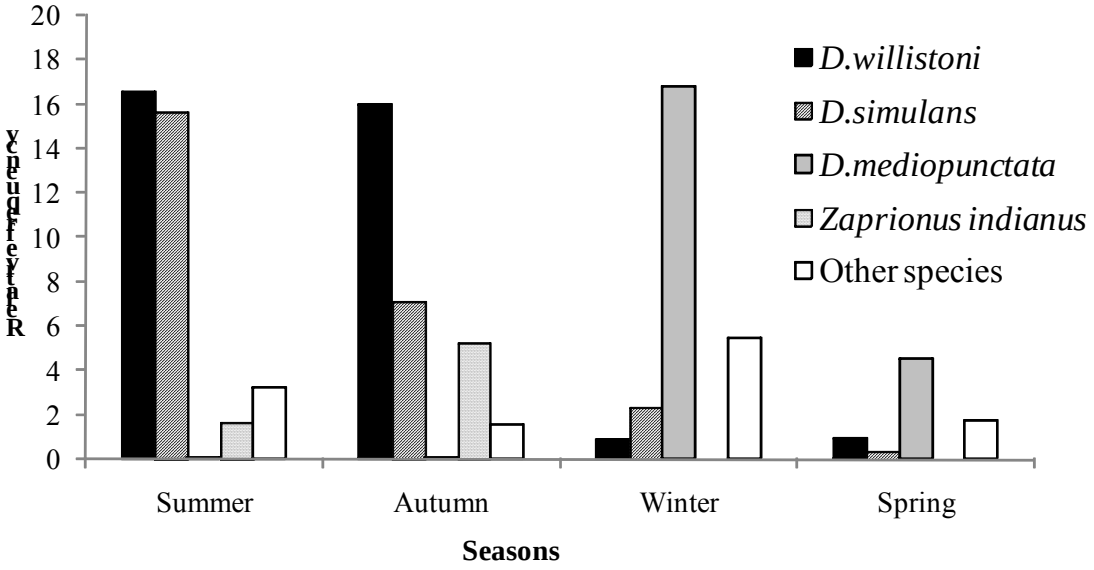

Figure 3. Relative frequency of the most abundant drosophilids collected along the four seasons of the year 2004 in Gabriel Knijnik Park, Porto Alegre, Brazil.

Several studies have been carried out in natural environments in southern Brazil (Frank and Valente, 1985; Valente and Araújo, 1991; Saavedra *et al.*, 1995; Döge, 2006), all of which pointed to peaks in *D. willistoni* abundances in autumns and summers, as observed in the present study. Our results for *D. mediopunctata* likewise agree with the literature data, which demonstrate that this species is often observed in winter (Sene *et al*., 1980; Saavedra *et al*., 1995). This species' maturity span is quite long, with the first eggs being laid on the seventh day after eclosion. Also, *D. mediopunctata* fecundity is low and longevity is high. Temperature increases have been pointed to affect positively the population size of *D. simulans* (Döge, 2006), an exotic species commonly observed in forests across Brazil and especially in disturbed environments (Saavedra *et al*., 1995). This is possibly due to the fact that this species' growth speed rises with temperature, reaching its optimum at 29ºC (Cohet *et al*., 1979). Similarly, in the present study *D. simulans* was at its most frequent in summer, when temperatures are higher.

According to the theoretical model proposed by Sevenster and Van Alphen (1993), short life cycle species prevail when resources abound, since these species consume these supplies more quickly, excluding other species by competition. This could be seen as an explanation for the overwhelming dominance of *D. willistoni* in summer and autumn. On the other hand, in a scenario of

paucity of resources species with longer life cycles are at an advantage, since they have higher longevity values and lower metabolic rates and, therefore, exhibit greater probabilities of success in finding an oviposition site. This explains the expansion and dominance of *D. capricorni* and of the species of the *tripunctata* group in winter.

As for the seasonal oscillations in *Z. indianus* frequencies, an exotic species that invaded the Brazilian territory in the 1990's (Vilela, 1999), several studies have demonstrated the species' limitation to colder temperatures. Under lower temperatures, *Z. indianus* abundances dwindle and may even disappear completely from one environment (Gottschalk *et al*., 2007; Silva *et al*., 2005a,b; Garcia *et al*., 2008). In a recent study also carried out in the city of Porto Alegre, Garcia *et al*. (2008) have observed that very low temperatures constitute a limiting factor concerning *Z. indianus* population size.

When the different environments sampled in Gabriel Knijnik Park are considered, the highest number of species was recorded for the inner forest (21), followed by the forest edge (16) and field (15). In all sampling periods specified in the present study, the highest number of individuals was likewise collected in the inner forest (552). Field and forest edge came next, producing 416 and 409 specimens, respectively. It was also observed that the most abundant exotic species, *Z. indianus* and *D. simulans*, exhibited a significant preference for the field areas ($\chi^2 = 302.64$; df = 3; $P < 0.0001$), while *D. mediopunctata* and *D. willistoni*, the most common native species in all sampling periods, were significantly the prevailing species in occupying the inner forest ($\chi^2 = 332.73$; df = 3; $P < 0.0001$). In the forest edge, the representativeness of *D. willistoni* was significantly higher ($\chi^2 = 200.01$; df = 3; $P < 0.0001$) (Figure 4).

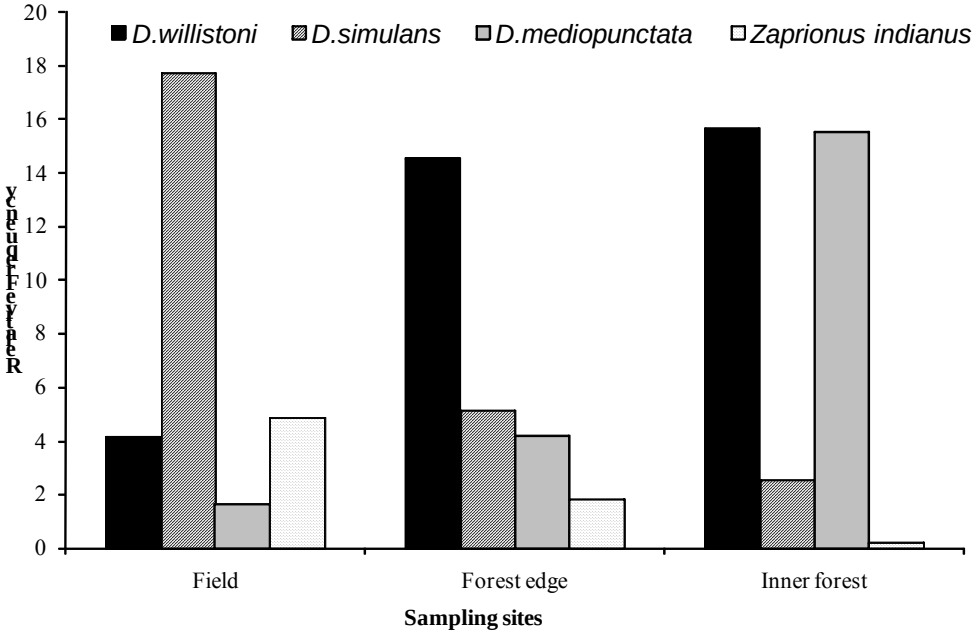

Figure 4. Relative frequency of the most abundant drosophilids collected in the three environments investigated in Gabriel Knijnik Park, Porto Alegre, Brazil.

Drosophila willistoni is the dominant species in tropical and subtropical forest areas in the Neotropical region (Dobzhansky and Pavan, 1950; Erhman and Powell, 1982; De Toni and Hofmann, 1995; Saavedra *et al*., 1995; Döge, 2006). This species occurs in higher numbers in more shady and

humid areas (Martins, 1987), which has also been observed in the present study, manifested in the preference for the forest edge and inner forest. The predilection for forest environments exhibited by *D. mediopunctata* was verified by Mata *et al.* (2008). *Zaprionus indianus* has been consistently observed in open environments (Tidon *et al*., 2003; Mata *et al*., 2008) and urbanized (Gottschalk *et al*., 2007), though it is rare in forests (Döge, 2006). *Drosophila simulans*, like *Z. indianus*, has higher relative frequency in open or dry environments as the Cerrado and the Caatinga, and thus it is considered adapted to arid conditions (Sene *et al*., 1980; Vilela *et al*., 1983; Tidon *et al*., 2003; Tidon *et al*., 2005).

As regards the species recorded at lower frequencies in the present study, as a rule some were rarely observed, considering the four seasons and the three environments altogether. Yet, they were more consistently represented when their populations were assessed considering a specific season of the year and/or one of the environments studied. For instance, in the forest edge *D. melanogaster* was recorded only in summer and was the third most common species in this environment and in this season, representing 4.83% of the drosphilids collected (Table 1). The species is appointed as characteristic of urban environments (Mata *et al*., 2008), and some authors have revealed that it is most abundant in summer (Parsons and Stanley, 1981). *Drosophila nappae* occurred only in the inner forest, in winter, when it was recorded at a frequency similar to 5%, being the third more frequent species in that environment and in that season. In a study conducted in the state of Santa Catarina, southern Brazil, Döge (2006) also observed the greater representativeness of the species in winter, in an inner forest environment.

Drosophila capricorni exhibited a higher occurrence in summer (2.15%) and, in this season, the species was detected in the forest edge as well as in the inner forest. Although the species has been associated to cold climates (Dobzhansky and Pavan, 1950), only four specimens were collected in winter in the present study, accounting for 1.14% of the individuals collected in this season. Also, these four *D. capricorni* specimens collected in winter were recorded only in the inner forest (Table 1).

The frequencies of the most abundant species recorded in the present study fluctuated consistently between seasons. Similarly, the structures of drosophild assemblages recorded in the inner forest, forest edge, and field were observed to be markedly different from one another. These results underline the importance of understanding the patterns of temporal and spatial population oscillations as a means to assist in the development of conservation strategies. Also, these findings may be used as a tool in the implementation of conservation areas and the design of parks with vegetal covers that are large enough to work as ecological sanctuaries to several species that, as a rule, tend to decrease in frequency due to the urbanization and the arrival of colonizing species.

References: Bächli, G., 2009, TaxoDros: The database on Taxonomy of Drosophilidae. Electronic Database accessible at http://www.taxodros.unizh.ch. Captured on 01 December 2009; Begon, M., J.L., Harper, and C.R. Townsend 1990, In: *Ecology, Individual,s Population,s and Communities*. Blackwell Scientific Publications, Melbourne, Australia, 876 p.; Cohet, Y., J. Vouidibio, and J.R. David 1979, J. Therm. Biol. 5: 69-74; De Toni, D.C., and P.R.P. Hofmann 1995, Rev. Bras. Biol. 55: 347-350; Dobzhansky, T., and C. Pavan 1950, J. Anim. Ecol. 19: 1-14; Döge, J.S., 2006, M.Sc. Thesis. Universidade Federal do Rio Grande do Sul, pp. 1-196; Ehrman, L., and J.R. Powell 1982, In: *The Genetics and Biology of* Drosophila, vol. 3b. (Ashburner, M., H.L. Carson, and J.N. Thompson, jr., eds.). Academic Press, New York, pp. 345–384; Franck, G., and V.L.S. Valente 1985, Rev. Bras. Biol. 45: 133-141; Garcia, A.C.L., C. Rohde, G.F. Audino, V.L.S. Valente, and V.H. Valiati 2006, J. Zool. Syst. Evol. Res. 44: 212-216; Garcia, A.C.L, V.H. Valiati, M.S. Gottschalk, C. Rohde, and V.L.S. Valente 2008, Iheringia, Sér. Zool. 98: 329-338; Gottschalk, M.S., D.C. De Toni, P.R.P. Hofmann, and V.L.S. Valente 2007, Neotropical Ent. 36: 848-862; Lachaise, D., 1979, Ph.D. Thesis, Université Pierre et Marie Curie, Paris, pp. 1-1294; Martins, M.B.,

1987, Bolm Mus. Para. Emilio Goeldi 3: 195-218; Martins, M.B., 1989, Acta Amazonica 19: 265-271; Mata, R.A., M. McGeoch, and R. Tidon 2008. Biodivers. Conserv. 17:2899-2916; Parsons, P.A., and S.M. Stanley 1981, In: *The Genetics and Biology of Drosophila*, vol. 3a. (Ashburner, M., H.L. Carson, and J.N. Thompson, jr., eds.) Academic Press, New York, pp. 349-429; Pavan, C., 1959, Bolm Fac. Filos. Ciênc. S. Paulo 11: 1-81; Powell, J.R., 1997, *Progress and Prospects in Evolutionary Biology, The* Drosophila *Model* (Powell, J.R., ed.), New York, Oxford University Press; Purvis, A., and A. Hector 2000, Nature 405: 212-219; Saavedra, C.C.R., S.M. Callegari-Jacques, M. Napp, and V.L.S. Valente 1995, J. Zool. Syst. Evol. Res. 33: 62-74; Sene, F.M., F.C. Val, C.R. Vilela, and M.A.Q.R. Pereira 1980, Pap. Avul. Zool. 33: 315-326; Sevenster, J.G., and J.J.M Van Alphen 1993, J. Anim. Ecol. 62: 720-736; Silva, N.M., C.C. Fantinel, V.L.S. Valente, and V.H. Valiati 2005a, Iheringia, Sér. Zool. 95: 233-240; Silva, N.M., C.C. Fantinel, V.L.S. Valente, and V.H. Valiati 2005b, Neotropical Ent. 34: 363-374; Throckmorton, L.H., 1975, In: *Handbook of Genetics* (King, R.C., ed.). Plenum Press, New York, pp. 421-467; Tidon-Sklorz, R., and F.M. Sene 1999, In: *Biodiversidade do Estado de São Paulo, Brasil, síntese do conhecimento ao final do século XX. Invertebrados terrestres* (Brandão, C.R., and E.M. Cancello, eds.), pp. 245-261; Tidon, R., D.F. Leite, and B.F.D. Leão 2003, Biol. Cons. 112: 299-305; Tidon, R., D.F. Leite, L.B. Ferreira, and B.F.D. Leão 2005, In: *Ecologia e biodiversidade do Cerrado* (Scariot, A., J.M. Felfili, and J.C. Souza-Silva, eds.), pp. 337-352; Valente, V.L.S., and A.M. Araújo 1991, Rev. Bras. Entomol. 35: 237-253; Vilela, C.R., 1999, Dros. Inf. Serv. 82: 37-39.

Expression of Gal4 alone alters DNA replication and causes cell death in ovarian follicle cells.

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Drosophila melanogaster is a powerful model organism for biological research in large part because of the many versatile genetic tools available to the fly geneticist. One of the most powerful tools is the Gal4-UAS system, which uses the yeast transcription factor Gal4 to drive expression of transgenic constructs within the developing organism (Brand and Perrimon 1993). This system has been expanded and modified to allow exquisite spatial and temporal control of expression (McGuire et al 2004). The importance of the Gal4-UAS system cannot be overstated. However, the utility of the system depends on the expression of Gal4 alone having no confounding effects on the cellular or developmental process being studied.

We have found that expression of Gal4 in ovarian follicle cells can result in disrupted developmental gene amplification, cell death, and altered egg chamber morphology. During oogenesis, the oocyte is surrounded by a layer of epithelial cells known as follicle cells which secrete proteins essential for chorion (eggshell) synthesis (see Calvi, 2006, for review). To support rapid eggshell synthesis, the DNA copy number of chorion and other genes are amplified by repeated rounds of DNA re-replication, a process known as developmental gene amplification. At Stage 10B of oogenesis, genomic DNA replication shuts down, and amplification begins at six discrete sites within the genome (Claycomb *et al.,* 2004). This amplification can be seen as nuclear foci of BrdU incorporation from stage 10B to 13 (Figure 1A, Calvi *et al.,* 1998). Surprisingly, we found that induction of an *Hsp70:Gal4* on the 3rd chromosome (Brand and Perrimon, 1993) alters BrdU

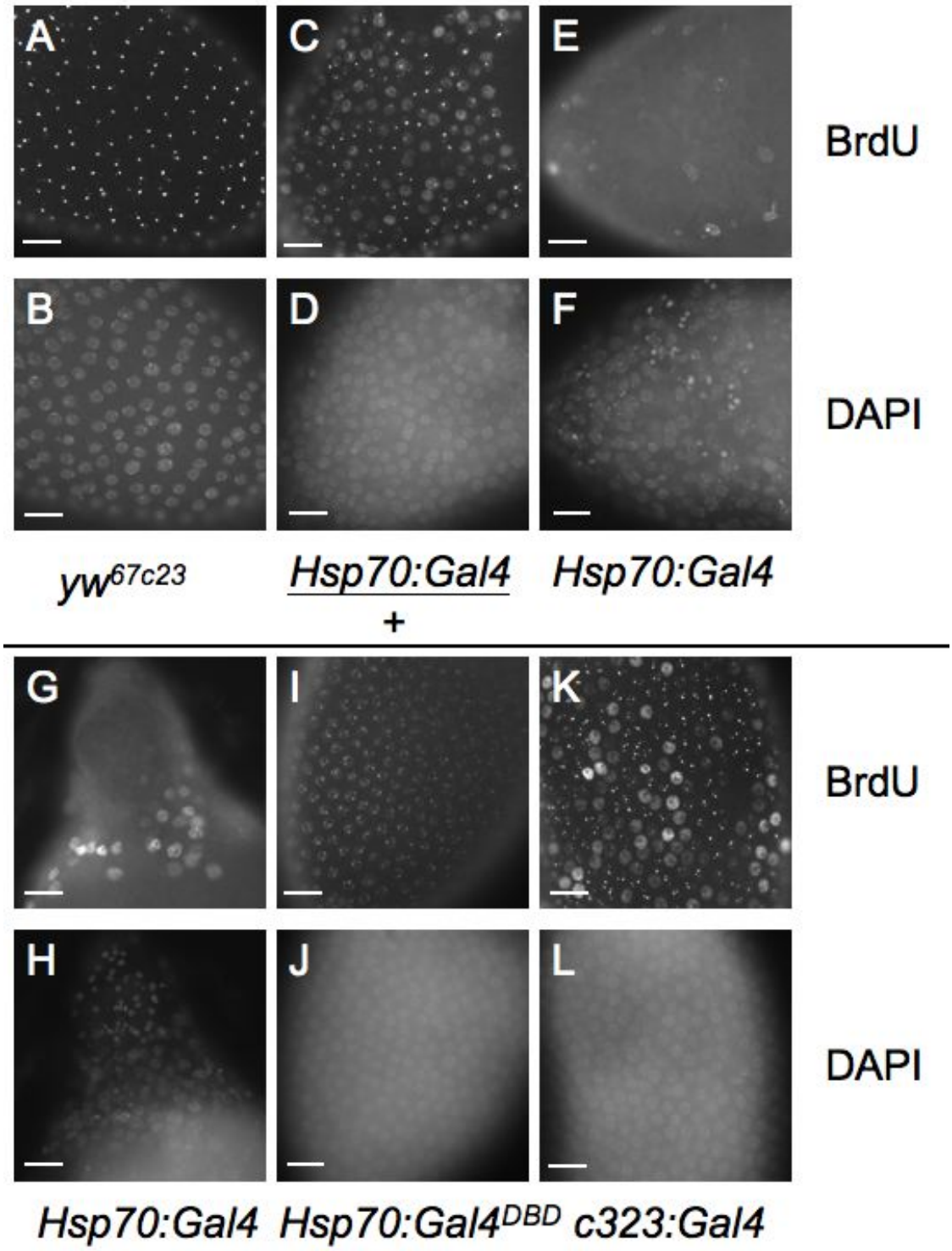

Figure 1. Adult females were heat-shocked for 30 minutes at 37°C, and allowed to recover for 14 hours at 23°C. Ovaries were dissected and labeled with BrdU (A, C, E, G, I, K) and DAPI (B, D, F, H, J, L) as described (Calvi *et al.,* 1998). Labeled ovaries were analyzed with a Leica DMRA2 fluorescent microscope. Genotypes analyzed include *yw*^{67c23} (A, B), *Hsp70:Gal4/+* (C, D), *Hsp70:Gal4* (E, F, G, H), *Hsp70:Gal4*^{DBD} (I, J), and *c323:Gal4* (K, L). Scale bars represent 10 □m in all pictures.

incorporation patterns 14 hours after heat shock induction. With one copy of *Hsp70:Gal4*, stage 10B egg chambers displayed a mixture of BrdU incorporation patterns in the follicle cells (Figure 1C). Approximately half of the cells displayed the normal BrdU focal pattern, while the remainder showed incorporation throughout the nucleus. With two copies of *Hsp70:Gal4,* BrdU incorporation is abolished in almost all follicle cells, and labeling with the fluorescent DNA dye DAPI revealed multiple pycnotic nuclei, suggesting that Gal4 expression causes cell death (Figure 1E-F). In addition, a subset of stage 9 egg chambers appeared misshapen, what we refer to as a "conehead" phenotype. In these egg chambers, the follicle cells collect at the posterior to form a tapered tip, rather than uniformly surrounding the oocyte as they normally would (Figure 1G-H). In addition, they appear to pile up on top of each other, instead of forming a normal organized epithelial layer. This suggests that Gal4 alters follicle cell migration and epithelial integrity during stage 9. These phenotypes occur with some delay and were not observed sooner than 12 hours after heat induction of Gal4 (data not shown).

The unusual BrdU incorporation and egg chamber morphology are not due to the insertion site of *Hsp70:Gal4*, as we observed that induction of an independent *Hsp70:Gal4* insertion on the 2nd chromosome (Brand and Perrimon, 1993) resulted in the same phenotypes (data not shown). Altered BrdU patterns and cell death were not observed after induction of a *Hsp70:Gal4*^{DBD}, which retains the DNA binding domain but lacks the transcriptional activation domain (Aggarwal and Calvi, 2004) (Figure 1I-J). Finally, we observed the same mosaic effect on BrdU incorporation 14 hours after heat shock of *c323:Gal4*, an enhancer trap line that expresses Gal4 in stage 8 and later follicle cells (Manseau *et al.,* 1997) (Figure 1K). While the most dramatic disruption of amplification occurred after heat shock, we have also observed altered BrdU incorporation patterns in some *c323:Gal4* egg chambers that were not treated with heat shock. Heat shock of control strains that do not contain a Gal4 transgene did not display these phenotypes, indicating that they are not due to heat stress alone (Figure 1A).

It remains unclear by what mechanism Gal4 affects DNA replication, cell viability, and egg chamber morphology. We are confident that these effects are not due to altered expression of genes at the insertion site as multiple Gal4 constructs on separate chromosomes display these same phenotypes. These effects also require the transcriptional activation domain, suggesting that altered transcription of endogenous *Drosophila* genes may be responsible. Consistent with this interpretation, a recent microarray study showed that over 1000 genes have significantly altered expression levels in larval salivary glands after induction of *Hsp70:Gal4* (Liu and Lehmann, 2008). Although these experiments were performed in different tissues, it suggests that the follicle cell transcriptome could also be drastically altered after Gal4 expression, which may lead to aberrant BrdU incorporation, cell death, and altered egg chamber morphology. Another possibility is that Gal4 may have toxic effects on the cell that are independent of its transcriptional activity.

The effect of Gal4 alone on fly cells is not unprecedented. It was previously shown that *GMR:Gal4*, which is expressed in larval eye imaginal discs, causes an increase in apoptosis and a rough eye phenotype (Kramer and Staveley, 2003). This further illustrates that Gal4 expression alone can have unforeseen effects in certain developmental contexts, which may confound the interpretation of experimental data. Our results emphasize that, although the Gal4:UAS system remains a powerful tool in *Drosophila* research, it should be used with appropriate caution and controls.

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References: Aggarwal, B., and B.R. Calvi 2004, Nature 430(6997): 372-6; Brand, A.H., and N. Perrimon 1993, Development 118(2): 401-15; Calvi, B.R., M.A. Lilly, and A.C. Spradling 1998, Genes Dev. 12(5): 734-44; Calvi, B.R. 2006, *DNA Replication and Human Disease* (DePamphilis,

M.L., ed.) 233-256; Claycomb, J.M., M. Benasutti, G. Bosco, D.D. Fenger, and T.L. Orr-Weaver 2004, Dev. Cell. 6(1): 145-55; Kramer, J.M., and B.E. Staveley 2003, Genet. Mol. Res. 2(1): 43-7; Liu, Y., and M. Lehmann 2008, Fly 2(2): 92-98; Manseau, L., A. Baradaran, D. Brower, A. Budhu, F. Elefant, H. Phan, A.V. Philip, M. Yang, D. Glover, K. Kaiser, K. Palter, and S. Selleck 1997, Dev. Dyn. 209(3): 310-322; McGuire, S.E., G. Roman, and R.L. Davis 2004, Trends Genet. 20(8): 384-91.

Drosophila melanogaster mutant *tan*.

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Drosophila melanogaster gene *tan* was originally discovered in the early 20th century as a mutant strain lacking the dark pigment pattern of wild-type (wt) flies and, therefore, showing a light yellowish brown color (McEwen, 1918). Flies lacking Tan function also exhibited abnormalities in vision (Benzer, 1967; Inoue *et al.*, 1988; True *et al.*, 2005), and *tan* males displayed an abnormal courtship behavior (Cook, 1980; Tomkins *et al.*, 1982). tan^1 (t^1) and tan^3 (t^3) alleles were found as spontaneous mutations, t^3 mutant being apparently lighter than t^1 (Brehme, 1941). *tan* is the structural gene for N-β-alanyldopamine hydrolase (NBAD-hydrolase or Tan protein), the enzyme that generates dopamine (DA) from NBAD (Wright, 1987; True *et al.*, 2005). Tan is expressed as a precursor protein of 43.7 kDa. This precursor is cleaved into two subunits of 29.9 and 13.8 kDa that apparently conform together a heterodimeric active protein (Wagner *et al.*, 2007).

The enzyme that generates NBAD from DA, the opposite reaction to the one catalyzed by Tan, is the NBAD-synthase or Ebony protein (Wright, 1987; Pérez *et al*., 1997), which is codified by the gene *ebony*. Since both Tan and Ebony are involved in cuticle tanning, carcinine regulation, and NBAD metabolism in nervous tissue (Wright, 1987; Pérez *et al.*, 1997, 2004; Hovemann *et al.*, 1998; Borycz *et al.*, 2002; True *et al.*, 2005), it has been suggested that they function together in a system regulating the levels of dopamine during cuticle sclerotization and histamine in the visual metabolism (Borycz *et al.*, 2002; Pérez *et al.*, 2010).

During the last few years, several publications appeared regarding NBAD-synthase (Wappner *et al*., 1996a, b; Pérez *et al*., 1997, 2002, 2004, 2010; Hovemann *et al.*, 1998; Borycz *et al.*, 2002; Wittkopp *et al.*, 2002; Schachter *et al*., 2007), but very little is known about *tan* (True *et al.*, 2005; Wagner *et al.*, 2007). Thus, it was important to further characterize the NBAD-hydrolase in *D. melanogaster* wt and in mutants t^1 and t^3.

Methods

All *Drosophila melanogaster* wt (*CS*) and mutant (t^1; t^3; $ebony^4$ (e^4); $white^{1118}$ (w^{1118})) strains were from Bloomington Stock Center. *Ceratitis capitata* wt (*Argentina-17*) was from INTA-Argentina Stock Center. To study NBAD-hydrolase activity we developed a heterologous coupled assay for sequential synthesis and hydrolysis of NBAD. We performed both assays in 50 mM Tris/ClH buffer, pH 7.5 at 22ºC. For NBAD synthesis the reaction mix contained dopamine (0.1

mM), $C^{[14]}$beta-alanine (5×10^4 cpm), Mg^{2+} (5 mM) and ATP (2 mM), which are essential requirements for NBAD-synthase (Pérez *et al.*, 2002). After 15 min of incubation with *C. capitata* protein extracts of 6 hours after onset of pupariation (Rabossi *et al.*, 1992), the reaction was stopped with 10 mM EDTA and the tube was boiled for 3 min. After centrifugation (10 min 14000 rpm), the supernatant was divided into two isovolumetric portions. The first one was set aside to quantify the synthesis of NBAD; the second moiety was used to develop the hydrolysis reaction (with the addition of 20 mM EDTA and *D. melanogaster* protein extract). The hydrolysis reaction was stopped after 10 minutes with 2.5% perchloric acid. Western blots were performed as described in Wittkopp *et al.* (2002). Rat Tan antibody was kindly provided by Dr. B. Hovemann, Ruhr University, Bochum, Germany. RNA was isolated from adult heads and then cDNA was synthesized using the SuperScript® II (Invitrogen) following the indications of the manufacturer. After cloning the cDNA into a T-easy vector (Promega), clones were sequenced in a 3130 Genetic Analyzer Applied Biosystem/Hitachi.

Results

We studied the activity of Tan in adults of *D. melanogaster* wt, t^1 and t^3 mutant strains in both epidermal and nervous tissues. We observed that the protein extracts from epidermis of wt, e^4 and w^{1118} flies hydrolyzed NBAD, while those from t^1 and t^3 extracts showed very low activity (Figure 1). When we studied NBAD-hydrolase activity in nervous tissue (heads), the results showed that the enzyme from wt, e^4 and w^{1118} strains hydrolyzed around 80% of total NBAD while, as expected, t^1 extracts were unable to hydrolyze NBAD (Figure 1). Surprisingly, t^3 head extracts were able to hydrolyze NBAD at almost the same rate than wt head extracts (Figure 1). We then performed western blots analysis of head and body extracts of wt, e^4, t^1 and t^3 strains (Figure 2). We found that Tan was synthesized in both epidermal and nervous tissues as precursor proteins of different sizes. The precursor protein of epidermal tissue exhibited an apparent MW of 43 kDa and the processed peptides behaved as species of 29 and 12 kDa apparent MW (Figure 2A, wt and e^4 strains). On the other hand, in nervous tissue the precursor protein had an apparent MW of 36 kDa and the processed fragments, 25 and 11 kDa, respectively (Figure 2B, wt and e^4 strains). The precursor protein was present in the protein extract of t^1, but no bands corresponding to the processed protein were found in both epidermal and nervous tissues (Figure 2A and 2B). Surprisingly, we found in epidermal extracts of t^3 the bands corresponding to both the precursor and the high MW processed peptide (Figure 2A). However, the low MW processed peptide band was not found (Figure 2A). In

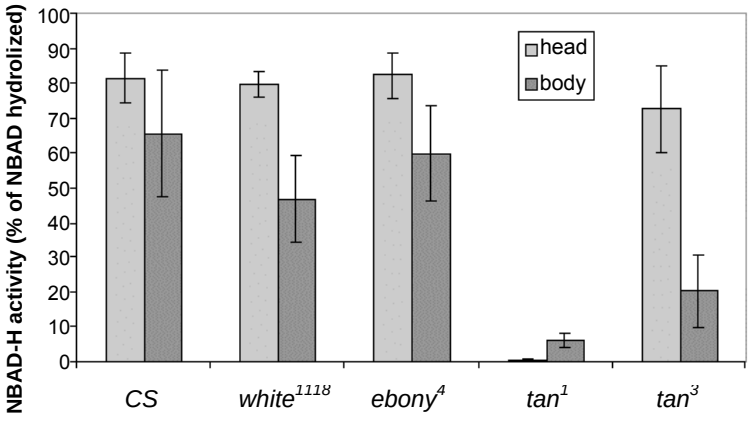

Figure 1. NBAD-hydrolase activity (% of NBAD hydrolized) in heads and bodies of wt (*CS*), w^{1118}, e^4, t^1 and t^3 strains. NBAD-hydrolase activity was normal in wt, w^{1118} and e^4; but residual in t^1 and surprisingly normal in heads of t^3 but weak in its bodies.

contrast, in head extracts of t^3 the three bands were found, although the precursor was very difficult to detect (Figure 2B).

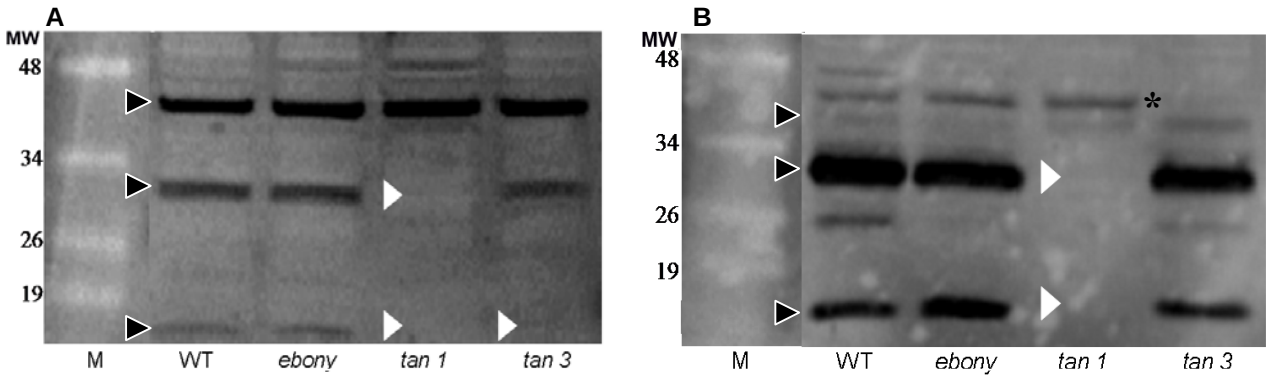

Figure 2. Western Blots analysis of bodies (A) and heads (B) extracts of wt, t^1 and t^3 strains using Tan antibody. The black arrow-heads point at the bands corresponding to the precursor protein and the processed peptides. The white arrow-heads point at the missing bands. In the left side are shown the MW (kDa) of the markers (M). Tan precursor from head epidermis was also detected (*).

In order to know the origin of the mutant defects, we isolated the coresponding mRNAs and synthesized the corresponding cDNAs. We then sequenced the cDNA of t^1 and t^3 mutants and found that t^1 had a point mutation resulting in the replacement of Arg_{217} for Pro_{217}. However, the cDNA sequence of t^3 was identical to that of wt (Pérez *et al.*, 2009).

Discussion

We developed a new method for measuring the NBAD-hydrolase activity, which consists in a coupled assay with heterologous homogenates, for sequential synthesis and hydrolysis of [^{14}C]NBAD. As far as we know, it was the first time that Tan activity was reported in adult flies, being a normally active protein in epidermal and nervous tissues. Western blot assays showed different apparent MWs for Tan in epidermis (43, 29 and 12 kDa) and nervous tissue (36, 25, and 11 kDa). This result suggests that Tan expression is regulated differentially in each tissue. As True *et al.* referred (2005), t^1 showed a point mutation, which we confirmed. We found that this mutation prevented Tan from being processed and hence from being active. Surprisingly, t^3 sequence showed no difference with wt cDNA, explaining the normal NBAD-hydrolase activity and western blot pattern in nervous tissue. Nevertheless, this mutant presented a weak NBAD-hydrolase activity in epidermis. This was in agreement with the western blot profile of t^3, where we were unable to observe the low MW processed peptide band. These results suggest that t^3 is a complex mutant with normal expression in nervous tissue and very scarce activity in epidermis.

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References: Benzer, S., 1967, Proc. Natl. Acad. Sci. 58: 1112-1119; Borycz, J., J.A. Borycz, M. Loubani, and I.A. Meinertzhagen 2002, The J. of Neurosci. 22: 10549-10557; Brehme, K.S., 1941, Proc. Natl. Acad. Sci. 27: 254-261; Cook, R., 1980, Biol. Cybern. 37: 41-51; Inoue, H., T. Yoshioka, and Y. Hotta 1988, J. Biochem. 103: 91-94; Hovemann, B.T., R.P. Ryseck, U. Walldorf, K.F. Stortkuhl, I.D. Dietzel, and E. Dessen 1998, Gene 221: 1-9; McEwen, R.S., 1918, J. exp. Zool. 25: 49-106; Pérez, M.M., A. Badaracco, and L.A. Quesada-Allué 2009, Genbank, GU144522; Pérez, M.M., J. Schachter, J. Berni, and L.A. Quesada-Allué 2010, J. Insect Physiol. 56: 8-13; Pérez, M.M., J. Schachter, and L.A. Quesada-Allue 2004, Neurosci. Lett. 368: 186-91; Pérez, M.M., N. Castillo-Marin, and L.A. Quesada-Allué 1997, Drosophila Information Service 80: 39-41; Pérez, M.M., P. Wappner, and L.A. Quesada-Allué 2002, Insect Biochem. Mol. Biol. 32: 617-625; Rabossi, A., P. Wappner, and L.A. Quesada-Allué 1992, Can. Ent. 124: 1139-1147; Richardt, A., T. Kemme, S. Wagner, D. Schwarzer, M.A. Marahiel, and B.T. Hovemann 2003, J. Biol. Chem. 278: 41160-41166; Schachter, J., M.M. Pérez, and L.A. Quesada-Allué 2007, J. Insect Physiol. 53: 1188-97; Tompkins, L., A.C. Gross, J.C. Hall, D.A. Gailey, and R.W. Siegel 1982, Behav. Genet. 12: 295-307; True, J.R., S-D. Yeh, B.T. Hovemann, T. Kemme, I.A. Meinertzhagen, T.N. Edwards, S.R. Liou, Q. Han, and J. Li 2005, Plos Genetics 1: 551-562; Wagner, S., C. Heseding, K. Szlachta, J.R. True, H. Prinz, and B.T. Hovemann 2007, J. Comp. Neurol. 500: 601-611; Walter, M.F., L.L. Zeineh, B.C. Black, W.E. McIvor, T.R. Wright, and H. Biessmann 1996, Arch. Insect Biochem. Physiol. 31: 219-233; Wappner, P., K.J. Kramer, T.L. Hopkins, J.L. Cladera, F. Manso, and L.A. Quesada-Allué 1996a, J. Insect Physiol. 42: 455-461; Wappner, P., K.J. Kramer, F. Manso, T.L. Hopkins, and L.A. Quesada-Allué 1996b, Insect. Biochem. Mol. Biol. 26: 585-592; Wittkopp, P., J.R. True, and S.B. Carroll 2002, Development 129: 1849-1858; Wright, T., 1987, Adv. in Genet. 24: 127-222.

Preliminary list of the fauna of Drosophilidae from Užice, Serbia.

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During the past forty years, fauna of Drosophildae has been collected and investigated at more than 60 geographic localities within the territory of former Yugoslavia (for review see Kekić *et al*., 1999). A total of 59 species classified into 9 genera have so far been determined; the majority of them (as much as 37) belongs to the genus *Drosophila* (Kekić, 2002). However, Serbia is not yet analyzed sufficiently with respect to Drosophilidae fauna; to be more precise, the fauna of some regions is better explored, for example, in Vojvodina and the area along the Danube river (Kekić *et al*., 1999; Kekić, 2002, 2009), while in some parts of the country faunistical reasearches were not carried out.

Here we report the results of the first faunistical research conducted in west Serbia (Užice). Užice is the administrative center of western Serbia (Zlatibor District); it is placed in the ravine, along the banks of Đetinja River, surrounded by hills.

Investigation of Drosophilidae fauna was performed during August; flies were caught in the central part of the city, around the house, in garden and orchard. According to classification of Drosophilidae habitats (the main criterion for this classification was the estimated extend of human influence on habitat). This habitat belongs to semidomestic type (locations constantly under the

human influence, Kekić, 2002). A mixture of seasonal fruit in the process of fermenting was put on plastic trays and distributed over the habitat. During the maximum of flies` activity (in the morning and evening), flies attracted by baits were captured by net. They were preserved in 70% ethanol, until species identification was done (according to Bächli and Burla, 1985; Bächli *et al*., 2004; Kekić, 2009).

At the studied locality, 10 species of Drosophilidae were determined: eight of them belong to genus *Drosophila*, one to genus *Scaptodrosophila*, and one to genus *Scaptomyza* (Table 1). Among collected species, *Drosophila melanogaster* is dominant, with almost 75% of collected individuals. This is not surprising, since ***D. melanogaster*** is cosmopolitan species, associated with man and occurs mostly near houses, being especially numerous in gardens and orchards (Shorrocks, 1974). The other cosmopolitan *Drosophila* species recorded in Užice were: *simulans*, *funebris* and *immigrans*. However, considering specific relief and immediate surroundings of this city, it is not unusual that some species usually found in woodland were observed, like ***D. helvetica*** or fungivorous ***D. kuntzei***.

Table 1. List of Drosophilidae species recorded so far in Užice, Serbia.

GENUS	SUBGENUS	SPECIES GROUP	SPECIES
Drosophila	*Drosophila*	*Drosophila funebris*	*funebris*
"	"	*Drosophila immigrans*	*immigrans*
"	"	*Drosophila quinaria*	*kuntzei*
"	*Sophophora*	*Drosophila melanogaster*	*melanogaster*
"	"	"	*simulans*
"	"	*Drosophila obscura*	*ambigua*
"	"	"	*helvetica*
"	"	"	*subobscura*
Scaptodrosophila		*Scaptodrosophila rufifrons*	*rufifrons*
Scaptomyza	*Parascaptomyza*		*pallida*

Since the city is located in the ravine, on both sides of the river that is surrounded by a specific mountain relief, we believe that the expansion of research in this region will confirm the presence of a larger number of Drosophilidae species. Those faunistic collections would yield more completed data about species composition in the studied area, and, what is more important, their distribution and relationships in broader ecological context, since Drosophilidae fauna may be used as an indicator of the environmental conditions (Milošević *et al*., 1997).

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References: Bächli, G., and H. Burla 1985, *Diptera, Drosophilidae*. Insecta Helvetica, Zürich; Bächli, G., C.R. Vilela, S.A. Escher, and A. Saura 2004, *The Drosophilidae (Diptera) of Fennoscandia and Denmark.* Fauna Entomol. Scand., v. 39. Leiden, Brill, 362 p; Kekić, V., G. Bächli, and S. Pavković-Lučić 1999, Drosophilidae fauna (Diptera) of former Yugoslavia. In: *Contributions to the Zoogeography and Ecology of the Eastern Mediterranean Region*, Vol. 1 (suppl.): 9-15; Kekić, V., 2002, The Drosophilae (Drosophilidae, Diptera) of Yugoslavia, In: *Genetics, Ecology, Evolution* (Ćurčić, B.P.M., and M. Anđelković, eds.), Monographs, Vol. VI, Institute of Zoology, pp. 109-120, Belgrade; Kekić, V., 2009, The Drosophilidae (Diptera) of Fruška Gora Mountain. In: *Invertebrates of the Fruška Gora Mountain*. II. (Ed. S. Šimić), Matica Srpska, Novi Sad, p. 63-84; Shorrocks, B., 1972, *Drosophila.* Ginn and Company Ltd., London, 144 pp.; Milošević, J., V. Kekić, D. Marinković, and S. Pavković-Lučić 1997, Fauna of the genus *Drosophila* as an indicator of the environment conditions. Biodiversity and ecological problems of Balkan fauna, 26. – 29. May, Sofia, Bulgaria. Abstracts, p. 74.

The effect of dietary restriction on developmental time in *Drosophila melanogaster* and its sibling *D. simulans.*

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Abstract

We investigated developmental time difference in response to dietary restriction (DR) in two sibling species, *Drosophila melanogaster* and *Drosophila simulans*, which were collected at the same time from two different localities in Turkey. Different diets used in this experiment were: standard (C), sugar reduced (DR-S), yeast reduced (DR-Y), and sugar with yeast reduced (DR-SY) diets. When the species developmental times in response to different DR were analyzed, both of the species showed the same pattern. We did not observe significant difference in relation to developmental time between different populations of *D. melanogaster* whereas two *D. simulans* populations showed significant developmental time differences. As a major result, egg-to-pupa developmental time was observed to be prolonged-mostly due to yeast restriction.

Introduction

D. melanogaster and *D. simulans* are sibling species of the *melanogaster* species complex. Previous studies with these two sibling species have shown many slight but significant differences due to the divergence of the ecological niches (*e.g*., David *et al.,* 2004). Although limited in scope and number, studies of life history traits in *Drosophila* have exposed considerable interspecific variability (Markow and O'Grady, 2006). The intra- and interspecific variability of life history traits can be explained not only by the genetic constitution of species or populations but also by environmental effects (food abundance, heat, etc.), and genotype by environment interaction (James *et al.*, 1997; Gibert *et al.*, 2004; Lazzaro *et al.*, 2008). Developmental time, a very important life history trait, is largely affected by environmental conditions (James and Partridge, 1995). Nutritional manipulation is one of the mostly used ways to expose the effects of food as an environmental variable on aging and development of the organisms. *Drosophila* is being increasingly used as a laboratory model for life history evolution (Powell, 1997). *Drosophila* is an organism that breeds and feeds in ephemeral substrates; therefore, the larval developmental time is a very important trait (Chippindale *et al.*, 1997; Soto *et al.,* 2006; Folguera *et al.,* 2008). Important levels of genetic variation in developmental time occur in natural populations (Cortese *et al.,* 2002; Fanara *et al.*, 2006).

The effects of DR have been investigated for more than 70 years in various organisms. Although DR is known to extend the life span of a wide range of organisms, species-specific effects of DR restriction have also been recorded (*e.g*., Mockett *et al.,* 2006). Restriction of yeast levels prolonged the life span in *D. melanogaster* (Chippindale *et al.*, 1993; Mair *et al.,* 2005; Min and Tatar, 2006; Piper and Partridge, 2007). There are various DR studies that were focused on the adult stage of *Drosophila*, but only a few studies were conducted to investigate the effects of DR on juvenile stages (Tu and Tatar, 2003).

Species that have the ability to move away from unsuitable conditions are unable to increase

their longevity with DR, because leaving an area is the best and easiest strategy (Bourg and Minois, 2005). The quality of the larval medium is very important with respect to developmental time (Chippindale *et al.*, 1997; Soto *et al.*, 2006; Folguera *et al.,* 2008), as larvae with limited dispersal ability should complete their development in the poor medium conditions.

In the scope of this study we addressed these questions: 1) What is the response of the two closely related species, *D. melanogaster* and *D. simulans*, to different DR regimes, which are applied at early developmental stages? Are there any differences between and within species? 2) Which component of the food medium affects the developmental time?

Materials and Methods

Culture and Diet

The stocks (*Drosophila melanogaster* and *Drosophila simulans*) used in this experiment were constructed from the samples taken in Edirne in September 2006 and in Antalya in May 2006 in Turkey (Table 1). The stocks have been maintained in half pint bottles with overlapping generations with a 12-12 h light-dark cycle at 25°C and 60% R.H.

Table 1. Geographical locations (as latitudes) of the populations of the two species and some relevant climatic parameters for the sampling sites.

Population	Latitude	T_{year} (C°)	R_{year} (mm)	H_{year} (%)
Edirne	41° 39’	13.56	585.9 mm	70
Antalya	36° 54’	18.17	1068 mm	64

T_{year}: Total yearly temperature; R_{year}: Total yearly rainfall; H_{year}: Total yearly humidity

Four different food types were used to measure developmental time differences due to DR. One of the food types was standard cornmeal medium, which we use to maintain our laboratory stocks. The other three food types were the restrictions with respect to the standard. The components of these food types are given in Table 2.

Table 2. Nutritional composition of experimental food types.

Food Type	Nutritional Composition (Grams of Components per Liter Water)	
	Sugar	*Yeast*
Control (C)	94 g	19 g
DR sugar / Control yeast (DR-S)	47 g	19 g
Control sugar / DR yeast (DR-Y)	94 g	9.5 g
DR sugar / DR yeast (DR-S/DR-Y)	47 g	9.5 g

Egg collection

About 500 flies were taken from each population to be the parents of the experimental flies and were transferred to 15 laying pots in approximately equal numbers containing yeasted cornmeal medium. After an acclimation period of 24 h at 25°C, flies were transferred to fresh medium for a 2 h pre-lay period and then transferred again to fresh medium for 4 h at 25°C for egg collection. Eggs were collected 4 h after the midpoint of the laying period. Eggs were placed in vials containing 7 mL food media, as five replicates consisted of fifty eggs per each experimental group.

Developmental time

Developmental time was measured as the mean egg-to-pupa developmental time, and numbers of pupae were scored every four hours a day until the number of the pupae in each vial did not change for 72 hours.

Statistical Analyses

First, we tested for differences in the mean egg-to-pupa developmental times among food types by the analysis of variance. Two way ANOVA between food type and populations within species was carried out, and also three-way ANOVA between species, population, and food type was applied to investigate the effect on developmental time.

In all cases the datasets were checked if the assumptions of normality and homogeneity of variances required for ANOVA were satisfied. All tests were done by using SPSS 15.0.

Results

DR applied in *Drosophila* by the simultaneous dilution of nutrient in the standard cornmeal medium in which the yeast was the only source of protein and sugar as the main source of carbohydrate. We tested the separate effects of sugar and yeast on egg-to-pupa developmental time and calculated average developmental time for each species and population. In this experiment, average egg-to-pupa developmental times for different populations and different food types varied between 126.08 and 214.89 hours (Table 3 and Table 4). A two-way ANOVA was carried out to evaluate the differences in the egg-to-pupa developmental time with respect to population and food type (Table 3 and Table 4). For both species a two-way ANOVA showed significant interactions between developmental time with yeast and sugar/yeast restriction. Table 3 shows the mean egg-to-pupa developmental time and significant comparisons of *D. melanogaster* populations. Developmental time of both populations that fed on DR-SY were observed to be prolonged significantly (Table 3). However, when we compared the developmental time of yeast restricted and sugar-yeast restricted populations, we found that yeast restrictions developed more slowly. In addition, developmental time of sugar restricted *D. melanogaster* Antalya population was significantly different from those of DR-Y and DR-SY groups ($p < 0.001$).

Table 3. Results of multiple comparisons of ANOVA and descriptive statistics for developmental time of *D. melanogaser*'s population.

Species / Population	Food Type	n	Mean ± S.E. (in hours)	Significant comparisons†
D. melanogaster ANTALYA	1. Control	221	142.70 ± 0.80	1-3***
	2. DR-S	219	138.32 ± 0.64	1-4*
	3. DR-Y	216	182.39 ± 1.28	2-3***
	4. DR-SY	226	163.84 ± 0.91	2-4***
D. melanogaster EDİRNE	1. Control	218	138.17 ± 0.72	
	2. DR-S	204	152.61 ± 1.13	1-3***
	3. DR-Y	211	169.00 ± 1.14	1-4*
	4. DR-SY	232	157.00 ± 0.84	

n = sample size; *S.E.*= Standard Error
*p<0.05, ***p<0.001
†significative values after Bonferroni correction for multiple comparisons.

D. simulans egg-to-pupa mean developmental times are given in Table 4. When compared to the control group, we found statistically significant differences in developmental times of all DR groups (DR-S, DR-Y, DR-SY). The Control and the DR-S groups developed faster than the DR-Y and DR-SY groups. Also the longest developmental time from egg-to-pupa was observed in the yeast-restricted group.

Table 4. Results of multiple comparisons of ANOVA and descriptive statistics for developmental time of *D. simulans*'s population.

Species / Population	Food Type	n	Mean ± S.E. (in hours)	Significant comparisons†
D. simulans ANTALYA	1. Control	209	158.01 ± 1.10	1-2**
	2. DR-S	172	135.88 ± 1.00	1-3***
	3. DR-Y	191	214.89 ± 1.73	1-4***
	4. DR-SY	194	184.52 ± 1.18	2-3***
				2-4***
				3-4***
D. simulans EDİRNE	1. Control	242	126.74 ± 0.76	1-3***
	2. DR-S	224	126.08 ± 0.82	1-4***
	3. DR-Y	185	172.84 ± 1.13	2-3***
	4. DR-SY	192	153.58 ± 1.10	2-4***
				3-4*

n = sample size; *S.E.*= Standard Error
*p<0.05, **p<0.01, ***p<0.001
† significative values after Bonferroni correction for multiple comparisons.

Table 5. Results of the analysis of variance for developmental time testing for differences between species, populations and food types.

Source of variation	*df*	*Mean Square*	*F*	*P-value*
Food	3	9363.356	152.2993	0.000
Population	1	4507.203	73.3117	0.000
Species	1	239.778	3.9001	0.053
Species*Population	1	3186.045	51.8225	0.000
Species*Food	3	957.387	15.5723	0.000
Population*Food	3	748.278	12.1711	0.000
Species*Population*Food	3	4.187	0.0680	0.978
Error	64	61.480		
Corrected Total	79			

df: degrees of freedom

Both populations of these sibling species were observed to have an extended and significant ($p < 0.001$) mean developmental time with respect to yeast restriction.

The effects of the food type, species, and population on egg-to-pupa developmental time were analyzed using a three-way analysis of variance (ANOVA) in which food type (C, DR-S, DR-Y, DR-SY), species (*D. melanogaster, D. simulans*), and population (Antalya, Edirne) constituted the three factors in the analysis (Table 5). Significant effects were established of food and population ($p < 0.001$) on developmental time. We found that the species did not affect developmental time significantly, but in margin ($p = 0.053$). There is a significant two-way interaction between species and food type ($p < 0.001$), species and population ($p < 0.001$), food type and population ($p < 0.001$), whereas we did not find any significant interactions between species, food type, and population ($p = 0.978$).

We found that yeast restriction had highly pronounced effect on developmental time and that yeast restriction put a high delay on larval development in all populations of the two species. Figure 1 shows the means of egg-to-pupa developmental times with respect to DR variation. Additionally,

the feeding in the DR-SY medium prolonged developmental time, too. The sugar-restricted groups have small, insignificant effects on developmental time.

The developmental time patterns within and between species are mostly similar (Figure 1). However, while there was a significant difference between two populations of *D. simulans* ($p < 0.001$), we did not find any significant difference between two populations of *D. melanogaster* ($p = 0.770$).

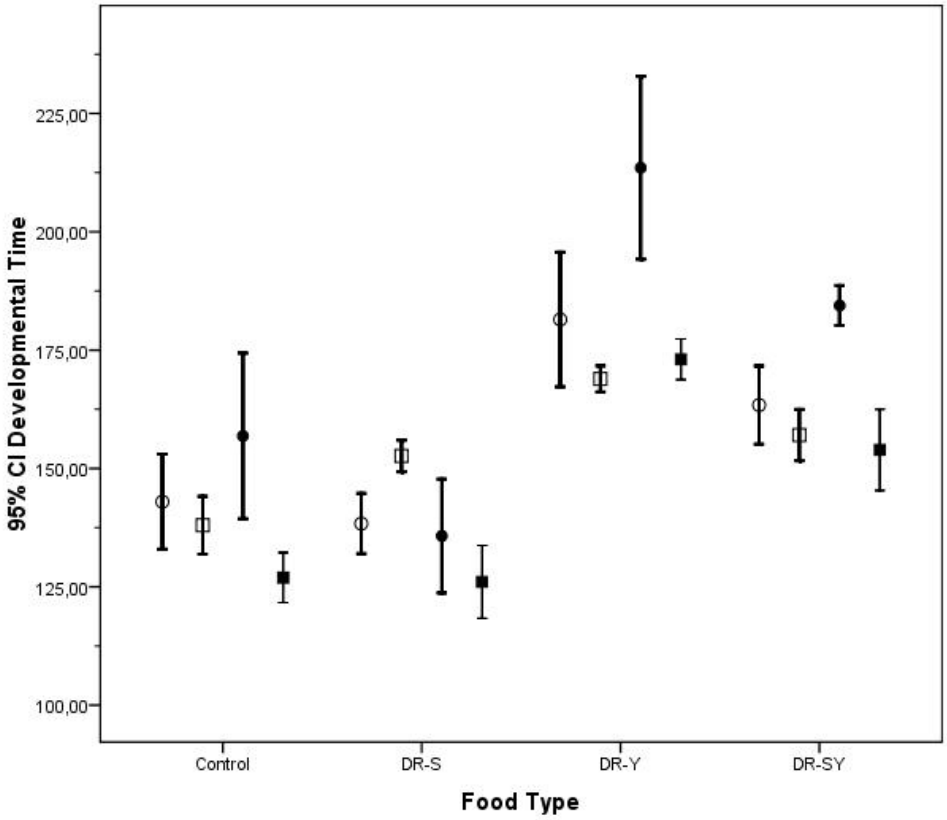

Figure 1. A 95% confidence intervals of egg-to-pupa developmental times in Antalya and Edirne populations of *D. melanogaster* and *D. simulans* feeding on different food types. (*D. melanogaster*, open labels; *D. simulans*, black labels; Antalya populations, round labels; and Edirne populations, square labels).

Discussion

As a major result of our study, developmental time is affected at-most with yeast restriction, and this observed pattern seems not to be depending on species or their populations. As seen in Tables 3 and 4, two populations of *D. melanogaster* have nearly the same overall average developmental time (slightly shorter in Edirne; not significant), but populations of *D. simulans* differ significantly from each other. The comparisons between the DR groups within population show us that restriction of yeast lengthened the developmental time at most, regardless of species or population. This may be due to the larval tendency to appropriate more protein from the yeast restricted food medium, which postpones pupation time (*e.g.*, Gebhardt and Stearns, 1993).

Nearly all of the DR studies are conducted at the adult stage. Only a few studies focused on juvenile DR and its effects (Tu and Tatar, 2003). In the study of Tu and Tatar, they found that although many adult traits were affected by larval nutrient conditions, nutrition seems to be affecting adult aging only when applied at the adult stage. They emphasize that *D. melanogaster* DR applied at the larval stage does not have an impact on adult aging, and larval yeast restriction did not cause an increasing rate of mortality. However, in our previous study we found that age-specific mortality and mean longevity is affected by larval dietary restriction depending on species, population, and sex (Onder *et al.*, 2009). There are also other evidences which indicates developmental time's correlation with some adult fitness components (Cortese *et al.*, 2002; Folguera *et al.*, 2008).

It is interesting to note that the developmental time patterns in response to DR in these siblings are quite similar. As mentioned before, the major component of food medium, which affects egg-to-pupa developmental time, is yeast. Grandison *et al.* (2009) find that dietary essential amino acids affected lifespan and fecundity in *D. melanogaster*. Especially adding methionine alone increases fecundity without shortening lifespan by female flies. The results of Grandison *et al.* imply that further investigations should focus on the effects of variable amino acid concentrations on developmental time. We conclude that further dietary restriction studies should focus on the relationship between developmental time and some other life history traits.

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References: Bourg, E.L., and N. Minois 2005, Ageing Res. Rev. 4: 409-421; Chippindale, A.K., J.A. Alipaz, H. Chen, and M. Rose 1997, Evolution 51: 1536-1551; Chippindale, A.K., A.M. Leroi, S.B. Kim, and M. Rose 1993, J. Evol. Biol. 6: 171-193; Cortese, M.D., F.M. Norry, R. Piccinali, and E. Hasson 2002, Evolution 56: 2541-2547; David, J.R., R. Allemand, P. Capy, M. Chakir, P. Gibert, G. Petavy, and B. Moreteau 2004, Genetica 120: 151-163; Fanara, J.J., G. Folguera, P.F. Iriarte, J. Mensch, and E. Hasson 2006, J. Evol. Biol. 19: 900-908; Folguera, G., S. Ceballos, L. Spezzi, J.J. Fanara, and E. Hasson 2008, Biol. J. Linn. Soc. 95(2): 233-245; Gebhardt, M.D., and S.C. Stearns 1993, Journal of Evolutionary Biology 6: 1-16; Gibert, P., P. Capy, A. Imasheva, B. Moreteau, J.P. Morin, G. Petavy *et al.* 2004, Genetica 120: 165-179; Grandison, R.C., M.D.W. Piper, and L. Partridge 2009, Nature 462: 1061-1064; James, A.C., and L. Partridge 1995, J. Evol. Biol. 8: 315-330; James, A.C., R.B.R. Azevedo, and L. Partridge 1997, Genetics 146: 881-890; Lazzaro, B.P., H.A. Flores, J.G. Lorigan, and C.P. Yourth 2008, PLoS Pathog. 4(3): e1000025; Mair, W., M.D.W. Piper, and L. Partridge 2005, PLoS Biol. 3(7): 1305-1311; Markow, T., and P.M. O'Grady 2006, Drosophila: *A Guide to Species Identification and Use*. Academic Press, London; Min, K.-J., and M. Tatar 2006, Mech. Ageing Dev. 127: 93-96; Mockett, R.J., T.M. Cooper, W.C. Orr, and R.S. Sohal 2006, Biogerontology 7: 157-160; Önder, B.Ş., M. Yilmaz, and E.D. Özsoy 2009, Hacettepe Journal of Biology and Chemistry 37(1): 1-11; Piper, M.D.W., and L. Partridge 2007, PLoS Genetics 3(4): 461-466; Powell, J.R., 1997, *Progress and Prospects in Evolutionary Biology: The* Drosophila *Model*, Oxford Univ. Press, New York; Soto, I., M. Cortese, V. Carreira, G. Folguera, and E. Hasson 2006, Genetica 127(1-3): 199-206; Tu, M.-P., and M. Tatar 2003, Aging Cell 2: 327-333.

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The mitochondrial *COI* gene fails as DNA barcoding in the sibling species of *Drosophila buzzatii* cluster.

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Abstract

The DNA barcoding using part of the *COI* region from the mitochondrial DNA was proposed to be a faster alternative method to identify species. In this study, we used this methodology to identify the seven cryptic and cactophilic species of the *Drosophila buzzatii* cluster. The identification of these species is made by comparisons of the aedagus morphology, the male reproductive structure, that make impossible to identify immature forms and females. Using three methods of molecular classification (Maximum Likelihood, Maximum Parsimony and Neighbor-Joining), we test the DNA barcoding in identification of 48 samples from these species and *D. martensis* and *D. richardsoni* as outgroups. Our results showed that the use of the DNA barcoding in the fast identification of the *D. buzzatii* species has several limitations and must be used with caution.

Introduction

The identification of species is a ubiquitous problem, considering the different fields and biological groups. The use of DNA-based analysis for species identification, the so called DNA barcoding, was proposed as an alternative path for taxonomic identification and could allow a faster characterization of the species biodiversity than the taxonomic traditional methodologies (Hebert *et al.,* 2003). For animal taxa, fragments of the *cytocrome C oxidase subunit I* (*COI*) mitochondrial gene has gained the designation of "global standard" sequence as the barcoding region for animals (Hebert *et al.,* 2003; Hajibabaei *et al.,* 2007). However, the use of DNA barcoding is controversial, including arguments agreeing (Hebert *et al.,* 2003; Tautz *et al.,* 2003; Hajibabaei *et al.,* 2007) and against (Will and Rubinoff, 2004; Meier *et al.,* 2006; Solé-Cava, 2008) the use of this technique in modern systematics.

The *Drosophila buzzatii* cluster (*D. repleta* group, *D. buzzatii* complex) is composed of seven sibling and cactophilic species: *D. buzzatii*, *D. serido, D. antonietae, D. seriema*, *D. gouveai*, *D. borborema,* and *D. koepferae* (see Manfrin and Sene, 2006). The monophyly and the intra cluster relationship were proposed based on chromosomal inversion and molecular markers (Ruiz *et al.,* 1982; Ehrman and Wasserman, 1987; Tosi and Sene, 1989; Ruiz and Wasserman, 1993; Manfrin *et al., 2001*; Manfrin and Sene, 2006). The cluster is endemic of South America, with the exception of *D. buzzatii* species, which was introduced to other continents along with its host cactus *Opuntia ficus-indica* (Barker *et al.,* 1985).

The species of the *D. buzzatii* cluster are cryptic and their identification is done using comparative analysis of the morphology of the male reproductive apparatus, the aedagus (Vilela and Sene, 1977). This fact has at least three consequences: 1) the immature individual could not be identified; 2) the female identification is possible only by their male progeny with the establishment of female isolines at the laboratory; 3) differences between species are obtained only by morphometrics analyses.

Considering the potential of *COI* gene as DNA barcoding (Hebert *et al.,* 2003; Tautz *et al.,* 2003; Hajibabaei *et al.,* 2007), we test this gene as diagnostic character in *D. buzzatii* cluster and, moreover, it was briefly discussed its use for barcoding in the *Drosophila* species.

Material and Methods

A total 576bp from the 5' end of the *COI* mitochondrial gene of 48 flies from *D. buzzatii* cluster were analyzed: *D. koepferae* (1), *D. antonietae* (7), *D. borborema* (10), *D. buzzatii* (10), *D. gouveae* (6), *D. serido* (8), and *D. seriema* (4). This sample includes 38 *COI* sequences isolated in previous works (Manfrin *et al.,* 2001; Franco 2009) and 10 new sequences. We also included in the analysis *Drosophila martensis* (1) and *D. richardsoni* (1) as outgroups. The template for the mitochondrial DNA was obtained isolated by PCR using the primers TY-J-1460 (Simon *et al.,* 1994; De Brito *et al.,* 2002) and 2191r (De Brito *et al.,* 2002). PCR products were sequenced using the ABI PRISM BigDyeTM Terminator Cycle Sequencing Ready Reaction Kit in an ABI automatic sequencer. The DNA sequences were checked by eye and aligned using the program BioEdit v. 7.0.9.0 (Hall, 1999). The data were analyzed using three algorithms. The first one was made by the program TCS v 1.21 (Clement *et al.,* 2000). This program uses statistical parsimony to construct a haplotype network. The second was a Maximum Parsimony tree (100000 reply) performed by the program PAUP v. 4.0b10 (Swofford, 2001). The third one was a Neighbor-Joining tree (10000 reply) performed by the program Mega v. 4.1 (Tamura *et al.,* 2007).

Results and Discussion

Thirty two (32) haplotypes were generated in network analysis. These haplotypes were allocated in three clades and three isolated haplotypes (Figure 1). The Clade 01 is composed only by samples of *D. buzzatii* species. The Clade 02 comprises all the samples from *D. antonietae* and two *D. gouveae* samples. In the Clade 03 was allocated samples of *D. gouveai*, *D. seriema*, *D. borborema,* and *D. serido* species. This result suggests the absence of diagnostic character for *D. buzzatii* cluster species in this genetic region. *Drosophila koepferae, D. martensis, and D. richadsoni* formed isolated clades.

The MP and NJ trees presented similar topologies, and in both of them we confirm the monophyletic status of the *D. buzzatii* cluster with high bootstrap values (Figures 2 and 3). Two main *COI* lineages were formed within the *D. buzzatii* cluster clade. The first group is formed by *D. buzzatii* and *D. koepferae* (bootstrap value of 79%), and *D. buzzatii* clearly was separated from *D. koepferae* with bootstrap value of 100% (Figure 2). The second subdivision could be divided in two considering the bootsprap values. One division comprises the same species of the Clade 02 generated by the TCS program, with bootstrap values of 91, including all the *D. antonietae* species and three *D. gouveai* samples. The second division is a polytomy including all other species of the group; in this polytomy a division with high bootstrap support (90%) includes the subsample of *D. seriema*, *D. serido,* and *D. gouveai*.

Neighbor-Joining analyses show the same consistency considering the division of the *D. buzzatii* cluster from the external group with a bootstrap value of 100 (Figure 3). Two main groups inside *D. buzzatii* cluster were formed: one containing *D. buzzatii* and *D. koepferae* samples (74% bootstrap) and the other with *D. antonietae*, *D. gouveai*, *D. seriema*, *D. serido,* and *D. borborema* (bootstrap 99). *Drosophila buzzatii* and *D. koepferae* comprised a separate group (bootstrap 100). The samples of *D. antonietae* and three samples of *D. gouveae* were grouped together (bootstrap 92),

as in the TCS and Maximum Parsimony analyses (Figures 1 and 2). The *D. borborema* species encompasses a group with one individual of *D. seriema* (bootstrap value of 63%). The species *D. seriema*, *D. serido,* and *D. borborema* could not be separated.

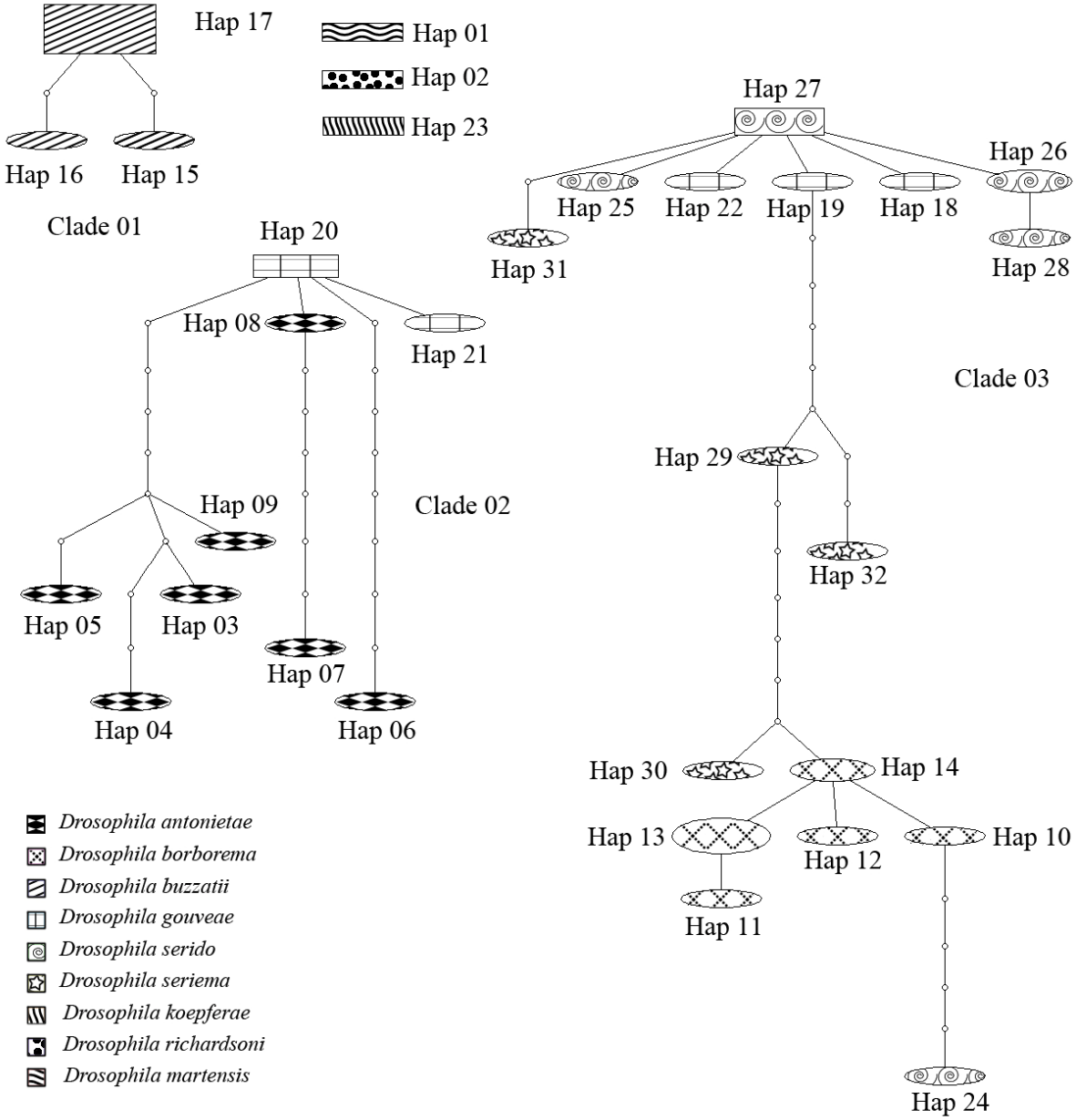


Figure 1. Haplotype network generated by the TCS program. The size of the ellipses represent the frequency of the haplotype. Retangular forms represents ancestral haplotype and the white dots the supposed and not found haplotype.

The results of these analyses are similar. The samples of *D. buzzatii* species, always grouped together as a cohesive entity, even being from different populations, and is more basal on the topology. This is according to other sets of data as morphology and molecular data (Manfrin and

Sene, 2006) that considering this species the more differentiated and most basal composing a more ancient linneage in the cluster (Manfrin and Sene, 2006). In this case, the information in the *COI* mtDNA is a good diagnostic and could be used as a DNA barcoding.

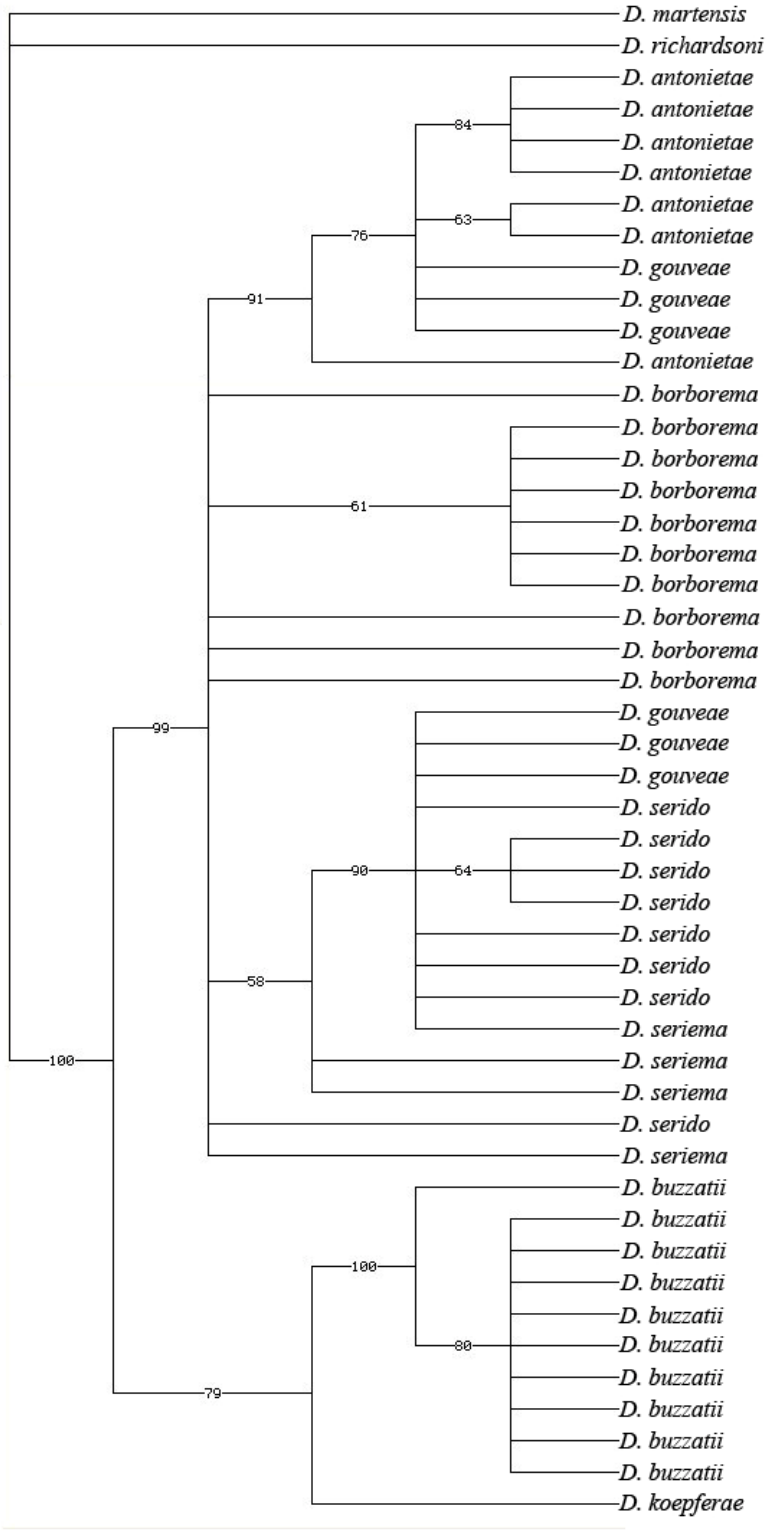
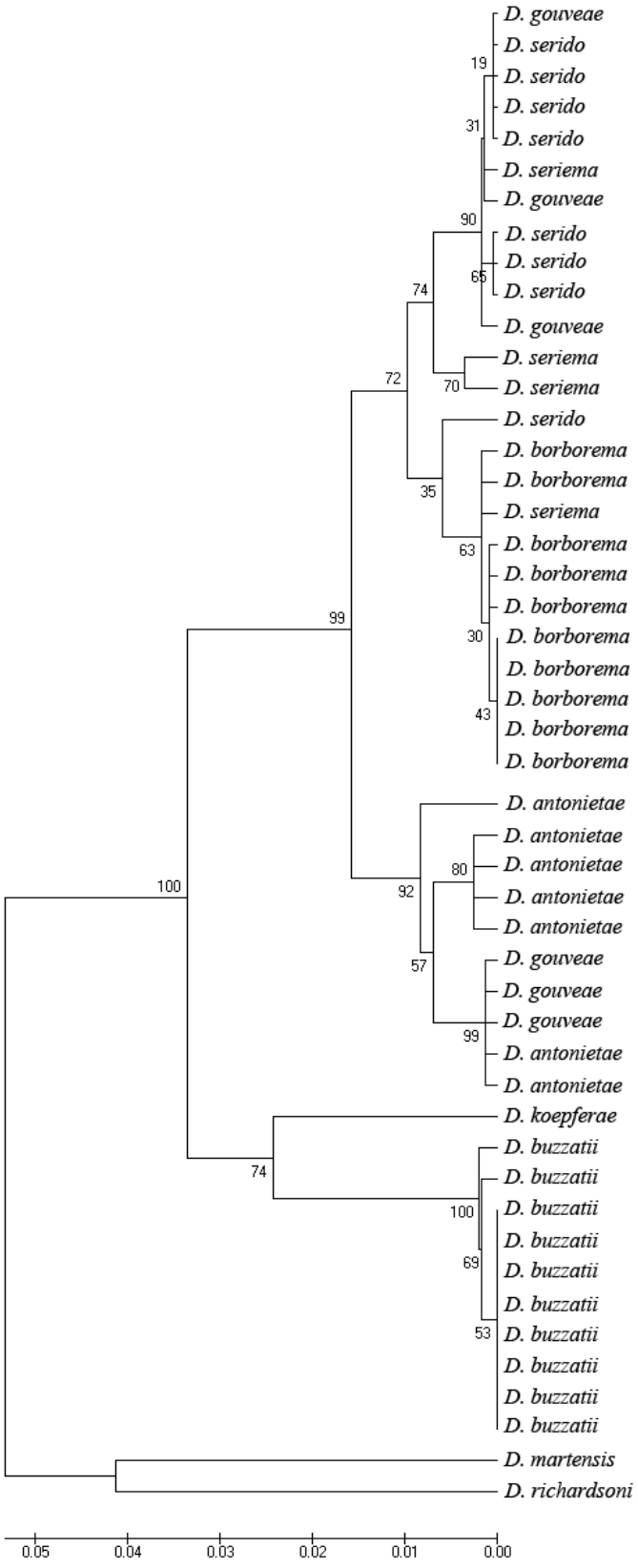


Figure 1. Parsimony tree generated by the program PAUP, using the Maximum Parsimony approach. The bootstrap values were obtained using 10000 replications.

The sample for *D. koepferae* was small and it is a limit to discuss the use the *COI* sequence as a DNA barcoding for this species. However, considering the entire previous data that proposed that this species is older in the cluster (Manfrin *et al.*, 2006), we believe that the data are a good indicator for the presence of diagnostic characters in sequence and it could be used as a DNA barcoding.

The results for *D. antonietae* samples from different geographic areas showed that they encompass a consistent group indicating that the sequence of *COI* has diagnostic character for the species being a DNA barcoding. The hypotheses to explain the clustering of the sequences of three individuals of *D. gouveai* together with *D. antonietae* is asymmetrical introgressive hybridization resulting from secondary contact between populations of this species (Manfrin *et al.*, 2001). This information confirmed that mtDNA sequences can have diagnostic character for one species, but we have to use it with caution.

The species *D. borborema, D. serido, D. seriema,* and *D. gouveai* were grouped together but with unresolved relationships



among them. These results show that the sequences of *COI* could not be used as a DNA barcoding for these species. One possible explanation for this fact is incomplete lineage sorting as the cladogenetics events are recent for this species and do not have time to acquire reciprocal monophyly (Manfrin *et al.,* 2001; Franco, 2009), this way, to have a DNA barcoding is a time depending event. As these species are sympatric in several locations we also could assume that the sharing of mtDNA sequences could be the result of introgression. In this sense, other molecular markers are required to evaluate introgressive events and give support or not to the sequences of *COI* to be used as DNA barcoding. Besides this possibility, assuming that the divergent times of cladogenetics events among these species are recent (3-6 Myr according to Manfrin *et al.,* 2001), the sharing of DNA sequences could be an ancestral polymorphism shared by those species which obscures any historical patterns maintained in the nuclear genome.

Figure 2. Similarity tree generated by the program Mega, using the Neighbor-Joining approach and bootstrap values using 10000 replications.

In conclusion, our data suggest that the mt*COI* has a limited use as a DNA barcoding and should be used with caution. In the *D. buzzatii* cluster these sequences have diagnostic information allowing its use as a DNA barcoding in species that split for long evolutionary period of times, but not when they are recent. Moreover, the so common event of introgression (Manfrin *et al.,* 2001) should be considered when DNA barcoding testing is done, which could generate many erroneous results and could not be performed for these purposes.

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References: Barker, J.S.F., F.M. Sene, P.D. East, and M.A.Q.R. Pereira 1985, Genética 67: 161-170; Clement, M., D. Posada, and K.A. Crandall 2000, Molecular Ecology 9(10): 1657-1659; De Brito, R.A., M.H. Manfrin, and F.M. Sene 2002, Genetics and Molecular Biology 25(2): 161-171; Ehrman, L., and M. Wasserman 1987, Evolutionary Biology 21: 1-20; Franco, F.F., 2009, História evolutiva do "cluster" *Drosophila buzzatii* (grupo *D. repleta*): eventos históricos e diversificação de espécies no Brasil. Ribeirão Preto, Universidade de São Paulo Faculdade de Medicina de Ribeirão Preto: 197; Hajibabaei, M., G.A.C. Singer, P,D.N. Hebert, and D.A. Hickey 2007, Trends in Genetics 23(4): 167-172; Hall, T., 1999, BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT; Hebert, P.D.N., A. Cywinska, S.L. Ball, and J.R. DeWaard 2003, Proceedings of the Royal Society of London Series B-Biological Sciences 270(1512): 313-321; Manfrin, M.H., R.A. De Brito, and F.M. Sene 2001, Annals of the Entomological Society of America 94(3): 333-346; Manfrin, M.H., and F.M. Sene 2006, Genetica 126(1-2): 57-75; Meier, R., K. Shiyang, G. Vaidya, and P.K. Ng 2006, Syst. Biol. 55(5): 715-28; Ruiz, A., A. Fontdevila, and M. Wasserman 1982, Genetics 101: 503-518; Ruiz, A., and M. Wasserman 1993, Heredity 70: 582-596; Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook 1994, Annals of the Entomological Society of America 87(6): 651-701; Solé-Cava, A.M., 2008, Código de Barras do DNA: o rabo que abana o cachorro. Ciência Hoje. Brasil, Ciência Hoje. 41: 65-67; Swofford, D., 2001, PAUP* 4.0 beta 5: Phylogenetic Analysis Using Parsimony (and other methods), Sinauer Associates Sunderland, MA; Tamura, K., J. Dudley, M. Nei, and S. Kumar 2007. Molecular biology and evolution 24(8): 1596; Tautz, D., P. Arctander, A. Minelli, R.H. Thomas, and A.P. Vogler 2003, Trends in Ecology & Evolution 18(2): 70-74; Tosi, D., and F.M. Sene 1989, Revista Brasileira de Genética 12: 729-745; Vilela, C.R., and F.M. Sene 1977, Papéis Avulsos de Zoologia 30: 295-299; Will, K.W., and D. Rubinoff 2004, Cladistics-the International Journal of the Willi Hennig Society 20(1): 47-55.

Studies on Drosophilids (Diptera: Drosophilidae) of Gujarat State in India.

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Introduction

During recent years, considerable data have been accumulated regarding faunal composition of drosophilid species as a result of extensive field collections in different ecological habitat by Ayala (1970). The drosophilid flies thus obtained have been utilized for various studies *viz*., taxonomic, ecological, genital, behavioral, and its distribution record. As a result more than 290 drosophilid species have been reported so far from different ecogeographical areas in India (Gupta, 2005), and most of them are new to the world of science. These data prompted us to undertake such studies from previously unexplored forest region of Gujarat state in India.

Materials and Methods

Collections were made twice, once in the month of September 2003, 2004 and other in February 2004, 2005 in Gujarat state forest and wild areas (Figure 1) of Jamnagar, Junagarh (Girnar Hill, agricultural area), Sassan Gir forest (Tallala, Dewalia), Navsari and Valsad (Ramnagar, Atul and Baletha), Chharodi (Ahmedabad), Panchmahal (Deroli, Virpur, Rarodra and Akrund), and Dangs (Dolwan, Vyara, Dhangdhar) by using all the three methods of collection, *i.e*. Bail trap, Net sweeping and Aspirator with the help of various fermenting fruits as bait. A total of 7693 drosophilid flies (including 4118 males and 3575 females) were collected during 2003-2004, and 5460 flies (including 2090 males and 3370 females) were collected during 2004-2005. 6208 male flies were preserved in 70% alcohol and all the male flies were grouped on the basis of distinguished morphological characters and analyzed taxonomically on the basis of genital structure (McAlpine, 1981; Okada, 1966).

Figure 1. Map showing collection Areas in Gujarat State in India.

Results and Discussion

The field collection from different ecological habitats of drosophilid flies from the Gujarat state in India in different seasons have yielded a total of 7693 flies during year 2003-2004 (4118 males and 3575 females) and 5460 flies during year 2004-2005 (2090 males and 3370 females) (Table 1). These males were grouped on the basis of morphological characters and analyzed taxonomically on the basis of genital structure. The taxonomic analysis on the basis of genital structure has helped us to identify several species of drosophilids being reported for the first time from Gujarat state. The identified species are *D. punjabiensis*, *D. bipectinata*, *D. parabipectinata*, *D. biarempis*, *D. takahashii*, *D. melanogaster*, *D. kikkawai*, *D. jambulina*, *D. tristipennis*, *D. ananassae*, *D. suzuki*, *D. malerkotliana*, *Zaprionus indionus*, *D. latifshahi*, *D. repleta*, *D. immigrans*,

Liodrosophila quadrimaculata, *L. globosa*, *Scaptomyza clavata*, and *S. pallida*. Out of them, 12 species belong to subgenus *Sophiphora*, 3 to subgenus *Drosophila* of the genus *Drosophila* and 5 species belong to genera *Zaprionus, Liodrosophila, Parascaptomyza*, and *Scaptomyza* (Table 1).

Table 1. List of *Drosophila* species collected & identified during September and February months from Gujarat forest regions in India.

Si. No.	Name of species	Genus	Subgenus	Collection time				Total no. of males
				Sep.03	Feb.04	Sep.04	Feb.05	
1	*D.punjabiensis*	*Drosophila*	*Sophophora*	385	75	435	143	1038
2	*D.bipectinata*	,,	,,	135	78	180	67	460
3	*D.parabipectinata*	,,	,,	100	65	95	59	319
4	*D.biarempis*	,,	,,	65	30	85	58	238
5	*D.takahashii*	,,	,,	95	72	78	92	337
6	*D.melanogaster*	,,	,,	115	68	135	109	427
7	*D.kikkawai*	,,	,,	215	190	228	156	789
8	*D.jambulina*	,,	,,	70	45	95	65	275
9	*D.tristipennis*	,,	,,	12	7	15	8	42
10	*D.ananassae*	,,	,,	85	67	132	90	374
11	*D.suzuki*	,,	,,	-	107	-	178	285
12	*D.malerkotliana*	,,	,,	108	80	145	64	397
13	*D.latifshahi*	,,	*Drosophila*	85	56	72	47	260
14	*D.repleta*	,,	,,	50	35	47	31	163
15	*D.immigrans*	,,	,,	-	205	-	245	450
16	*Zaprionus indianus*	*Zaprionus*	-	102	-	113	-	215
17	*Liodrosophila quardrimaculata*	*Liodrosophila*	-	20	-	34	-	54
18	*Liodrosophila globosa*	,,	-	15	-	20	-	35
19	*Scaptomyza clavata*	*Scaptomyza*	--	13	-	18	-	31
20	*Scaptomyza pallida*	,,	-	7	-	12	-	19
							Total	6208

Among the species lured to the fermenting fruits, *D. punjabiensis*, *D. takahashii*, and *Zaprionus indianus* were collected large numbers. Another interesting feature noticed during these collections were occurrence of *D. immigrans* and *D. suzukii* only during February, whereas other species of the subgenus *Sophophora* and *Drosophila* of the genus *Drosophila* were collected in all the collections. The species belonging to other genera were only collected during September. Most surprisingly, the cosmopolitan species *viz*. *D. melanogaster* and *D. ananassae* could not be recorded from wild area but only from urban regions perhaps due to better food resources availability (Bock and Parsons, 1977). These observations clearly indicate that the variation in species spectrum and species distribution depends largely upon capabilities of resource utilization, *i.e*. fermented fruits and natural decays in different environmental conditions and in different seasons (Dobzhansky and da Cunha, 1955; Gupta, 2005). The species belonging to genus *Drosophila* were found to occur in enormous number carrying to its efficiency in better resource utilization.

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References: Ayala, F.J., 1970, Competition, coexistence and evolution. In: *Essays in Evolution and Genetics in Honour of Theodosius Dobzhansky* (Hecht, M.K., and W.C. Steere, eds.). Appleton-Century-Crofts, New York): 121-158; Bock, I.R., and P.A. Parsons 1977, J. Biogeography 4: 203-213; Dobzhansky, Th., and A.B. da Cunha 1955, Ecology 36(1): 34-36; Gupta, J.P., 2005, A monograph on Indian Drosophilidae. J. of Scientific Research (B.H.U.) 51: 1-252; Okada, T.,

1966, Entomol. Suppl. 6: L1-129; McAlpine, J.F., 1981, Morphology and terminology adult IV. In: *Manual of Nearetic Diptera* (McAlpine, J.F., *et al*., eds.). 1: 9-63. Agriculture Canada Monograph No. 27. Biosystematics Research Institute, Ottawa, New York and London.

"Lights-Off" phenotype in *Drosophila* containing an UAS-Aβ42 transgene.

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Summary

The UAS-Aβ42$^{H29.3}$ transgenic *Drosophila* line used to model Alzheimer type neurodegeneration and other phenotypes falls down when transitioned from a light to a dark environment. This unusual phenotype is not dependent on the presence of a Gal4 driver and is thus unrelated to Aβ42 induced proteotoxicity or neurodegeneration. We propose that it may instead be the result of disruption in another gene function caused by the P element insertion site. Since this transgenic line is used by several groups to analyze neurological phenotypes associated with Aβ42 neuronal expression, the results of these studies must be interpreted with caution.

Introduction

Drosophila has been used to model neurodegenerative aspects of Alzheimer disease (AD) by expressing the neurotoxic human Aβ42 peptide in neurons (Crowther, *et al*., 2005; Crowther, *et al*., 2004; Crowther, *et al*., 2006; Finelli, *et al*., 2004; Iijima, *et al*., 2008; Iijima, *et al*., 2004; Ling, *et al*., 2009). As part of the usual phenotypic characterization of these flies, they are often examined for neurological phenotypes, such as locomotor functions or learning and memory tasks. We recently characterized phenotypes in one of the *Drosophila* AD models that has a UAS-Aβ42 transgene (UAS-Aβ42$^{H29.3}$) inserted on the 2nd chromosome. When Aβ42 expression was driven in either cholinergic, GABAergic, or glutaminergic motor neurons, flies show a variety of phenotypes. This includes reduced locomotor function, increased autophagy activity, and shortened lifespan. All of these phenotypes are dependent on the presence of a Gal4 neuronal driver as well as the UAS-Aβ42 responder. Since neurological phenotypes are an excellent way to analyze the consequences of transgene expression before extensive neurodegeneration takes place, we have been interested in defining additional phenotypes. We noticed that when light-adapted UAS-Aβ42$^{H29.3}$ flies are challenged with a sudden transition to darkness (*i.e*. we turn off the lights), they become disoriented and fall to the bottom of their vials. Further analysis of this phenotype, however, revealed that it was not dependent upon the presence of a Gal4 driver.

Materials and Methods

Light to Dark Sensitivity Assay

Flies containing the UAS-Aβ42$^{H29.3}$ transgene were obtained from Novartis (Finelli, *et al*., 2004) and stocks were carried over *CyO*. Expression was driven in cholinergic neurons using a 7.4 kb Cha-Gal4 transgene, and stocks also contained a UAS-GFP(S65T) reporter gene ([Cha-Gal4], UAS-GFP(S65T)/*CyO*; line 19B). Adult flies (one to four weeks old) of mixed gender were housed in groups of 100-200 in glass milk bottles at room temperature on standard *Drosophila* medium. In a darkened room, groups of 10 flies were placed in a 100 ml glass cylinder and conditioned to light by placing them directly below a fluorescent desk lamp equipped with 2 model F15T8 daylight balanced fluorescent bulbs (18 inches long, 15 watts) for 10 minutes. The light was then turned off for 10 seconds and back on for 30 seconds, and this cycle was repeated 5 times. Flies that dropped to the bottom of the cylinder each time the light was turned off were counted.

Western Blot

Expression of Aβ42 only in flies containing the Gal4 driver was confirmed by Western blotting using a protocol adapted from Ijima *et al*. (2004). Proteins were electroblotted to a PVDF membrane, which was boiled for 5 minutes in PBS and stained with anti-Aβ mouse monoclonal antibody 6E10 (Covance, 1:500 dilution). This was visualized using a horseradish peroxidase coupled goat anti-mouse second antibody (Bio-Rad, 1:40,000 dilution) followed by luminol detection (Pierce, Picoquant) using X-ray film.

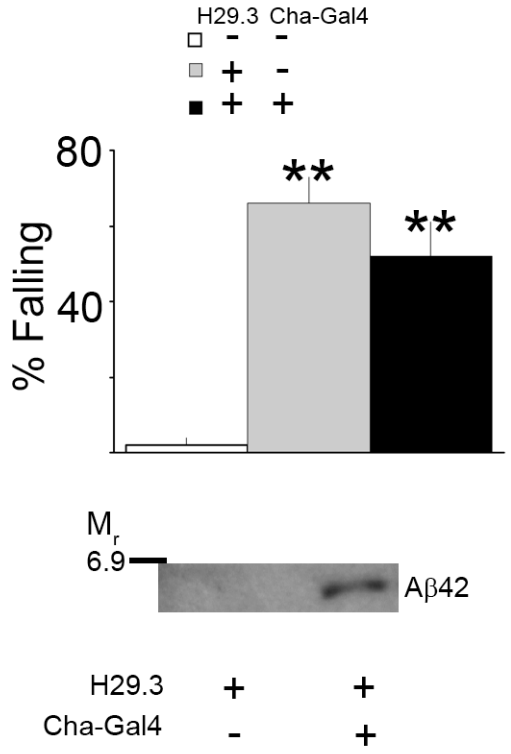

Figure 1. "Lights-Off" phenotype depends on the presence of the H29.3 UAS-Aβ42 transgene. Top: Control flies with a Cha-Gal4 driver, but no responder, rarely fall when lights are turned off. The presence of either the H29.3 responder alone or in combination with the Cha-Gal4 driver results in more than 50% of flies with the "Lights-Off" phenotype. Values are the means (± SEM) for 5 trials of groups of 10 individuals. Statistical significance was determined by individual t tests (**, $p < .01$). Bottom: Western blot showing Aβ42 expression only when the responder and driver are present together. Adult fly heads were homogenized in RIPA/SDS extraction buffer, and soluble proteins were immunoblotted with an anti-Aβ42 monoclonal antibody (Iijima, *et al*., 2004).

Results

All transgenic fly stocks were constructed by P-element transformation using a w^{118} genetic background. UAS-Aβ42$^{H29.3}$/*CyO* flies show no obvious abnormal locomotor or behavioral phenotypes and have a similar lifespan to CantonS or w^{118} flies. When stocks containing the UAS-Aβ42$^{H29.3}$ transgene are recombined with the 7.4kb Cha-Gal4

cholinergic neuron driver, significant phenotypes have been observed, including decreased lifespan and locomotor function.

As shown in Figure 1 (Top) 52-66% of light-adapted UAS-Aβ42$^{H29.3}$/*CyO* or UAS-Aβ42$^{H29.3}$ stocks recombined with the Cha-Gal4 driver fall to the bottom of the cylinder when the lights are turned off. In contrast only an occasional control fly (19B) shows this unusual behavior in response to lights off. Otherwise wild type stocks containing the *CyO* marked balancer chromosome also do not fall down in response to lights off (data not shown). This unusual "Lights-Off" behavior thus appears to be a result of the UAS-Aβ42$^{H29.3}$ insertion and does not depend on the presence of the Gal4 driver.

Western blot analysis confirms that only stocks where the UAS-Aβ42$^{H29.3}$ responder has been recombined with the Cha-Gal4 driver express detectable levels of Aβ42 protein (Figure 1 (Bottom)). The "Lights Off" phenotype is thus also unrelated to Aβ42 protein accumulation in neurons.

Discussion

H29.3 flies display a "Lights-Off" phenotype characterized by spontaneous falling to the bottom of a cylinder in response to a sudden light to dark transition. This unusual behavior is not dependent on the presence of a Gal4 driver, occurs in flies with no detectable Aβ42 expression, and is not seen in control flies. The phenotype thus appears to be independent of Aβ42 expression and depends instead on the presence of the H29.3 transgene insertion. We propose that this phenotype is related to disruption of an unknown genetic function by the P-element mediated insertion site of the H29.3 transgene. Caution should be used when interpreting neurological phenotypes of stocks containing the H29.3 transgene, especially for visually mediated phenotypes.

References: Crowther, D.C., K.J. Kinghorn, E. Miranda, R. Page, J.A. Curry, F.A. Duthie, D.C. Gubb, and D.A. Lomas 2005, Neuroscience 132: 123-135; Crowther, D.C., K.J. Kinghorn, R. Page, and D.A. Lomas 2004, Curr. Opin. Pharmacol. 4: 513-516; Crowther, D.C., R. Page, D. Chandraratna, and D.A. Lomas 2006, Methods in Enzymology 412: 234-255; Finelli, A., A. Kelkar, H.J. Song, H. Yang, and M. Konsolaki 2004, Mol. Cell. Neurosci. 26: 365-375; Iijima, K., H.C. Chiang, S.A. Hearn, I. Hakker, A. Gatt, C. Shenton, L. Granger, A. Leung, K. Iijima-Ando, and Y. Zhong 2008, PLoS ONE 3, e1703; Iijima, K., H.P. Liu, A.S. Chiang, S.A. Hearn, M. Konsolaki, and Y. Zhong 2004, Proc. Natl. Acad. Sci. USA 101: 6623-6628.; Ling, D., H.J. Song, D. Garza, T.P. Neufeld, and P.M. Salvaterra 2009, PLoS ONE 4: e4201.

Analysis of morphometric traits among few species of *Drosophila*.

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Understanding the microevolutionary basis of macroevolutionary change has been challenging evolutionary biologist for decades. Recently fresh attention is given to the origin and control of morphological variation (Hallgrimsson and Hall, 2005), since accumulation of small variation for several generations provides the raw material for natural selection and understanding of

the factors which promote/buffer it. It is a new area for understanding how morphological evolution proceeds. To understand accumulation of smaller variation, the closely related species provides an excellent material. So as the *montium* species are proved to be very closely related by different authors by analyzing them karyotypically, biochemically, enzyme and also at molecular level. But there are no reports on morphometric traits. By recognizing this lacuna, the present work has been undertaken to study morphometric traits, such as sternopleural bristle number, wing length, and wing width in the *montium* group a subgroup of *melanogaster* and *D. melanogaster* used for comparison.

Fly stocks used for the present analysis were *D. melanogaster, D. kikkawai* and *D. jambulina*. Experimental stocks used were established by the 50 isofemale lines collected from nature and maintained in the laboratory since 15 years under constant temperature 22±1°C and 70% relative humidity. The eggs were collected by following the method of Delcour (1969). Approximately 150 eggs were placed in quarter pint milk bottles containing wheat cream agar medium. When adults emerged, virgin females and males were isolated within 3 hr of eclosion and used for wing length/wing width and sternopleural bristle number measurement. Male and female flies were measured separately using the method of Hegde and Krishna (1999). The sternopleural bristles on left sternal plates were counted and recorded; 30 replicates were maintained for each metric character and data were subjected to Student 't' test.

The metric characters measured for three species are depicted in Table 1. The sternopleural bristle number for all three species ranges between 5-11. Sexwise difference ranges between 6-11, 5-10, 7-10 in males, and in females it is 6-10 in *D. melanogaster,* and it is 7-10 in both *D. kikkawai* and *D. jambulina,* respectively (Figure 1a). Intra and interspecies difference is non significant. However, sternopleural bristle number is higher in females compared to males in all the three species.

Table. 1. Mean ± SE for the metric characters analyzed for three species of *Drosophila.*

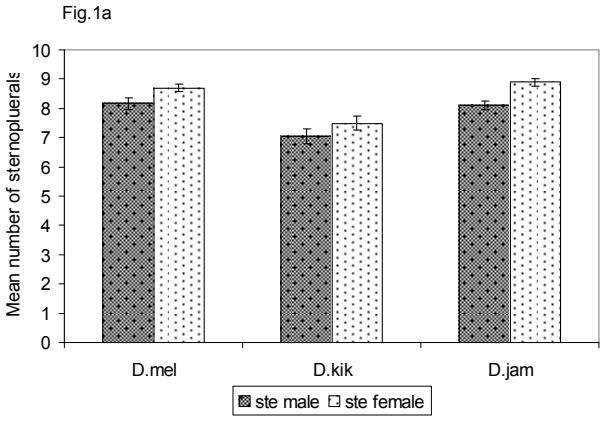
Species	Sternopleurals		Wing length		Wing width		WL/WW ratio	
	males	females	males	females	males	females	males	females
D. melanogaster	8.1667 ± 0.1982	8.7000 ± 0.1369	1.8843 ± 0.0122	2.0110 ± 0.0112	0.8770 ± 0.0079	0.9277 ± 0.0080	2.1500 ± 0.0089	2.1710 ± 0.0185
D. kikkawai	7.0333 ± 0.2514	7.5000 ± 0.2236	1.6593 ± 0.0144	1.8153 ± 0.0140	0.7403 ± 0.0093	0.8110 ± 0.0080	2.2470 ± 0.0205	2.2410 ± 0.0161
D. jambulina	8.1000 ± 0.1385	8.9000 ± 0.1300	1.5753 ± 0.0103	1.6723 ± 0.0104	0.6993 ± 0.0077	0.7560 ± 0.0103	2.2561 ± 0.0204	2.2217 ± 0.0245

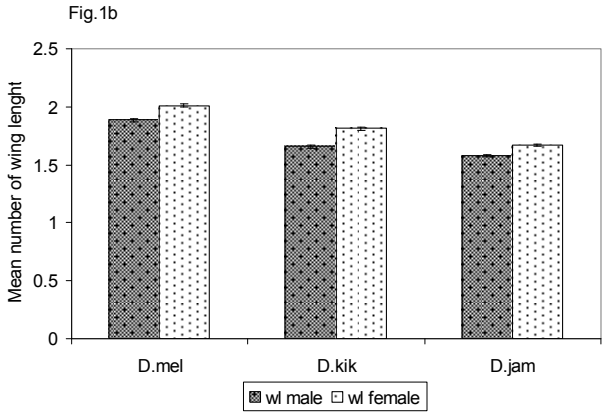
Wing length among three species range between 1.42-1.65 mm. There is a difference in the wing length between male and female in all the three species (Figure 1b). Inter- and intra-species differences are non-significant. Wing width on the other hand ranges between 0.56-0.83mm (Figure 1c). The greater wing width is observed in *D. melanogaster* compared to other two species. WL/WW ratio is depicted in the Table 1. Further no difference was observed among the species studied could be due to breeding of species for a long time in almost constant laboratory environment. The difference observed was larger in the natural population.

The present basic work enabled us to study the metric characters in entire *Drosophila* group is with less variable. Literature survey revealed the phenotypic variation increase in case of variation in temperature (Precht *et al.,* 1973; Cossins and Bowler, 1987; Leather *et al.,* 1993). Stress increases the phenotypic variance of most quantitative traits (Hoffmann and Parsons, 1997; Hoffmann and Hercus, 2000). But the variance in the wild collected individuals is greater than that of laboratory

grown flies (Coyne and Beecham, 1987; David *et al.,* 1997; Gibert *et al.,* 1998). This could be genetic or non genetic.

In the present study, the species are closely related and interesting to ecologists for many reasons, and it could be the accumulation of divergence leading them to diverge further from the main stem. Though they achieved a species level, the difference is non-significant for all the metric characters measured. The parameters, such as sternopleural bristle number, wing length, and wing width measured among three species have revealed that range of bristle number, length and width varies, but not at the significant level. Interspecies comparison shows that sternopleural bristle number is more in *D. melanogaster* compared to other two species. Mean difference is statistically non-significant. It has been also reported by the other authors that geographic clines lack clear genetic divergence in morphology in different strains and species of *Drosophila* (Sokoloff, 1966; Kitagawa *et al.,* 1982).





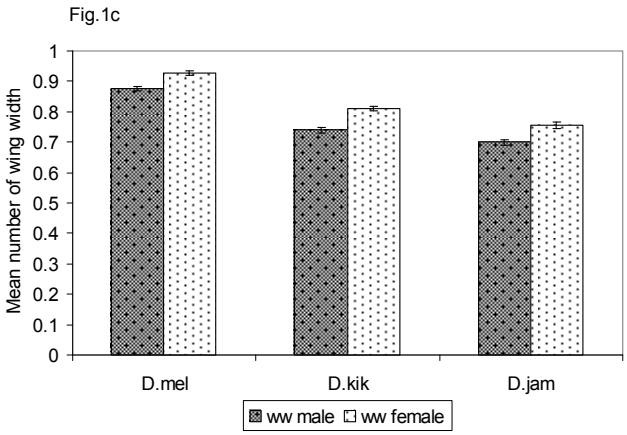


Figure 1. Morphometric traits in males and females of *D. melanogaster, D. kikkawai and D. jambulina*; a) sternopleural bristle number, b) wing length, c) wing width.

In *Drosophila,* females are larger than males for most body dimensions and also differ in pigmentation, the number of abdominal segments structure, genitalia, behaviour, and many other features. In the present study there is a slight variation in wing length, wing width, and number of sternopleural bristles among females and males. Females show slightly higher values than males. Further, Church and Robertson (1996) demonstrated that male and female flies diverge at the beginning of the third larval instar for total body content of DNA, RNA, and protein. Though larger, as adult females emerge earlier than males suggesting different rate of development is 4% faster than males. The analysis of the mouth parts showed that they are able to consume more food in a given time than males. Further, they have more feeding time than males, and also female beings emerged first, they are the first one to utilize the resources when compared to males. There must be the large

mouth parts, which are advantageous to the female beings to develop as normal. So as in the other metric traits. Perusal of literature reveals, the species under study are closely related. The present study also showed there is a similarity among the species with respect to metric traits.

References: Church, R.B., and F.W. Robertson 1996, Ecology 37: 544; Cosins, A.R., and K. Bowler 1987, *Temperature Biology of Animals*. London: Chapman & Hall; Coyne, J.A., and E. Beecham 1987, Genetics 177: 737; David, J.R., P. Gibert, E. Gravot, G. Petavy, J.P. Morin, and D. Karan *et al.,* 1997, J. Therm. Biol. 22: 441-451; Delcour, L., 1969, Dros. Inf. Serv. 44: 133-134; Gibert, P., B. Moreteau, J.C. Moreteau, and J.R. David 1998, Heredity 80: 326-335; Hallgrimsson, B., and B.K. Hall 2005, *Variation*. Academic Press, San Diego; Hegde, S.N., and M.S. Krishna 1999, Current Science 77: 1346-1347; Hoffmann, A.A., and P.A. Parsons 1997, *Extreme Environmental Change and Evolution*. Cambridge: Cambridge University Press; Hoffmann, A.A., and M.J. Hercus 2000, Bioscience 50: 217-226; Kitagawa, O., K.I. Wakahama, Y. Fuyama, Y. Shimada, E. Takanashi, M. Htsumi, M. Umabo, and Y. Miitay 1982, Jpn. J. Genet. 57: 141; Leather, S., K. Walters, and J. Bale 1993, *The Ecology of Insects Overwintering*. Cambridge: Cambridge University Press; Precht, H., J. Christophersen, H. Hensel, and W. Larcher 1973, *Temperature and Life*. Berlin: Springer Verlag; Sokoloff, A., 1966, Evolution 20: 49-71.

The effects of dibutyl phthalate on the development of *Drosophila melanogaster.*

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Abstract

In this study, the developmental effects of a well-known plasticizer dibutyl phthalate (DBP) were determined in wild type *Oregon* strain of *Drosophila melanogaster*. The 72 h larvae of *D. melanogaster* were exposed to four concentrations of DBP-acetone solution. After 72 hours, the pupae were counted. The developmental times of DBP exposure groups were found extended comparing to the control group. The reductions were found statistically significant compared to the control ($P < 0.05$).

Key-words: Dibutyl phthalate, plasticizer, *Drosophila melanogaster*, development, mean pupation time.

Introduction

In recent years, the contamination with phthalate esters (1,2-benzendicarboxylates) (PAEs) turned into ubiquitous in the soil, water, and air (Steiner *et al.*, 1998). The increased contamination with phthalate is a common problem worldwide due to its widespread utilization areas. Therefore, the concerns about possible harmful health effects have been raised (Swan *et al.,* 2005; Hauser *et al.,* 2006).

PAEs including dibutyl phthalate (DBP) are widely used in PVC industry and to a lesser extent in the non-polymer industry products (children's toys, sealants, paints, printing inks, cosmetics, varnishes, shampoos, cables or fabrics, insect repellents etc.) (Heudorf *et al.*, 2007). PAEs

are oily substances and slightly soluble in water. They are not bound to plastics covalently (Mikula *et al.,* 2005). So, they can be leached from these products to the environment (Steiner *et al.,* 1998).

PAEs are known to disrupt the endocrine system of animals (Fisher, 2004) and hence its exposure may cause potential health problems (Prasanth *et al.,* 2009). DBP is a component of PAEs and has been reported a potential for endocrine disrupting effects on vertebrates (Toppari *et al.,* 1996; Keith, 1997; Wine *et al.,* 1997).

The toxic effects of DBP were investigated in many organisms including rat, abalone, freshwater rotifer etc. The researchers found that DBP can cause reproductive tract malformations in male rats (Wölfle *et al.,* 2009), fertility reductions and ovarian malfunctions in female rats (Gray *et al.,* 2006). The developmental effects of DBP were found significantly toxic (malformation of embryos, low larval settlements) in the exposed larvae of abalone *Haliotis diversicolor supertexta* (Zhihui *et al.,* 2009). In addition, it is shown that DBP significantly prolonged the generation time of freshwater rotifer *Brachionus calyciflorus* (Zhao *et al.,* 2009).

To the best of our knowledge, there are no reports in the open literature on the effects of DBP on *D. melanogaster*. Therefore, the major aim of the present study is to assess the effects of DBP on development of the fruit fly *D. melanogaster*.

Materials and Methods

In order to determine the mortality of DBP (CAS No: 84-74-2), wild type *Oregon* strain of *D. melanogaster* was used. First, *Oregon* adult flies were crossed in culture bottles containing Standard *Drosophila* medium at 25 ± 1°C culture room. After 72 ± 2 hours, individuals were removed from bottles and the 3rd instar larvae were collected. Ten larvae were placed into each vial. The DBP was firstly diluted in 0.1 ml acetone and after 100 ml sucrose solution was added. The solution and 1.5 gr *Drosophila* medium were added in the vials. The treatment doses were 1 ml/L, 0.1 ml/L, 0.01 ml/L and 0.001 ml/L. Each dose had two replicates. Statistical differences between the transition period of control and treated larvae were examined with two-variable *t* test using SPSS 11.5. The homogeneity of variances of data was tested with Levene statistics, and the variances were found homogeneous ($P > 0.05$).

Table 1. The pupation percentages of the 3rd instar larvae of *Drosophila melanogaster* after treated with DBP.

No	Doses (ml/L)	*n*	24 h	48 h	72 h
1	Control	60	23.3	66.67	96.67
2	0.001	60	5	61.6	88.33
3	0.01	60	3.33	51.6	98.33
4	0.1	60	5	60	95
5	1	60	1.67	58.33	96.67

n: Number of larvae.

Results

The developmental and mortality effects of DBP were investigated in 72 h larvae of *D. melanogaster*. After the exposure with DBP, pupation was recorded at 24 hours, 48 hours, and 72 hours. After 24 hours, pupation percentages were found to be 23.3% in control group, 5% in 0.001 ml/L dose, 3.33% in 0.01 ml/L dose, 5% in 0.1 ml/L dose, and 1.67 in 1 ml/L dose. After 48 hours, pupation percentages were found to be 66.67% in control group, 61.6% in 0.001 ml/L dose, 51.6% in 0.01 ml/L dose, 60% in 0.1 ml/L dose, and 58.33 in 1 ml/L dose. After 72 hours, pupation percentages were found to be 96.67% in control group, 88.33% in 0.001 ml/L dose, 98.33% in 0.01 ml/L dose, 95% in 0.1 ml/L dose, and 96.67% in 1 ml/L dose (Table1).

Table 2 shows mean pupation times (hour) of the control and exposure groups. The mean pupation time of control group was found to be 57.4 of DBP on development of *D. melanogaster*. The mean pupation times were found to be 57.4 in control group, 60.9 in 0.001 ml/L dose, 62.9 in 0.01 ml/L dose, 61.7 in 0.1 ml/L dose, and 62.5 in 1 ml/L dose. As seen in Table 2, there was a statistically significant reduction in the mean pupation times in the treated groups ($P < 0.05$) compared to the control group.

Table 2. The effect of DBP treatment on the mean pupae numbers of *Drosophila melanogaster* in 72 hours.

No	Doses (ml/L)	*n*	No. of pupae	Mean Pupation Time (hour) ± SE	SD	Significant differences of the means*
1	Control	60	58	57.4 ± 1.77	16.85	1-2*, 2-3*
2	0.001	60	53	60.9 ± 1.4	13.5	2-1*, 2-5 *
3	0.01	60	59	62.9 ± 1.32	12.75	3-1*, 3-4*
4	0.1	60	59	61.7 ± 1.35	13.35	4-1*, 4-5*
5	1	60	57	62.5 ± 1.28	12.33	5-1*

n: Number of Larvae, SE: Standard Error, SD: Standard deviation; *p < 0.05

Discussion

In the present study, the developmental effects of plasticizer DBP were determined in wild type *Oregon* strain of *Drosophila melanogaster*. The pupation percentages of four treatment groups and control group were determined and compared. As seen in Table 1, the pupation percentages of treatment groups (5%, 3.33%, 5%, 1.67%) in first 24 hours were found to be dramatically delayed when compared to that of control group (23.3%). After 48 hours, the differences between control and treatment groups were found stable.

The mean pupation time (hour) of the four treatment groups were calculated and compared with control group. As seen in Table 2, the mean pupation times of the treatment groups (60.9, 62.9, 61.7, 62.5, respectively) were markedly prolonged when compared to the that of control group (57.4). The results showed significant reductions among the treatment groups and control group ($P < 0.05$). These significant delays show that the pupation of *D. melanogaster* was markedly affected by DBP. Similarly to our findings, Zhao *et al.* (2009) determined a significantly prolonged generation time in freshwater rotifer *Brachionus calyciflorus* with DBP exposure.

It is reported that DBP treatment can cause fertility reductions, reproductive malformations in rats and low larval settlements in *H. diversicolor supertexta* (Gray *et al.,* 2006; Wölfle *et al.,* 2009; Zhihui *et al.,* 2009). Besides, the researchers found that several phthalates including DBP or their metabolites, respectively, can cross the placenta barrier and reach the human fetus (Wittassek *et al.,* 2009).

In conclusion, the present study has revealed that DBP can cause statistically significant reductions in pupation time of *D. melanogaster*. Concerning the potential estrogenic activity of DBP, further research is required.

References: Fisher, J.S., 2004, Reproduction 127: 305-315; Gray, L.E., Jr., J. Laskey, and J. Ostby 2006, Toxicological Sciences 93(1): 189-195; Hauser, R., J.D. Meeker, S. Duty, M.J. Silva, and A.M. Calafat 2006, Epidemiology 17: 682-691; Heudorf, U., V. Mersch-Sundermann, and J. Angerer 2007, Int. J. Hyg. Environ. Health 210: 623-634; Keith, L.H., 1997, *Environmental Endocrine Disruptors—A Handbook of Property Data*, Wiley-Interscience: New York; Mikula, P.,

Z. Svobodova, and M. Smutna 2005, Czech. J. Food Sci. 23: 217–223; Prasanth, G.K., L.M. Divya, and C. Sadasivan 2009, Toxicology 262: 38-42; Steiner, I., L. Scharf, F. Fiala, and J. Washuttl 1998, Food Addit. Contam. 15: 812; Swan, S.H., K.M. Main, F. Liu, S.L. Stewart, R.L. Kruse *et al.,* 2005, Environ. Health Perspect. 113: 1056–1061; Toppari, J., J.C. Larsen, P. Christiansen, A. Giwercman, and P. Grandjean 1996, Environ. Health Perspect. 104: 741; Wine, R.N., L.H. Li, L.H. Barnes, D.K. Gulati, and R.E. Chapin 1997, Environ. Health Perspect. 105: 102-107; Wittassek, M., J. Angerer, M. Kolossa-Gehring, SD. Schafer, W. Klockenbusch *et al.,* 2009, Int. J. Hyg. Environ. Health 212: 492–498; Wölfle, D., K. Pfaff, T. Platzek, and A. Luch 2009, Toxicology Letters 189S: S57-S273; Zhao, L.L., Y.L. Xi, L. Huang, and C.W. Zha 2009, Aquat. Ecol. 43 (2): 395-402; Zhihui, Y., Z. Xiangjing, and C. Zhonghua 2009, Chinese Journal of Oceanology and Limnology 27 (2): 395-399.

Vitamin A deprivation does not decrease fluorescence of ARF72-RFP, a label for Golgi apparatus, in *Drosophila* visual receptors.

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Each ommatidium of the *Drosophila* compound eye has 8 photoreceptors (retinula cells); the rhabdomere of each is the specialized organelle that houses rhodopsin and the visual transduction molecules. One of the primary functions of the retinula cell is maintenance of the rhabdomere, including turnover of membrane and protein (Lee *et al.,* 1996). Vitamin A deprivation reduces or eliminates rhodopsin in *Drosophila* rhabdomeres (Harris *et al.,* 1977). Vitamin A replacement synchronizes *de novo* synthesis and export of rhodopsin (Sapp *et al.,* 1991).

Although the quality of fixation of *Drosophila* photoreceptors has always been variable, electron micrographs from our lab archives showed that vitamin A deprived flies are more likely to be plagued by what we refer to as "ghosty cytoplasm" (marked with asterisks [*], Figure 1, Top left) than vitamin A replete controls (Figure 1, Middle left). (Electron micrographs are labeled thus: R is rhabdomere, G is Golgi, > is desmosome, N is nucleus, and PG is pigment granule.) We hypothesized that cytoplasmic organelles dedicated to biosynthesis, rough endoplasmic reticulum and Golgi apparatus, might be reduced by vitamin A deprivation. We tested our hypothesis using a fly stock we had been using to visualize Golgi apparatus, ARF72-RFP (ADP ribosylation factor tagged with red fluorescent protein).

Flies were lightly etherized and fixed to a glass slide for visualization of the deep pseudopupil. A typical fluorescence micrograph is presented (Figure 1, Top right); the blurry appearance compared with rhabdomere fluorescence (Stark and Thomas, 2004) is explained since Golgi apparatus is distributed throughout retinula cells. Fluorescence was quantified using a fluorescence microscope with a photometer system (Stark *et al.,* 1985). The pseudopupil image was delimited by the photometer and fed to the photomultiplier tube. Rhabdomeres were excited with a calibrated amount of 488 nm light, and a voltage response proportional to the level of fluorescence being emitted was recorded by a computer. Flies were raised at room temperature either on our yellow cornmeal food (supplemented with beta-carotene, vitamin A replete) or on Sang's medium lacking vitamin A. A strong correlation between age and level of fluorescence was noted (Figure 1, Middle right). Thus, for control, measurements were performed on flies within 12 hr of eclosion.

RFP fluorescence of vitamin A deprived flies (n = 28) was the same as that of flies reared on vitamin A replete medium (n = 27), as witnessed, since the error bars (95% confidence intervals)

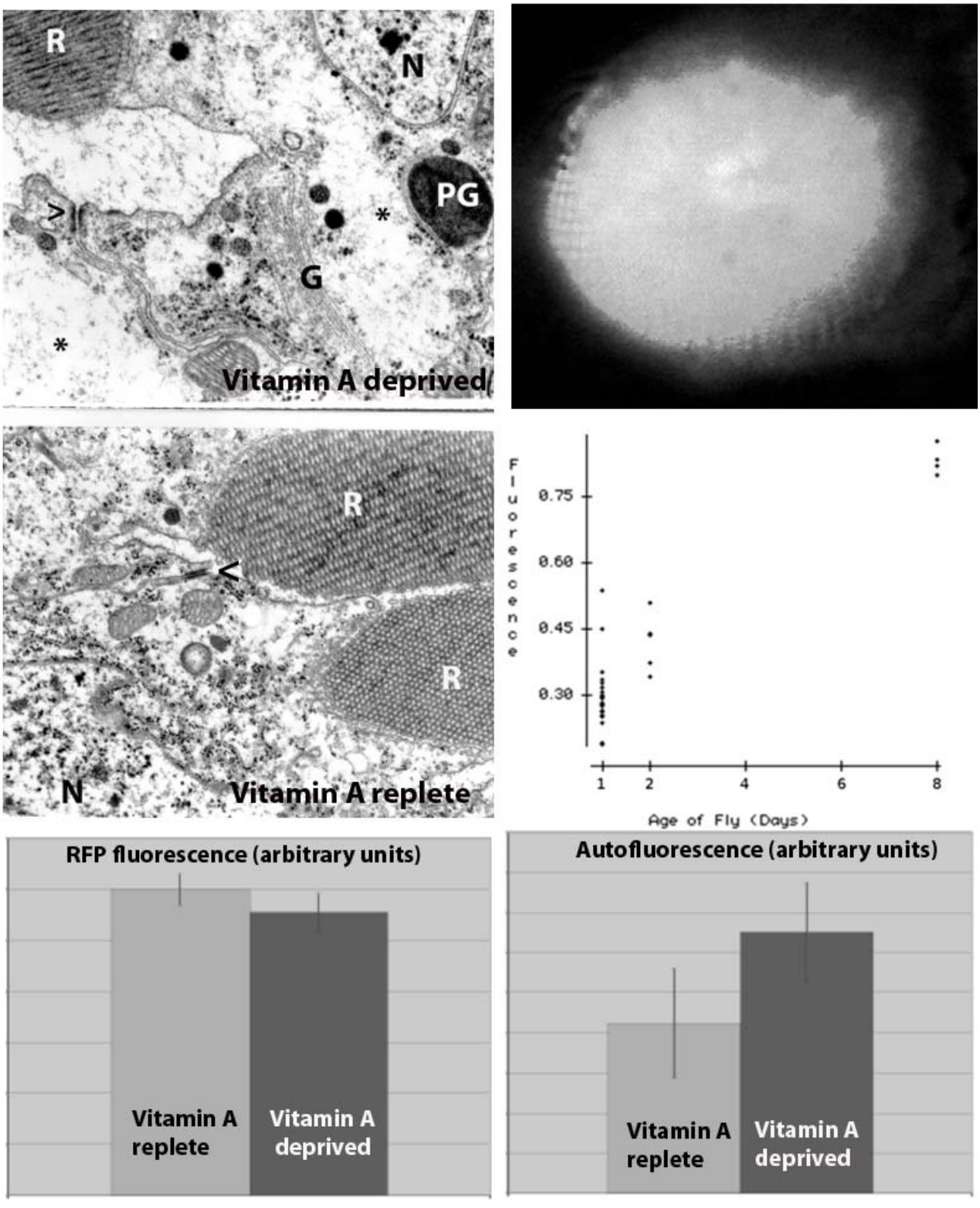
R
N
PG
G
Vitamin A deprived
R
R
N
Vitamin A replete
Fluorescence
0.75
0.60
0.45
0.30
1
2
4
6
8
Age of Fly (Days)
RFP fluorescence (arbitrary units)
Vitamin A replete
Vitamin A deprived
Autofluorescence (arbitrary units)
Vitamin A replete
Vitamin A deprived

Figure 1.

overlap (Figure 1, bottom left). The stock of flies was not genetically pure; only 28.7% of the ARF72-RFP flies raised on the replete medium and 18.3% of the flies raised on the deficient food showed RFP fluorescence. Furthermore, we noted a variation in eye color – ranging from slightly pink to pure white (in the white-eyed stock). However, there was no correlation found between eye color and whether a fly showed RFP fluorescence. Measuring at a higher sensitivity to quantify background autofluorescence among flies that were negative for RFP, the level was higher in vitamin A deprived flies (n = 125) than in those raised on replete food (n = 67) (Figure 1, Bottom right); however, again, this difference was not statistically significant as witnessed by the overlapping 95% confidence intervals. The purpose of this last control was to verify the expectation that background fluorescence did not predominate in our measurements of RFP fluorescence.

In conclusion, we reject our hypothesis that Golgi apparatus, as quantified by ARF72-RFP fluorescence, is higher in vitamin A replete *Drosophila* than in vitamin A deprived flies; further, we conclude that any difference in quality of fixation between replete *vs* deprived *Drosophila*, if real, cannot be attributed to different amounts of Golgi complexes in retinula cells.

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References: Harris, W.A., *et al.,* 1977, Nature (Lond.) 266: 648-650; Lee, R.D. *et al.,* 1996, Micros. Res. Tech. 35: 418-430; Sapp, R.J. *et al.,* 1991, Exp. Eye Res. 53: 73-79; Stark, W.S. *et al.,* 1985, Curr. Eye Res. 4: 1059-1075; Stark, W.S., and C.F. Thomas 2004, Molec. Vision 10: 943-955 on line at http://www.molvis.org/molvis/v10/a113/).

Mechanosensation diversity across and within *Drosophila* species.

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Introduction

Mechanosensation remains a largely ignored area of organismal perception. Smell, taste, vision, and hearing are well characterized in *Drosophila*, yet we know little about mechanosensation and the somatosensory system in general. Knowledge of the genetics, morphology, and molecular biology of olfaction (smell) and gustation (taste) in *Drosophila* have in particular fueled an incredible diversity of important work in many fields. In evolutionary biology, we know that olfactory and gustatory receptors have evolved as adaptations within *Drosophila* species (McBride, 2007; Kopp *et al.*, 2009), and many other non-*Drosophila* species (for example, Hayden *et al.*, 2009; Steiger *et al.,* 2009). We have little information, however, on the role of mechanosensation in evolution.

Detecting the attack of a parasitoid may be an important role for mechanosensation. In wild *Drosophila* populations, depending on the season, as many as 35-85% of individuals can be parasitized by a variety of wasp species, and it is thought that wasps could play a major role in controlling fly population size (Carton *et al.,* 1986). Hwang *et al.* (2007) showed that nociceptive neurons, a type of mechanosensory perception, are important for larval rolling behavior, a defensive response to parasitoid wasp attack. Apart from cellular immunological response, larval rolling behavior may be *Drosophila'*s main defense against parasites. Critical to this response is immediate and accurate mechanosensation.

If mechanosensory response contributes largely to parasite avoidance, and if parasitic wasps exert strong selective pressure, then mechanosensation may serve as an adaptation. We do not know

how frequently different *Drosophila* species encounter wasps, but it is possible that if mechanosensation is adaptive then divergent fly populations that experience differential parasite loads will have differential mechanosensation.

To get an initial picture of phenotypic variation in broadly defined mechanosensory response across *Drosophila*, we measured the touch response of eight *Drosophila* species and 26 of the 50 *D. melanogaster* lines from the *Drosophila* Population Genomics Project (DPGP), potentially capturing both inter- and intra-specific variation. Our results show small but significant genotypic variation within *D. melanogaster* and no significant variation between tested species. Our data are weakened by low replicate sizes within genotypes/species and by a significant effect of who performed the experiment.

Materials and Methods

We measured mechanosensory response in eight *Drosophila* species (*D. ananassae, D. erecta, D. parabipectinata, D. melanogaster, D. sechellia, D. simulans, D. santomea, D. yakuba*), ranging in divergence from ~0.5 to 15 million years. Multiple lines of *D. melanogaster* were used to uncover any intraspecific variation (w^{1118}, *yw,* and 26 Raleigh lines collected from Raleigh, NC by T. MacKay). Flies were reared on cornmeal-yeast-agar medium at 20°C with 12-h light-dark cycle. Tactile response was measured after Caldwell *et al.* (2003). Late 2nd and early 3rd instar larvae were placed in the center of a 1% agar/distilled water 55mm petri dish after gentle washing with distilled water at room temperature. Once individuals began unidirectional motion they were gently stroked along their 2nd or 3rd thoracic segment with an eyebrow hair. Responses were scored: 0, showing no response; 1, showing brief hesitation and continued motion in the same direction; 2, anterior recoil and continued motion in the same direction; 3, turning between 0 and 90 degrees; 4, turning greater than 90 degrees. Individuals were tested 4 times with ~5 seconds between stroking. Larvae that did not move were discarded. Overall scores for individuals were summed, and responses ranged from 0 to 16. Measurements within genotype/species were taken over multiple days at various times by both EE and BW. Mean and standard error were measured for each genotype or species.

Our results did not fit a normal distribution and are heteroscedastic. Thus we used non-parametric analyses to see if genotypes/species had significantly different touch responses. We used Kruskal-Wallis rank sum test (KW) and a general linear model (GLM). In our model, our total response by test (y) could be influenced by variance in genotype/species (G), experimenter (E), and their interaction(G×E).

$$y = b_0 + Gx + Ex + GxEx + \varepsilon$$

We analyzed *D. melanogaster* (*D. mel*) and *D. simulans* (*D. sim*) independently, sorting by genotype. For cross-species comparisons, all *D. mel* and *D. sim* genotypes were taken to calculate their species' respective values. All statistical analyses were performed with R (2005).

Results/Discussion

We found variation in touch response within both genotype (*D. mel* and *D. sim*) and species (Figure 1a-c). *D. melanogaster,* in particular, had a broad range of responses, with some genotypes

contributing heavily to the species range (307, 315, and 357). Within *D. simulans* and within other species responses were much less varied.

Within *D. melanogaster*, genotype contributed significantly to total response (KW: $\chi^2 = 51.5$, p = 0.003) with genotypes 774 (p = 0.032), 786 (p = 0.034), w^{1118} (p = 0.003), and *yw* (p = 0.011) showing significantly different contributions to overall variance in touch response. Overall, the experimenter did not have a significant influence on touch response (p = 0.556), but the interaction of genotype with experimenter did on genotypes 315 (p = 0.002), 357 (p = 7.2e-06), and 399 (p = 0.032). *D. simulans* did not show any difference between genotypes (KW: $\chi^2 = 1.88$, p = 0.596), and no genotype, experimenter, or interaction contributed to overall variance. Across all species, we found no significant differences in response due to species alone (KW: $\chi^2 = 9.42$, p = 0.224). We did find a significant effect of experimenter; however, (KW: $\chi^2 = 29.82$, p = 4.74e-08). No genotype, experimenter, or G×E interaction contributed significantly to overall response variance.

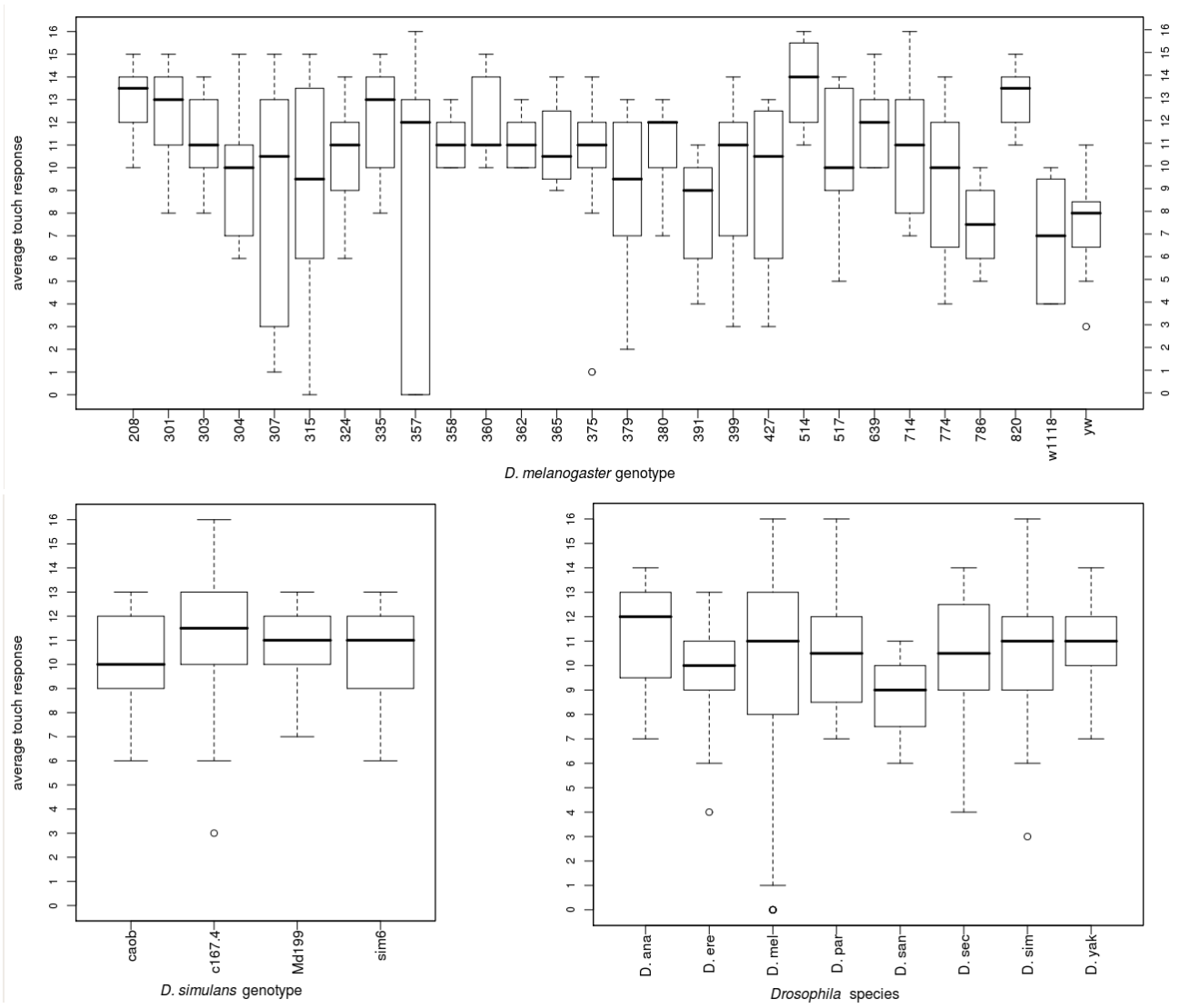

Figure 1. Box-and-whisker plot surveying mechanosensation across, a) *D. melanogaster* Raleigh inbred lines, b) *D. simulans* genotypes, and c) multiple *Drosophila* species. Aggregate results from *D. melanogaster* and *D. simulans* are contained within their species' averages in c.

Our replicate sizes were not equal across tests. For example, we tested 196 *D. melanogaster* larvae but only 8 *D. sechellia*. When standard deviation for response mean was regressed against sample size for each species, we found a positive correlation ($R^2 = 0.311$), and we found an even stronger positive correlation between inter-quartile range for response means and species sample size ($R^2 = 0.575$). The correlation coefficients, however, are somewhat low, and when *D. melanogaster* is removed this correlation disappears.

We found weak but significant differences in genotype touch response within *D. melanogaster*. Interestingly, we found strong genotype by experimenter effects in certain genotypes. Two factors may account for this. First, we attempted to elicit larval response as uniformly as possible, but subtle differences in technique could have driven dynamic responses in some genotypes. Thus, the interaction could be real, and certain genotypes are more sensitive to the quality of touch than others (for example, accidentally poking instead of stroking). Second, this interaction could also be an artifact of varied replicate sizes for each experimenter within genotype. The large influence of experimenter on species-wide response variance could also be due to this asymmetry.

We chose the method of Caldwell *et al.* (2003), because it appeared to elicit consistent responses in our pilot experiments with *D. simulans* even with multiple experimenters. Expanding our tests to other species uncovered unexpected variation, and this could easily have been due to technical error (for example, elicitation differences and unequal sample sizes). However, when using this test or others like it in the future, the unconscious influence of experimenter should be tested and controlled.

Mechanosensation could contribute to larval fitness. Parasitoid wasps encounters are common in many *Drosophila* populations, and rolling behavior is a typical defense behavior in response to ovipositor touch. In biomedical research, *Drosophila* offers great opportunity to dissect the genetic and molecular mechanisms of mechanosensation. Understanding the genetic variation existing across *Drosophila* species would thus inform a variety of fields. While we found some evidence of genetic variation in mechanosensation within *D. melanogaster*, some confirmation is required. Future tests may take "high" and "low" genotypes, having responses on the extreme ends, and cross them to see if touch response is heritable.

References: Caldwell, J.C., M.M. Miller, S. Wing, D.R. Soll, and D.F. Eberl 2003, PNAS 100: 16053-8; Carton, Y., M. Boulétreau, J.J.M. van Alphen, and J.C. van Lenteren 1986, The *Drosophila* parasitic wasps. In: *The Genetics and Biology of* Drosophila, 3e (Ashburner, M., H.L. Carson, and J.N. Thompson, jr., eds.), pp.347-394. Academic Press, London; Kopp, A., O. Barmina, A.M. Hamilton, L. Higgins, L.M. McIntyre, and C.D. Jones 2008, Mol. Biol. Evol. 25(6): 1081-1092; Hayden, S., M. Bekaert, T.A. Crider, S. Mariani, W.J. Murphy, and E.C. Teeling 2009, Genome Res.[epub ahead of print]; Hwang, R.Y., L. Zhong, Y. Xu, T. Johnson, F. Zhang, K. Deisseroth, and D. Tracey 2007, Curr. Biol. 17(24): 2105-16; McBride, C.S., 2007, PNAS 104: 4996-5001; R Development Core Team 2005, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org; Steiger, S.S., A.E. Fidler, J.C. Mueller, and B. Kempenaers 2009, J. Hered. [epub ahead of print].

Technique Notes

Potential use of marker pen ink as a marking method for drosophilids.

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Introduction

Mark-release-recapture methods have been widely employed to estimate absolute parameters like dispersal and density in mobile animal populations (Southwood, 1978). These parameters are primordial to elucidate the population structure, that has been used from conservation purposes to evolutionary researches and has long been recognized as a key factor for understanding genetic variation in nature (Wright, 1938) and for estimating the size of panmictic units (Begon, 1976). Mark-release-recapture methods are also the basis for the classic theories of population gene flow (Dobzhansky and Wright, 1943), based on the dispersal of *Drosophila pseudoobscura* Frolova, 1929, and for a unifying mathematical relationship between density and distance of dispersing insects (Taylor, 1978, 1980).

A prerequisite for the use of mark-release-recapture methods is a technique for marking animals, which has important implications for the reliability of the results. Desirable attributes for marking methods are the retention of marks for a sufficient period of time and that the marker does not adversely affect the biology of the organism altering, for example, the mortality rate of the species. Besides, the material needs to be inexpensive, nontoxic to the environment, clearly identifiable (on recapture), and easily applied (Southwood, 1978).

Many materials and methods have been proposed to mark insect since the seminal studies of Geiger *et al.* (1919) and Dudley and Searles (1923), in which paints, dyes, and stains were used to investigate population dynamics. Such materials, as well as micronized dusts, are the most successfully, commonly used and inexpensive materials for marking insects (Southwood, 1978; Wineriter and Walker, 1984). Tags are also promising marking methods for arthropods because they are inexpensive and can identify organisms on an individual basis; however, they are generally too large and heavy for small specimens like drosophilids, and their application is tedious and time-consuming (Hagler and Jackson, 2001). Mutilation, on the other hand, can be applied only for large or heavily sclerotized insects (as beetles) or insects with large wings (as butterflies and dragonflies) and, therefore, can not be applied for drosophilids. Recently, methods like Elemental Marking, Protein Marking, Genetically Engineered Marking and Biochemical Genetic Markers have been used, but they are usually expensive, time-consuming, and require an arduous workout for researchers and the death of the specimens after recapture. In addition, in some cases, specimens must be reared in the laboratory and markers may promote non-visible but detrimental mutations or physiological changes affecting fitness (Hagler and Jackson, 2001).

For wild drosophilids micronized fluorescent dusts have been by far the most successfully and commonly used marking method (Crumpacker, 1974; Begon *et al*. 1975; Begon 1976; Hagler and Jackson, 2001). In this paper, we test for drosophilids the effectiveness of a marking method commonly used for lepidopterans – which consists in marking the body of the insect with marker pen ink – and discuss the advantages and disadvantages of this method compared to micronized dusts.

Material and Methods

We investigate the effect of marking specimens with marker pen ink on persistence of marks and longevity – since some paints and inks are toxic to insects (Southwood 1978) – of South American strains of *Drosophila simulans* Sturtevant, 1919, *D. malerkotliana* Parshad and Paika, 1964, and *Zaprionus indianus* Gupta, 1970. Virgin flies, anesthetized with ethylic ether, were painted in the thorax with a marker pen. The number of spots (one to seven), their position (among ten possibilities) and the assignment of a specimen to the marked or non-marked categories were randomly determined.

After awakening, ten marked flies of the same sex and species were reared with ten non-marked flies – handled in the same way (control) – in vials with cornmeal medium (Marques *et al*., 1966), in a controlled chamber (25 ± 1^{o}C, 60% r.h.). Four vials for males and four for females were utilized for each species. Every two days, food (*Saccharomyces cerevisae*) and water were added to the medium. Every four days, the flies were transferred to a new vial with culture medium to maintain the quality of resource and to avoid toxic effects of metabolites. The vials were inspected daily to detect dead flies, which were removed of the vial. After removal, information on the sex, marking category (marked or non-marked), arrangement of dots for marked specimens (for assessment of the persistence of dots) and longevity were compiled.

A one-way ANOVA was performed using Statistica 5.1 software (StatSoft, Inc, 1998) to detect differences of longevity between marked and non-marked flies ($p \leq 0.05$) for each sex of each species. Data transformation was required for males of *D. malerkotliana* (√x), *D. simulans* (√x), and *Z. indianus* (sin (x)) to fit the ANOVA assumptions. Normality and homoscedasticity were assessed by Lilliefors and Bartllet tests, respectively, using the Genes 2007.0.0 software (Cruz, 2006). During the transference procedures, some flies escaped and were not considered for the analysis.

Results and Discussion

Longevity did not differ between marked (mean ± sd = 22.2 ± 12.3 days, N = 32) and non-marked (20.5 ± 12.9 days, N = 38) *D. simulans* females ($F = 0.325$, $p = 0.57$, df = 1), but it differed between marked (12.7 ± 7.6 days, N = 35) and non-marked (17.9 ± 11.1 days, N = 34) *D. simulans* males ($F = 4.841$, $p = 0.03$, df = 1). For *D. malerkotliana*, longevity differed neither between marked (21.7 ± 11.8 days, N = 30) and non-marked (24.8 ± 14.2 days, N = 31) females ($F = 0.854$, $p = 0.36$, df = 1) nor between marked (23.8 ± 9.2 days, N = 31) and non-marked (26.5 ± 10.0 days, N = 34) males ($F = 1.147$, $p = 0.29$, df = 1). In the same way, longevity differed neither between marked (27.0 ± 7.4 days, N = 40) and non-marked (32.9 ± 11.1 days, N = 32) *Z. indianus* females ($F = 3.668$, $p = 0.06$, df = 1) nor between marked (25.9 ± 18.9 days, N = 36) and non-marked (18.7 ± 1.8 days, N = 38) *Z. indianus* males ($F = 3.137$, $p = 0.08$, df = 1).

Our data suggest that marker pen ink is a reliable method for marking drosophilids, as also suggested by Morton (1982), who marked the upperside forewing and the underside hindwing of the marbled white butterfly *Melanargia galathea* (L.) (Satyridae). Although our results suggest almost

no relevant effect of such marker on longevity, a high variability in the data set as observed in our study increases the probability of error Type II, *i.e.*, a false negative and, consequently, caution is required in its use. Since longevity is naturally highly variable, we encourage further analysis.

The use of dust is frequently preferred for marking insects due to its inexpensiveness, environmental safeness, and the easy and rapid application (by shaking a container in which both dust and insects are put together) and detection (Hagler and Jackson, 2001). Several laboratory studies suggest that micronized dusts have a negligible effect on the viability (Crumpacker, 1974) and mortality (Crumpacker, 1974; Moth and Barker, 1975) of drosophilids and other dipterans, such as *Liriomyza trifolli* (Jones and Parrela, 1986). It has been also observed for drosophilids, in field work, that multiple marking have no significant effect compared to single marking (Rosewell and Shorrocks, 1987) and that different colors and compositions of dust do not affect the recapture rate (Begon, 1976).

Inks are also an inexpensive, clearly identifiable (on recapture), and rather easily applicable for marking insects. Furthermore, such material can not be transferred from marked to unmarked insects, which can occur for dust particles in the field or in traps and sweep nets used for sampling (Miller, 1993), affecting the results. Besides, ink is applied on specific structures while dust particles do not, producing adverse behavioral effects (as on dispersion) (Chang, 1946) through dust impregnation on sense organs (Cook and Hain, 1992).

Ink marks persist for a longer period of time than dusts. Several authors report that inks are very durable, mainly the non-water-soluble inks (Wineriter and Walker, 1984). For drosophilids we observed 100% of retention of marks after ten days and almost 85% at the end of the experiment (65 days). Laboratory experiments point out from 11 to 28 days of persistence for dusts (Crumpacker, 1974; Moth and Barker, 1975) with l00% retention after three days and 80% retention after 10 days (Shorrocks and Nigro, 1981). Such shorter period of persistence of dusts can be inappropriate for long-term studies.

Maybe the major advantage of inks compared to dust is the possibility to mark individual (but also groups) insects. Simple numbering systems (Southwood, 1978) or elaborate coding systems (Opp and Prokopy, 1987) can be used to differentiate them. For the studied flies, we considered viable the use of at least ten dots, which in a dot binary-coding system using just one color allows the recognition of 1023 (2^{10}-1) individuals. Distinct colors of dusts can be used in the same order of using distinct dots, but the differentiation of several colors in the same individual is more arduous (personal observations). For the marking procedure, we spent around three seconds per dot, what we consider viable. However, depending on the number of dots per fly and the number of flies to be marked, the process can be very time-consuming, in contrast to the use of micronized dusts.

Finally, it is important to emphasize that prior to such experiment, we tried to mark the specimens on the wings but the ink was removed by cleaning activities of the flies up to one week after marking, sometimes damaging the wings. Another consideration is that for species with thorax partially or totally dark, this method was not effective due to the hard detection of dots, as tested for *D. mercatorum* Patterson and Wheeler, 1942. For this species, we also set marks with a correction white water-soluble fluid but marks were easily missed after death due to the medium moisture. Thus, this ink was not necessarily ineffective for marking drosophilids, but our method to detect the dots only on dead flies did not allow us to make a reliable estimation of longevity.

Marker pen ink is inexpensive, clearly identifiable on recapture, and, has no relevant effect on the longevity of three species of Drosophilidae (*D. simulans*, *D. malerkotliana*, and *Z. indianus*). Since this material allows marking a higher number of individuals, does not impregnate sense organs throughout the body and is more persistent on the insect, we suggest its use for individual-based studies, long-term studies, and those in which sense organs are determinants for trustworthy results.

For other studies, the use of dust is preferable, since it is less time-consuming and a more conventional marking technique for insects (Hagler and Jackson, 2001).

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References: Begon, M., 1976, J. Anim. Ecol. 45: 441-456; Begon, M., O. Milburn, and D. Turner 1975, J. nat. Hist. 9: 315-20; Chang, H.T., 1946, Mosq. News 6: 122-25; Cook, S.P., and F.P. Hain 1992, J. Entomol. Sci. 27: 269-279; Crumpacker, D.W., 1974, Am. Midl. Nat. 91: 118-29; Cruz, C.D., 2006, Programa Genes: Análise multivariada e simulação. Editora UFV. Viçosa (MG). 175p.; Dobzhansky, T., and S. Wright 1943, Genetics 28: 304-340; Dudley, J.E., and E.M. Searles 1923, J. Econ. Entomol. 16: 363-68; Geiger, J.C., W.C. Purdy, and R.E. Tarbett 1919, J. Am. Med. Assoc. 72: 844-47; Hagler, J.R., and C.G. Jackson 2001, Annu. Rev. Entomol. 46: 511-43; Jones, V.P., and M.P. Parrella 1986, Ann. appl. Biol. 109: 33-39; Marques, E.K., M. Napp, H. Winge, and A.R. Cordeiro 1966, Dros. Inf. Serv. 41: 187; Miller, L.R., 1993, Sociobiology 23: 127-34; Morton, A.C., 1982, Oecologia 53: 105-110; Moth, J.J., and J.S.F. Barker 1975, J. Nat. Hist. 9: 393-396; Opp, S.B., and R.J. Prokopy 1987, Fla. Entomol. 70: 449-56; Rosewell, J., and B. Shorrocks 1987, Biol. J. Linn. Soc. 32: 373-384; Shorrocks, B., and L. Nigro 1981, Biol. J. Linn. Soc. 16: 293-301; Southwood, T.R.E., 1978, Absolute population estimates using marking techniques. In: *Ecological Methods*, pp. 70-129, New York: Chapman and Hall. 524p.; StatSoft, Inc. 1998, STATISTICA for Windows [Computer program manual]. Version 5.1. URL: http://www.statsoft.com; Taylor, R.A.J., 1978, Ecol. Entomol. 3: 63-70; Taylor, R.A.J., 1980, Nature 286: 53-55; Wineriter, S.A., and T.J. Walker 1984, Entomol. News 95: 117-23; Wright, S., 1938, Science 87: 430-431.

Effect of Fly Nap® on ovipositing and fertility in *Basc* mutant and wild type *Drosophila melanogaster*.

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Abstract

The objective of this study is to determine whether wild and *Basc* mutant genotypes of *Drosophila melanogaster* treated with the anesthetic Fly Nap® displayed significant differences in their egg deposition and subsequent egg fertility when compared to etherized wild type and *Basc* mutants. Some mutant genotypes are more sensitive to environmental insult than are the wild type (Nguyen *et al.*, 1979). We were particularly curious to note any significant effects of treatment with Fly Nap® on the *Basc* mutant as compared to the wild genotype. Flies were kept in egg-laying chambers at 25ºC, and ovipositing was quantified for each treatment group by counting the number of eggs deposited on agar at post-treatment intervals of 16, 24, 40, 48, 64, and 72 hours. Ovipositing of *Basc* mutants exposed to Fly Nap® was significantly ($p < 0.05$) lower than etherized *Basc* during the first 24 hours post-treatment. After 40 hours the most significant ($p < 0.01$) difference appeared between *Basc* mutants and wild type flies both treated with Fly Nap®. Wild type flies treated with Fly Nap®, while initially displaying lower egg deposition than etherized wild flies, recovered to control levels after 64 hours. The ovipositing of *Basc* mutants treated with Fly Nap® remained significantly ($p < 0.01$) lower than that of similarly anesthetized wild types from 40 hours post-

treatment through the remainder of the post-treatment time course. Fertility was assessed by taking 25 eggs from each group at post-treatment intervals, then counting the number of flies that had fully eclosed as adults. This adult to egg ratio was expressed as percent viability, with Fly Nap®-treated *Basc* mutants showing significantly ($p < 0.01$) lower egg viability than wild type flies treated concurrently with Fly Nap®. These results indicate that ovipositing of the *Basc* mutant, while characteristically lower than the wild type, is even further reduced after exposure to Fly Nap® anesthetic, with an accompanying significant reduction in viability in offspring.

Introduction

Certain mutants of *Drosophila melanogaste*r have shown higher sensitivity to certain environmentally-introduced chemicals than wild-type strains. Delayed maturation, decreased fertility and longevity, and significantly reduced mating success are just some of the detrimental effects of such chemicals. It is especially problematic when this sensitivity is found to include certain anesthetics, which are commonly employed in laboratory and educational settings (Tinklenberg *et al*., 1991; Walcourt and Nash, 2000; Barron, 2000; Weber *et al*., 2009). These are often used for the simplicity and speed in which they render test subjects more easily manipulated (Greenspan, 1997). Such responses to anesthetic can significantly affect behavior to such a degree that test scoring of flies is questionable at best. The detrimental effects of carbon dioxide, chilling, and even rough handling (*e.g*., aspiration) are well documented (Van Kijken *et al*., 1977; Kaiser, 1995; Nilson *et al*., 2006).

During semester-long experiments in which undergraduate genetics students measured changes in gene frequencies among two populations of *Drosophila*, wild-type and *Basc* mutants, we observed a significant reduction in the stabilization frequency of the Bar-eyed mutant alleles after replacement of ether anesthetic with Fly Nap®. *Basc* gene frequencies consistently stabilized at between 0.32 and 0.34 when populations were anesthetized with ether, but dropped to 0.10 or lower after switching to Fly Nap®. While most anesthetics have been shown to cause a reversible loss of mobility and coordination in wild-type and mutant flies (Champion De Crespigny and Wedell, 2008), it is our hypothesis that Fly Nap® causes a more prolonged course of such deficits. In *Basc*, an outbred mutant, that is already known to have comparatively lower fitness (*i.e*., reduced fertility and viability) (Volkova and Vorobjova, 2005), such detrimental effects may prove irreversible.

The objective of this study is to measure ovipositing between two treatment groups. Each treatment group is composed of wild-type and *Basc* mutants. One group was anesthetized with ether and the other with Fly Nap®. A random sample of eggs deposited by each treatment group was sampled and the number of eclosed adults was used as an indicator of fertility and offspring viability. As both strains have been drawn from populations selected to respond to ether anesthetization, the ether-treated group is essentially functioning as a control group.

Materials and Methods

Treatment of Flies

Two samples were taken from each stock population of wild-type and *Basc* mutants, and each placed in 1 in. × 4 in. clear plastic vials. Each treatment group received one sample of wild-type and one sample of *Basc* mutant flies. The first treatment group was anesthetized with ether, and each sample strain sexed. Fifty females were removed from each treated strain and placed in a separate

plastic vial. Five males from each strain were also added to the appropriate vial of like females. The etherized wild-type were designated "ether wild" (EW) and the etherized *Basc* mutants were designated "ether *Basc*" (EB). The second treatment group was anesthetized with Fly Nap®, and each sample sexed and segregated similarly to the first group. Fly Nap®-anesthetized wild-type were designated "Nap wild" (NW) and *Basc* mutants designated "Nap *Basc*" (NB). Each vial of treated flies was inserted and mounted in an egg-laying chamber so that flies could remain isolated from chamber media until they recovered sufficient mobility to enter the chamber. The chambers were then kept at 25°C.

Counting of Eggs

Eggs were harvested from egg-laying chambers maintained at 25°C, by inserting a petri-dish containing nutrient agar tinted with blue food dye. The agar surface was "roughed" and a thin layer of yeast slurry applied down the center of the plate. Flies were allowed to oviposit on agar medium overnight. After 16 hours post-treatment, the oviposited agar was removed and a new petri-dish inserted. Flies were then allowed to oviposit for eight hours during the day. This "recharging" of the egg-laying chambers was carried out for a total of three days, with petri dishes collected and deposited eggs counted at 16, 24, 40, 48, 64, and 72 hours post-treatment.

Removal of Eggs for Viability Testing

At each time point indicated above, 25 eggs were randomly sampled from each agar plate using a metal probe and transferred to plastic vials containing standard cornmeal food agar. Vials were then kept at 25°C. Eggs were allowed to develop until they eclosed after eight to ten days. Only eggs that began their developmental cycle (*i.e.*, developed into instar larvae) were scored as fertile, and all eclosed adult flies were scored and viability expressed as the percentage of full-term adults (including moribund) arising from the original 25 eggs seeded per vial.

Results

Etherized wild-type deposited significantly *($p < 0.05$) more eggs during initial 16 hours post-treatment (Figure 1). There remained significantly **($p < 0.01$) lower egg deposition between Fly Nap®-treated and etherized flies throughout the 48 hour post-treatment interval. Significant ($p < 0.05$) differences remained between etherized wild-type and *Basc* during this period, as well. At 64 hours, differences between wild-type treated with Fly Nap® and ether controls were no longer observed, while Fly Nap®-treated *Basc* deposition remained significantly **($p < 0.01$) lower than for both etherized strains. At 72 hours post-treatment, Fly Nap®-treated *Basc* ovipositing remained significantly *($p < 0.05$) lower than all other treatment conditions.

Offspring viability was quantified as the number of fully-developed adult flies arising from 25 eggs randomly sampled from each treatment condition and expressed as percent viability. *Basc* mutants from both treatment groups exhibited significantly *($p < 0.05$) lower offspring viability than wild-type (Figure 2).

Discussion

In this study, we provide evidence to support the hypothesis that anesthetization with Fly Nap® causes significant differences in post-treatment behaviors, such as ovipositing, and that these changes are more prolonged in the already fitness-stressed *Basc* mutant. Ovipositing in flies treated

with Fly Nap® is reduced by 79% (avg.) in wild-type and 92.4% (avg.) in *Basc* for up to 48 hours post-treatment, when compared with ether controls. After 72 hours post-treatment, ovipositing of wild-type treated with Fly Nap® recovered to within 16% of etherized wild-type. Fly Nap®-treated *Basc* ovipositing remains markedly lower (3.8% of etherized *Basc*) than all other treatment groups.

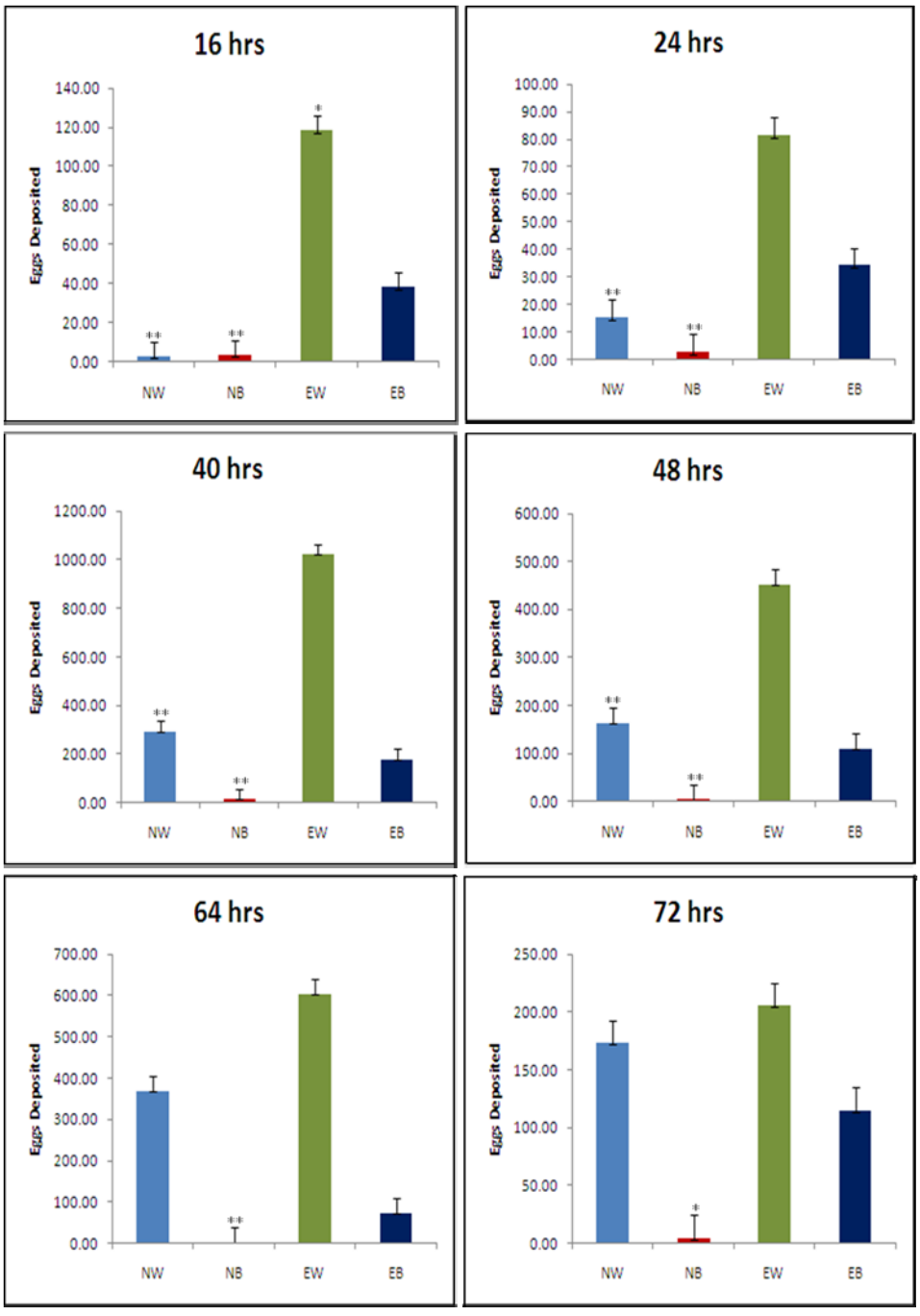

Figure 1. Effects on egg deposition of wild-type and *Basc* mutants anesthetized with Fly Nap® (NW and NB) or ether (EW and EB) at 16, 24, 40, 48, 64, and 72 hour intervals.

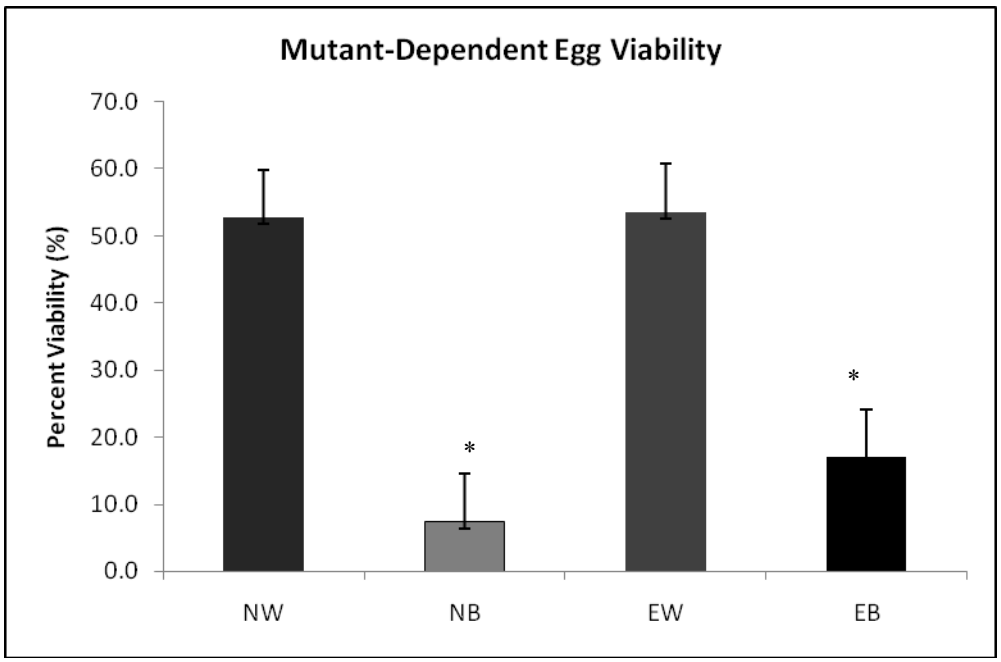

Figure 2. Viability of offspring from wild-type and *Basc* mutants treated with either Fly Nap® or ether anesthetic.

While the level of detrimental effects of Fly Nap® on post-treatment viability of *Basc* offspring remains unclear, mutants from both treatment groups displayed significantly lower ($p < 0.05$) viability than wild-type from either group. These results suggest that treatment with Fly Nap® results in a significant ($p < 0.05$) reduction in egg-laying in both wild-type and *Basc* mutant groups, and that the *Basc* mutants are more highly sensitive to such pertubations from which they fail to recover.

References: Barron, A.B., 2000, J. Insect Physiol. 46: 439-442; Champion De Crespigny, F.E., and N. Wedell 2008, Physiological Entomology 33: 310-315; Greenspan, R.J., 1997, *Fly Pushing: The Theory and Practice of* Drosophila *Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor; Kaiser, M., 1995, Dros. Inf. Serv. 76: 92-93; Nilson, T.L., B.J. Sinclair, and S.P. Roberts 2006, J. Insect Physiol. 52(10): 1027-1033; Tinklenberg, J.A., I.S. Segal, T.Z. Guo, and M. Maze 1991, Ann. NY Acad. Sci. 625: 532-539; Van Dijken, F.R., M.J.P.W. Van Sambeck, and W. Scharloo 1977, Experientia 33: 1360-1361; Volkova, N.E., and L.I. Vorobjova 2005, Russian Journal of Genetics 41(5): 490-494; Walcourt, A., and H.A. Nash 2000, J. Neurobiol. 42: 69-78; Weber, B., C. Schaper, D. Bushey, M. Rohlfs, M. Steinfath, G. Tononi, C. Cirelli, J. Scholz, and B. Bein 2009, Anesthesiology 110: 313-316.

Notes regarding the collection of African *Drosophila melanogaster.*

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Below I describe some techniques and observations concerning the collection of *Drosophila melanogaster* from sub-Saharan Africa. My most recent trip brought me to Ethiopia, Rwanda, and Kenya. Prior to that, I had also collected flies from Cameroon. I find that I'm still improving my collection techniques, but I would like to share some methods that have worked for me (reflecting both my own ideas and many suggestions by colleagues).

Geographical sampling strategy – which towns to collect from:

It seems likely that *D. melanogaster* originated in eastern Africa, so I visited the first three countries named above in the hope of finding genetically diverse populations that would inform us about the history of the species. Within any given country, it is advisable to collect flies from town areas, unless you harbor hopes of being the first person to discover a true wilderness population of *D. melanogaster*. I try to collect from towns of moderate size, avoiding tiny outposts where fly populations might go through frequent local bottlenecks, but also bypassing large cities and ports that may allow introgression from distant populations. In each country I've visited, I've collected at least two samples. This is partly in case one sample has poor population genetic properties (reduced diversity from a recent local bottleneck, or cosmopolitan admixture). An additional way to make the second sample worthwhile is to collect from sites with some environmental contrast, potentially enabling studies of local adaptation. In terms of climate, one might tend to head for a warm, humid area to find lots of flies, potentially in a fruit-growing area. But I haven't found this to be the best strategy. In warmer sites with moderate to high rainfall, *D. melanogaster* sees more competition from other Drosophilids, especially *D. ananassae* (an invader from southeast Asia), and often comprises a smaller portion of the total flies caught (but usually still fairly common). To enrich for *D. melanogaster*, I've found it very effective to either (1) go higher, or (2) go drier. Compared to most tropical Drosophilids, *D. melanogaster* has good resistance to both cold and desiccation. I've found repeatedly that when I collect from either cool mountain towns above 2000m, or dry and dusty lowland towns where you wouldn't expect to find any flies at all, I get a much higher proportion of *D. melanogaster*. I've read some reports of *D. melanogaster* not being found above a given altitude, but in general this seems to reflect the upward limit of human cultivation in those areas – if there's a town of any decent size up there, you're likely to find *D. melanogaster*. As for when to collect: most parts of sub-Saharan Africa have a dry season, which makes travel more practical. If the fly populations are somewhat reduced in this season, at least they're more likely to visit your traps if it's not raining, and if other food is scarce your traps may do all the better.

Collecting the flies – how to construct traps:

The traps I use are of a simple design. I obtain some empty half-liter plastic bottles (this is easy because I go through bottled water at a prodigious rate in Africa). I slice a window into the side of each bottle: about halfway between top and bottom, cutting about 3 cm along the sides and bottom of a square window. Then I add bait – banana is always available and works well for me (best if they're fairly ripe). I slice each banana into two or three sections (depending on length), then cut each section down the middle. I add a pinch of baker's yeast to each banana section, then cover the exposed fruit with a layer of damp cheesecloth (Figure 1A), and drop two banana sections into a trap. I also tie a half-meter string around the neck of the bottle to hang it from (hanging is advisable to avoid a trap full of roaches or ants; Figure 1B).

Local sampling strategy – where to place traps:

I always put traps indoors: partly to protect them from the elements, but also to enrich for *D. melanogaster* at the expense of other species that are less likely to go indoors, such as *D. simulans*. Having said that, I did obtain some *D. simulans* from most towns. Proportions of *D. simulans* (in

terms of the mel+sim total) ranged from zero to about one third. In any town, my strategy is to walk around and identify businesses that seem like promising trap sites. This includes fruit stands (if covered/enclosed), restaurant and café kitchens (mainly if they have fruit or tomatoes around), and most of all, bars. In one Kenyan town, the weather had turned rainy and the singular trap that saved the collection was placed in a beverage distributorship: right next to crates containing thousands of empty beer bottles. Once I've identified a business, I talk to the person in charge, explaining why I'm there and asking if I can hang this bottle-with-banana somewhere inside. I usually offer a small amount of money as compensation (up to a few dollars). As for how long to leave the traps out: even one day can be productive, though a second day might yield more flies per trap. Traps can be left for at least four or five days, depending on how dry the weather is (leaving the trap window mostly closed can help slow the bait drying out). One travel strategy is to leave traps in one town, move on to another town, and after finishing a collection there, come back for the traps in the first town. When picking up the traps, closing them without letting flies escape can be a bit tricky – I just try to close the window quickly with some wide tape (Figure 1C). Then I bring traps back to the hotel for processing. I don't recommend traveling very far with traps full of flies – this usually leads to significant mortality and/or escapes during transport.

Processing the flies – from traps to isofemale lines:

I've tried a couple techniques for getting flies out of the trap bottles. I've often used FlyNap (an anesthetic from Carolina Biological) to knock out a bottle of flies by setting a trap on its side and applying some of the liquid just inside the top. FlyNap puts the flies to sleep for a relatively long time (up to an hour or more, depending on exposure), which facilitates close examination, but direct contact with liquid Flynap is fatal to the flies. To expose flies to the vapors only, I apply Flynap to a large cotton ball that is in the middle of a 50mL Falcon tube (leaving a gap between the cotton and the top of the tube). Next I put a trap bottle on its side and cover the top half (encouraging the phototaxic flies to move away from the cap). Then I remove the cap and quickly replace it with a square of cheesecloth (Figure 1D) and then the Falcon tube with Flynap (Figure 1E). Vapors disperse through the cheesecloth into the trap bottle and safely anesthetize the flies. Then I slice the trap in half (with scissors, starting from the window; Figure 1F), remove flies with a paintbrush (Figure 1G), and examine them under a simple plastic photographer's loupe (15× lens; though clearly more sophisticated field scopes are available). Near the end of my last trip I ran out of FlyNap. Instead, I replaced a bottle's cap with a cotton ball, then used an aspirator/pooter to take out the flies one small batch at a time. Since these flies were awake, it was harder to look at them closely, but in general I was able to sort them by eye and I could still immobilize a fly by pinning it with a cotton ball against the side of a tube. Each putative *D. melanogaster* female then goes into a separate tube. The plastic tubes I used were 16×75mm test tubes (Lake Charles Manufacturing) with medium size cotton balls. These tubes fit into 3" tall freezer boxes (5×5" wide) with 7×7 dividers for space-efficient transport (Figure 1H). Before the trip, I wrapped about 3 ml of dry instant fly food (Carolina Biological) in a square of cheesecloth, then pushed this into the bottom of each tube. In the future, I may mix some dry antibiotic into the food before putting it in tube (choosing an antibiotic that doesn't kill yeast; bacterial growth was a problem during my last trip). In Africa, I added 3 ml of bottled water to each tube (sterility is important here), let it soak in for a couple days, then removed excess water and let it dry a bit before adding a few tiny pellets of yeast and then the flies. I'm mainly interested in starting isofemale lines, but I also bring back one or more vials containing multiple wild-caught males (they can occasionally be useful for "rescuing" an isofemale line that doesn't yield any male progeny, or for other experiments).

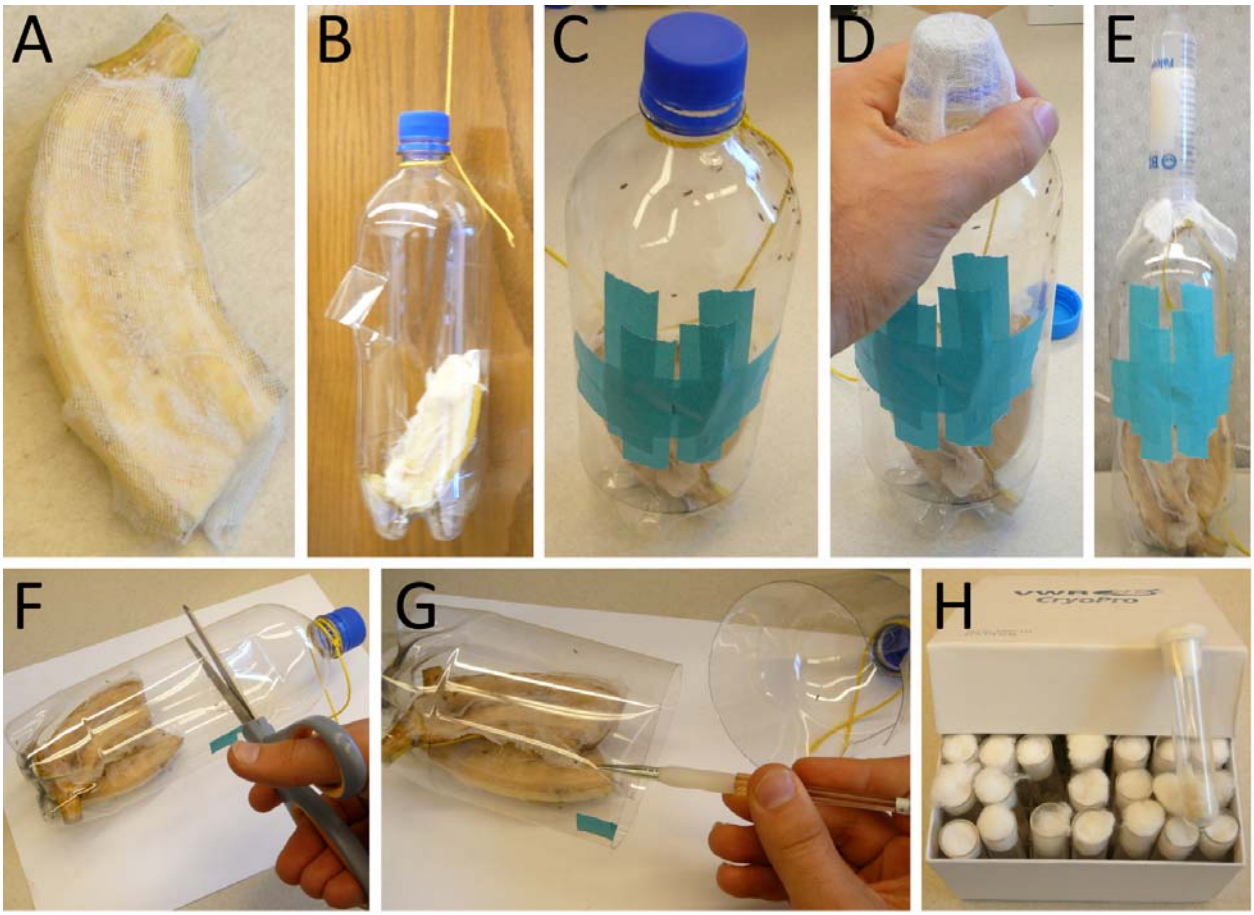

Figure 1. Photographs illustrating collection techniques, as described in the text.

From field to lab – getting the flies home:

Once in the tubes, flies travel quite well. For travel within Africa and for the journey back home, I always wrap the boxes of fly vials in a "space blanket". They're very light-weight, take up almost no space, cheap if ordered online, and highly insulative against heat and cold. I have two ways of getting flies from Africa back to the U.S., and I generally use both by duplicating each line in Africa (transferring each female to a new tube). One duplicate I ship back via DHL (they have a good African network and, last I checked, the best policy toward shipping insects). The other duplicate I travel with in my checked baggage. I haven't had any problem with things freezing in my checked bag, and the flies get less scrutiny there than they would in my carry-on.

Red tape:

Actually collecting *D. melanogaster* is about the easiest field work a biologist could ask for. But navigating layers of bureaucracy to get permission to take the flies home is a far more daunting task. I have succeeded in getting research and export permits a couple of times (Cameroon and Kenya), but this has always involved sacrificing a large portion of my time in Africa (even after initiating the process well before the trip) and barely receiving the export permit before my flight home. Also in both cases, I would not have been able to obtain permits without the help of local scientific contacts. In other countries, the permit process never became clear to me, even after

consulting researchers based there. When leaving a country, flies in my checked baggage have never received any interest at the airport. Once in Ethiopia, a baggage scanner wanted to look at the empty tubes of fly food in my wife's bag because she thought it looked like a carpet (perhaps those are subject to export tax), and was then suspicious about the tubes, but eventually they let us through (after I tasted the instant fly food in front of them and assured them that it was "flakes" and not "powder"). When shipping flies, convincing an African DHL office to accept a shipment of live flies is not guaranteed, but my success rate has been >90%. The primary documents of interest to DHL have been my U.S. import permit and an official-looking letter from my home institution). For bringing or shipping flies to the U.S., an import permit from the USDA PPQ office is needed, and can be applied for online. Note that if you're flying in with flies: you should let the USDA know when you're coming, you have to enter the U.S. at the "port of entry" listed on your permit, you must have one of your permit's "mailing labels" with the flies, and the flies should be sealed inside a second box.

Armchair collecting:

In case you'd like to have some flies collected but no one is available to make the trip, I've also had fairly good luck contacting researchers in Africa and asking if they'd be willing to send me some flies. Basically, I sent these kind people some simple collection equipment, compensated their travel expenses (a few hundred dollars at most), and they shipped flies to me. Results were variable but generally good, especially when I could find an entomologist to help. The instructions I sent to them resemble the methods described above. An alternative to the isofemale line preparation for less experienced collectors (which I have not yet tested) would be to receive a mixed-sex batch of flies, age and transfer the wild-caught females until they run out of sperm, and then pair each with a different wild-caught male.

Correction

Johnson, David A. 2007. Amplification of DNA from 30-year-old aceto-orecein stained salivary gland squash slides. Dros. Inf. Serv. 90: 156-158.

Two corrections are highlighted in bold below in the procedure steps 23 and 26. Step 23 should indicate –70°C, not 70°C; and step 26 omitted 70% before the word ethanol.

23) Incubate at **–70°**C for 5 minutes

26) Add 1000 µl **70%** ethanol, mix gently, and centrifuge for 1 minute at 13,000-16,000 ×g.

Call for Papers

Submissions to Drosophila Information Service are welcome at any time. The annual issue now contains articles submitted during the calendar year of issue. Typically, we would like to have submissions by mid-December to insure their inclusion in the regular annual issue. but articles can be accepted for this volume until 31 December. Details are given in the Guide to Authors or on the DIS web site: www.ou.edu/journals/dis.

New Species

Drosophila parapallidosa Tobari, sp. nov., is a new member of the *D. ananassae* species complex.

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Introduction

The *ananassae* species subgroup belongs to the *melanogaster* species group, and includes 22 species. In this subgroup, there are three species complexes: *ananassae*, *bipectinata* (Tobari, 1993), and *ercepeae* (Lemunier *et al.,* 1996). In the *ananassae* complex, which includes 10 species (Tobari, 1993), Tomimura *et al.* (1993) reported that, among 20 isofemale lines established from wild caught females from Kota Kinabalu, four lines are distinguishable from others by the composition of their inversions. These four isofemale lines do not carry the ST arrangement in the 2nd chromosome but do have *In(2L)B*, *In(2L)C*, and *In(2R)A*. They also have *In(3L)E* and *In(3R)B*, which have never been found elsewhere in the *D. ananassae* populations. We tentatively called these 4 lines Taxon-K. To investigate the phylogenetic relationships in the *ananassae* complex, analyses of mitotic and meiotic chromosomes, genomic DNA, mtDNA variation, and sterility of hybrid males were carried out. This paper diagnosed and describes Taxon-K, *Drosophila parapallidosa,* Tobari, sp. nov.**,** as a new member of the *ananassae* complex of the *ananassae* species subgroup.

Results

Taxonomy

Drosophila (Sophophora) *parapallidosa* Tobari sp. nov.

Diagnosis

The external morphology of *D. parapallidosa* is indistinguishable from that of *D. pallidosa*. The phallic organs of the three species are shown in Figure 1.

Description

It is very hard to distinguish *D. parapallidosa* from *D. pallidosa*, but differences in several morphological characters are listed in Tables 1 - 3. All type specimens have been deposited in the National Science Museum, Tokyo, Japan (NSMT).

Holotype

This is a male from the isofemale line T184, which was collected in August 1979 at Kota Kinabalu, Malaysia by Y. Fuyama, F. Hihara, and T.K. Watanabe.

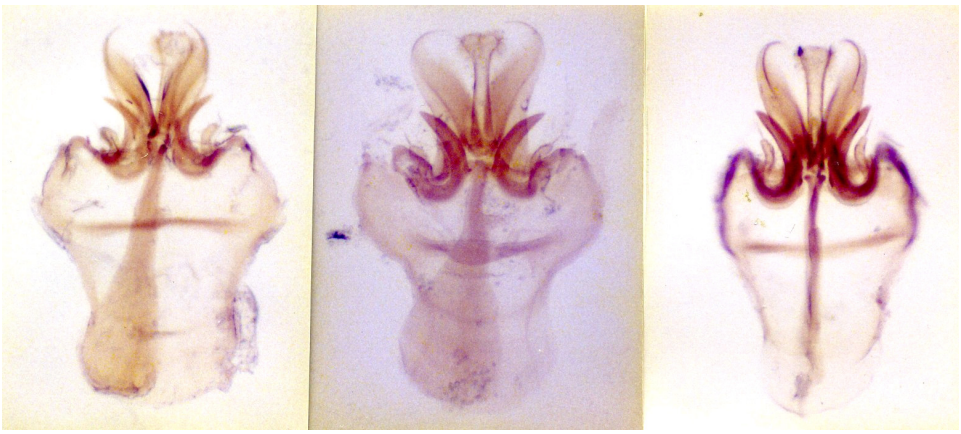


Figure 1. Phallic organs of three species, *D. parapallidosa*, *D. pallidosa*, and *D. ananassae.*

A. *D. parapallidosa* B. *D. pallidosa* C. *D. ananassae*

Table 1. Average number and teeth of the sex combs and their ranges in *D. parapallidosa* (T226), *D. pallidosa* (NAN24), and *D. ananassae* (HW).

Sex comb	Row	Species		
		D. parapallidosa	*D. pallidosa*	*D. ananassae*
metatarsus	1			1.0(0-3)
	2		1.4(0-2)	2.5(0-4)
	3	0.2(0-3)	4.0(1-6)	4.1(1-6)
	4	2.9(1-6)	5.1(3-7)	5.5(3-7)
	5	4.1(2-7)	4.9(4-7)	6.0(3-8)
2nd tarsus	1			
	2		0.1(0-1)	0.6(0-2)
	3	0.4(0-2)	2.2(1-3)	2.8(0-4)
	4	3.2(2-5)	3.5(3-5)	5.0(2-6)
	5	3.4(2-5)	4.0(3-5)	4.0(3-6)
No. of flies tested		140	160	50

Data from Appendix Table of Matsuda et al., (2009)

Paratypes

This group is comprised of 9 males and 10 females from the T184 line and 10 males and 10 females from the T226 line that was collected in August, 1979 at Kota Kinabalu, Malaysia by Y. Fuyama, F. Hihara, and T.K. Watanabe.

Distribution

KOTA KINABALU, Malaysia, LANYU, Taiwan, and OKINAWA, Japan.

Etymology

The name indicates the phenotypic similarity to *D. pallidosa*.

Table 2. Average values and ranges of taxonomic indexes of wings in *D. parapallidosa*, *D. pallidosa*, and *D. ananassae.*

Indexes	Species		
	D. parapallidosa	*D. pallidosa*	*D. ananassae*
C	1.3 (1.1-1.6)	1.4 (1.2-1.9)	1.4 (1.3-1.6)
4V	2.5 (2.0-3.0)	2.2 (1.7-2.8)	2.4 (2.0-3.1)
4C	2.0 (1.6-2.4)	1.7 (1.5-2.1)	1.8 (1.6-2.4)
5X	2.1 (1.5-3.0)	2.1 (1.4-2.7)	2.0 (1.5-2.6)
C3fg	0.54 (0.51-0.58)	0.52 (0.47-0.57)	0.51 (0.47-0.53)`
No. of flies tested	60	62	75

Data from Appendix Table of Matsuda et al., (2009)

Table 3. Major components of the cuticular hydrocarbons in *D. parapallidosa*, *D. pallidosa*, and *D. ananassae.*

Species	Major cuticular hydrocarbon
*D. parapallidosa**	C31: (Z,Z)-5,25-hentriacontadiene [(Z,Z)-5-25-$C_{31:2}$]
*D. pallidosa***	C33: (Z,Z)-5,27-tritriacontadiene [(Z,Z)-5,27,$C_{33:2}$]
*D. ananassae****	C31: (Z,Z)-5,25-hentriacontadiene [(Z,Z)-5-25-$C_{31:2}$]

* Data from Matsuda et al., (2009)
** Data from Oguma (1993) and Nemoto et al. (1994)
*** Data from Doi et al. (1997)

Morphological Characteristics

Sex combs

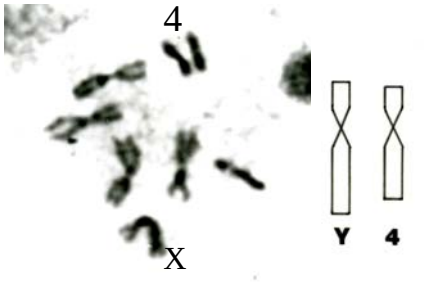
The number of teeth of the sex combs in *D. parapallidosa* is less than those in *D. ananassae* and *D. pallidosa* (Table 1).

Male terminalia

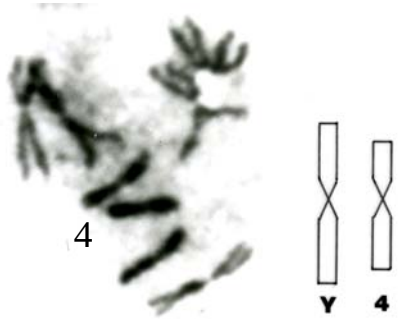
Periphallic and phallic organs of *D. parapallidosa* are difficult to distinguish from *D. pallidosa* males, as shown in Figure 1.

Wing index

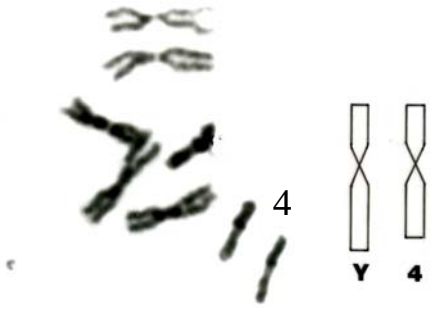
Ranges of four traits: Costal index, 4V, 4C, and 5X are shown in Table 2. C3fg values overlap completely among species, ranging from 0.42 to 0.63; average values are 0.51, 0.52, and 0.54 in *D. ananassae*, *D. pallidosa*, and *D. parapallidosa*, respectively. Other indices overlap as well in all three species, although the mean values are slightly different.



D. parapallidoas



D. pallidosa



D. ananassae

Figure 2. Male mitotic chromosome configurations of three species, *D. parapallidosa, D. pallidosa,* and *D. ananassae*.

Chromosome Configurations

Mitotic chromosomes

The Y and 4th chromosomes are slightly shorter in *D. parapallidosa* than those in *D. pallidosa*. Both chromosomes are sub-telocentric in *D. parapallidosa,* while the Y chromosome is submetacentric in *D. ananassae* and metacentric in *D. pallidosa* (Figure 2).

Polytene chromosomes

D. parapallidosa has no Standard arrangement on 2L and 2R, but has *In(2L)B, In(2L)C,* and *In(2R)A*. *D. parapallidosa* also has the *In(3L)E* arrangement on the 3rd chromosome, which has not been found in either *D. ananassae* or *D. pallidosa* (Tomimura *et al*., 1993. Matsuda *et al*., 2009).

Table 4. Hybrid male sterility and insemination success (%) among three species, *D. parapallidosa*, *D. pallidosa*, and *D. ananassae.*

Female \ Male	*D. parapallidosa*	*D. pallidosa*	*D. ananassae*
D. parapallidosa	-	F (68%)	F (44%)
D. pallidosa	S (82%)	-	F (67%)
D. ananassae	S (8%)	F (2%)	-

F: fertile, S: sterile (Data from Matsuda et al., 2009)

Cuticular Hydrocarbons

The main cuticular hydrocarbon of *D. parapallidosa* is the same as that of *D. ananassae*, but different from that of *D. pallidosa,* as shown in Table 3.

Interspecific Hybridization

Hybrid females are fertile in all interspecific crosses. F_1 males from crosses between *D. ananassae* or *D. pallidosa* females and *D. parapallidosa* males are sterile, while those from the reciprocal crosses are fertile (Table 4). Mating success between species is described in Matsuda *et al*. (2009) and summarized in Table 4. There are large differences in the reciprocal crosses between *D. ananassae* and *D. parapallidosa* (44% *vs.* 8%), but only slight differences in the reciprocal crosses between *D. pallidosa* and *D. parapallidosa* (82% *vs* 68%). Both pre-mating and post-mating isolations are found between *D. parapallidosa* and *D. ananassae* or *D. pallidosa*.

Table 5. Typical gene arrangements of three species, *D. parapallidosa*, *D. pallidosa*, and *D. ananassae.*

Gene arrangements	*D. parapallidosa*	*D. pallidosa*	*D. ananassae*
XLST	+	-	+
XLA	-	+	-
XRST	+	+	+
2LST, 2LA, 2LJ	-	-	+
2L(C+B)	+	-	-
2L(CD+B)	-	+	-
2RST	-	+	+
2RA		+	-
2RAB	-	+	-
3LST	+	+	+
3LA	-	-	+
3LE	+	-	-
3RST	+	-	+
3RA	-	-	+
3RB	+	+	-

Data from Tomimura et al., (1993), and Matsuda et al., (2009)
+ : present, - : absent

Discussion

The external morphology of *D. parapallidosa* is similar to *D. pallidosa* Bock and Wheeler, but they can be distinguished by the number of teeth of the sex comb, chromosome arrangements, karyotypes, hybrid sterility, cuticle hydrocarbons, and molecular variations. Although *D. parapallidosa* was first found in 1971 from Kota Kinabalu, Malaysia, and recurrently found in 1979 from the same area, it was not found in other populations until 1998. Since 1998, we have found *parapallidosa* in Ishigaki-jima, Iriomote-jima, and Hateruma-jima, Okinawa, Japan, and in Lanyu, Taiwan. Apparently, *D. parapallidosa* has recently migrated from a tropical area north to a sub-tropical area and expanded its habitat.

Acknowledgments: We greatly thank Dr. Gerhard Baechli for his valuable comments on the manuscript.

References: Doi, M., T. Nemoto, H. Nakanishi, Y. Kuwahara, and Y. Oguma 1997, J. Chem. Ecol. 23: 2067-2078; Lemunier, F., S. Aulard, M. Arienti, J.M. Jallon, M.L. Cariou, and L. Rsacas 1996, Ann. Entomol. Soc. Am. 90: 28-42; Matsuda, M., C-S. Ng, M. Doi, A. Kopp, and Y.N. Tobari 2009, Fly 3: 157-169; Nemoto, T., M. Doi, K. Oshio, H. Matsubayashi, and Y. Oguma 1994, J. Chem. Ecol. 3029-3037; Oguma, Y., 1993, In: *Tobari, 1993*, pp. 199-207; Tobari, Y.N., 1993, Drosophila ananassae: *Genetical and Biological Aspects*. Tokyo, Japan Scientific Society Press; Tomimura, Y., M. Matsuda, Y.N. Tobari, M.L. Cariou, J.L. Da Lage, W. Stephan, and C.H. Langley 1993, In: *Tobari, 1993*, pp. 139-198.

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)-325-4821; 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. 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.

Mutation Notes

Wing mutations detected in *Drosophila subobscura.*

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In the Málaga (Spain) population, crosses using the lethal balanced strain *Va*/*Ba* were carried out to obtain wild O chromosomes of *D. subobscura* in homozygous condition (Sperlich *et al.*, 1977; Mestres *et al.*, 1990). One of the chromosomal lines presented a mutation characterized by the lobulation of the distal area of the wing (Figures 1 and 2). It resembles the *dumpy* (*dp*) mutation of *D. melanogaster* (Lindsley and Zimm, 1992; Ranganath and Tanuja, 1999). This mutation has been previously described in *D. subobscura* (Krimbas, 1993). As in the F_3 we obtained wild, *dumpy*, *Varicose* and *dumpy Varicose* flies, it can be concluded that this mutation is recessive and not located in the O chromosome of the species. Its expressivity is variable. It can be qualified as rank RK1.

Figure 1. Ventral view of a *D. subobscura* female showing *dumpy* and *Varicose* mutations. The lobulation is clearly visible.

Figure 2. Wing detail of *dumpy* mutation.

Figure 3. General view of *D. subobscura* females showing the *ac* mutation. The drooping position of wings is apparent.

Figure 4. Female showing the drooping wing phenotype.

In another chromosomal line, a wing mutation characterized by the abnormal disposition of the wings, presenting them in a drooping position in reference to the body axis was obtained (Figures 3 and 4). This mutation has not been previously described in *D. subobscura* (Krimbas, 1993). We have called this mutation *ac* ("*ales caigudes*"). The mutation is recessive and located in the O chromosome. It can be qualified as rank RK1.

Acknowledgments: This work was supported by grants CGL2006-13423-C02-02 of M.E.C. (Spain), SGR2005 00995 from the Generalitat de Catalunya (Spain).

References: Krimbas, C.B., 1993, D. subobscura. *Biology, Genetics and Inversion Polymorphism*, Verlag Dr. Kovac, Hamburg, Germany; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster, Academic Press, San Diego, USA; Mestres, F., G. Pegueroles, A. Prevosti, and L. Serra 1990, Evolution 44: 1823–1836; Sperlich, D., H. Feuerbach-Mravlag, P. Lange, A. Michaelidis, and A. Pentzos-Daponte 1977, Genetics 86: 835–848; Ranganath, H.A., and M.T. Tanuja 1999, Resonance 4: 95–104.

New wing mutation in *Drosophila subobscura.*

Calabria, Gemma, and Francesc Mestres. Dept. de Genètica, Facultat de Biologia, Universitat de Barcelona. E-mails: gemmacalabria@ub.edu; fmestres@ub.edu

To obtain wild chromosomes in homozygous condition for the O chromosomes of *D. subobscura* from Málaga (Spain), appropriate crosses using the lethal balanced strain *Va*/*Ba* were carried out (Sperlich *et al.*, 1977; Mestres *et al.*, 1990). One of the chromosomal lines obtained presented a wing mutation. This was characterized by a reduction in length and changes in the shape (Figure 1). The vein pattern is altered forming new transverse veins (Figure 2a, 2b). This mutation has not previously been described in *D. subobscura* species (Krimbas, 1993). We have called this mutation *aa* (ales alterades). The mutation is recessive and located in the O chromosome. It can be qualified as rank RK1.

Figure 1. General view of a *D. subobscura* male and female with the *aa* mutation. The reduction of wing size is apparent.

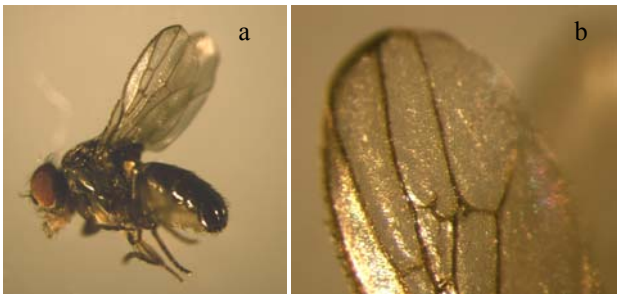


Figure 2. (a, left) Lateral view showing the altered vein pattern. (b, right) Detail of the wing where can be observed additional transverse veins.

Acknowledgments: This work was supported by grants CGL2006-13423-C02-02 of M. E. C. (Spain), SGR2005 00995 from the Generalitat de Catalunya (Spain).

References: Krimbas, C.B., 1993, D. subobscura. *Biology, Genetics and Inversion Polymorphism*, Verlag Dr. Kovac, Hamburg; Mestres, F., G. Pegueroles, A. Prevosti, and L. Serra 1990, Evolution 44: 1823-1836; Sperlich, D., H. Feuerbach-Mravlag, P. Lange, A. Michaelidis, and A. Pentzos-Daponte 1977, Genetics 86: 835-848.

Novel mutants in *D. simulans.*

Sousa-Neves, Rui, Joseph Schinaman, and Joyce Cater. Department of Biology, Case Western Reserve University, Cleveland, Ohio 44106.

Here we report five mutations in *Drosophila simulans*. All mutations appeared spontaneously in stocks recently established from natural populations of different localities. Below is a brief description of these mutants.

Results and Discussion

1. *orange glue*[1] (*ogl*[1]).

ORIGIN: Recessive mutation isolated in March 2009 from wild type Cayucos (California, 2008).
PHENOTYPE: The eyes are smaller than the wild type and rough. The orange eye pigmentation is usually unevenly distributed and more concentrated towards the center of the eye (Figure 1). The outer part of the eye varies from completely white to light orange. In males the eye color is usually more even and stronger than in females. The stock has normal fertility, but viability appears to be reduced.
LINKAGE: Chromosome 3. Placed on 3R due to the fact that *ogl* is balanced by In(3R)Ubx (81F1;84B1;84E1;89E1).

Figure 1. *orange glue* female. Note the color and distribution of the eye pigments of *ogl* when compared to the eyes of the females in Figure 2.

2. *blond*[1] (*bd*[1])

ORIGIN: Recessive mutation isolated in 2008 from wild type Fillmore (California).
PHENOTYPE: Adults have yellowish hairs and a cuticle slightly lighter than wild type (Figure 2A). The dark pigments in tergites vary from plain black to brown, and are usually darker than *D. melanogaster y*[1] or *D. simulans y*[2], which appear light brown. The wing surface appears to be thinner than the wild type and sometimes slightly ruffled. The wings are fragile and in old animals they frequently appear torn. The external cuticle of mutant pupae also appears more transparent than the wild type, and pharates are not as tanned as the wild type (Figure 2B). In addition, *bd*[1] males are not as successful in mating with females as wild type males, and both sexes appear less active than wild type.
LINKAGE: Chromosome 2.
NOTES: No detectable insertions/deletions were found within the transcription unit of all four *yellow*-related genes of the second chromosome (*yellow*-b, 36A14, *yellow*-c, 35B8, *yellow*-d, 59D9, and *yellow*-d2, 59D9). The *D. melanogaster* deficiencies Df(2R)or-BR6 (59D5-59D10;60B3-60B8) and Df(2L)TE35BC-24 (35B4-35B6;35E1-35E2) do not disrupt *bd*[1]. Blond macrochaeta, but not microchaeta, are greatly suppressed by *ebony*.

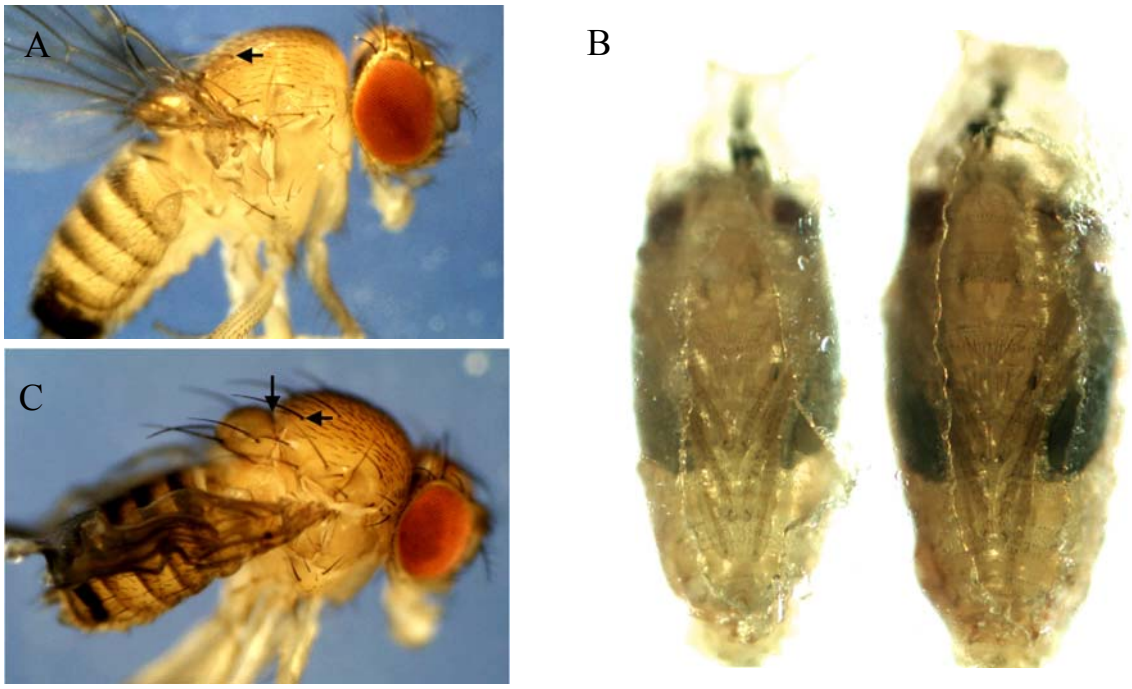


Figure 2. *blond*, *cy* and *tronc* mutants. A) *blond*[1] female. Compare the yellow hairs (arrows) and body color to the female shown in (C). B) *bd*[1] pupae. a *blond*[1] male (left) and a wild type male (right). Note the paler color of the wings of *bd*[1]. C) Double mutant *cy*[S]; *tronc*[1]**.** Note the enlarged thorax and deeper groove between the thorax and scutellum (arrow) when compared to the female in Figure 2. Double mutants frequently fail to extend wings.

3. *troncudo*[1] (*tronc*[1])

ORIGIN: Recessive mutation isolated in 2007 from wild type Sacra Família (State of Rio de Janeiro, Brazil). *tronc*[1] appeared in the F2 of another mutant found in Sacra, *cy*[S]. The original stock was *cy*[S] *tronc*[1], but *cy*[S] is separable from *tronc*[1].

PHENOTYPE: The thorax and scutellum are enlarged and the groove between the scutellum and the thorax is deeper than in the wild type (Figure 2C). Extensive lethality occurs at embryonic stages as judged by the number of dead embryos. *tronc*^{1} mutant flies are flightless and move slower than wild type.
LINKAGE: Chromosome 3. Placed inside In(3R)Ubx (81F1;84B1;84E1;89E1) due to the fact that the mutation is balanced by this inversion.
NOTES: Since the heterozygotes In(3R)Ubx /*tronc*^{1} are far healthier than homozygous *tronc*^{1}, they completely dominate the culture. *tronc*^{1} *ebony* flies are poorly viable and larvae develop black pseudo-tumors. Larvae eventually die completely black. In contrast *cy*^{S} *bd*^{1}; *tronc*^{1} have a better viability.

4. *curly* of Sacra (*cy*^{S}).

ORIGIN: Sacra Familia Stock
PHENOTYPE: Flies with curly wings, recessive.
LINKAGE: Chromosome 2 based on the failure to complement *cy*^{NC}.

5. *small wings*^{1} (*swg*^{1}).

ORIGIN: Recessive mutation found in November 2009 after crossing *bd*^{1} to *net pm*; *st e*.
PHENOTYPE: Wings are slightly darker, less transparent, and are reduced to approximately 60% of the wild type size (Figure 3). Also, wings often appear arched downwards. *swg*^{1} shares a great resemblance with *miniature* (*m*). Wing miniaturization is somewhat enhanced by the presence of *net*. These animals are viable and fertile.
LINKAGE: Not yet determined.

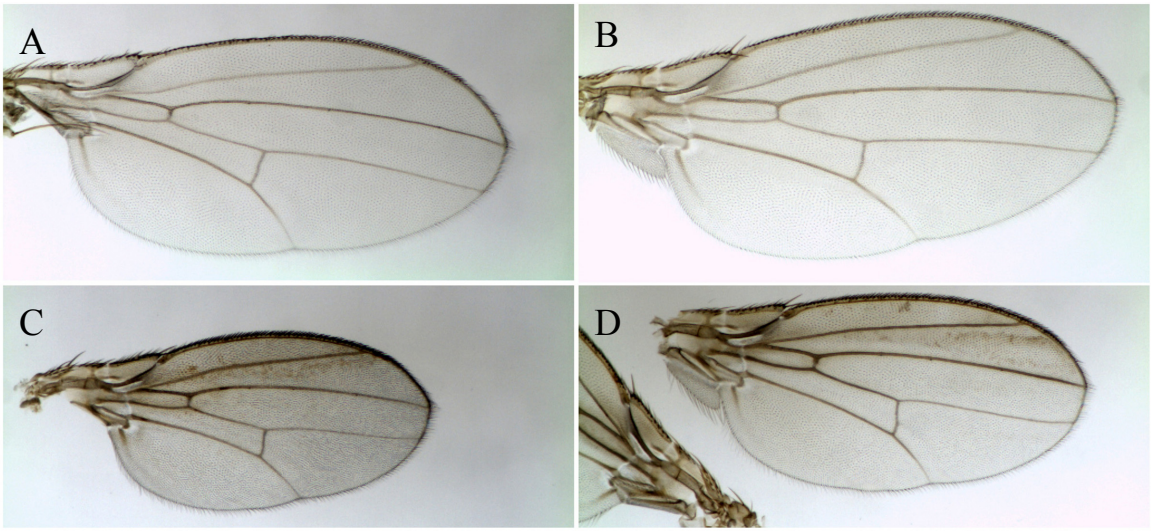


Figure 3. *swg* wings compared to the wild type. A-D same magnification. E and F, same magnification. A, wild type wing size of a *bd*^{1} male. B, wild type wing size of a *bd*^{1} female. C, wing size of a *swg*^{1} male. D, wing size of a double mutant, *swg*^{1} *bd*^{1}. Note the reduction in size of the wings in C and D when compared to A and B.

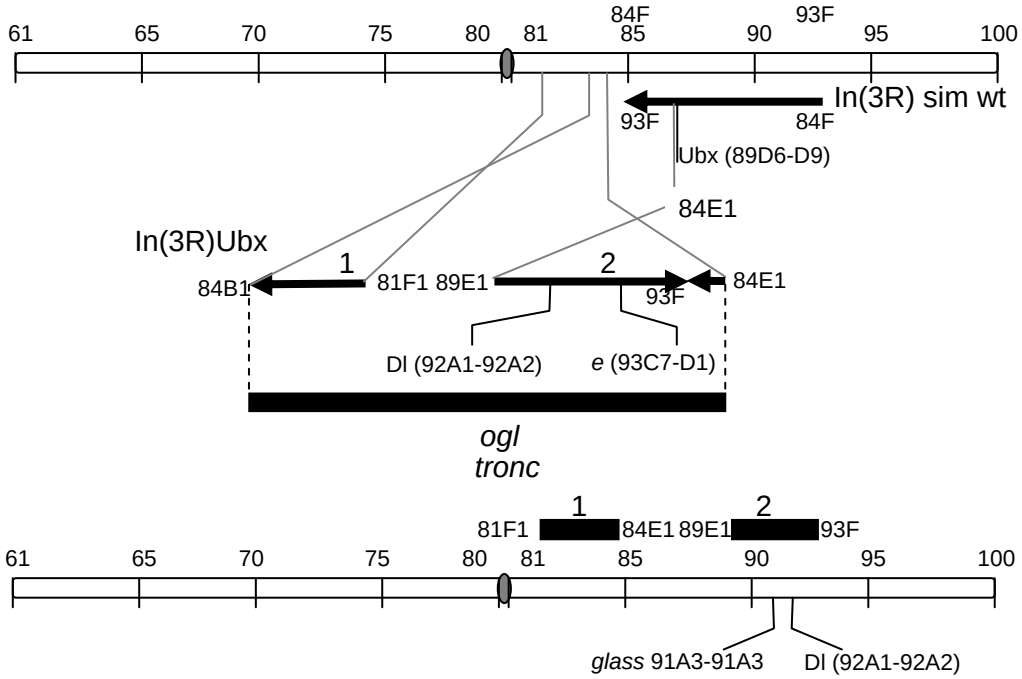


Figure 4. Estimated position of *ogl* and *tronc* based on the breakpoints of In(3R)Ubx**.** Chromosome 3 is represented at the top and bottom of this figure with some divisions of *D. melanogaster*. The wild type inversion In(3R) is shown right below the top chromosome (right to left arrow). Below this inversion is the mutant inversion In(3R)Ubx according to the breakpoints computed by Coyne and Sniegowski. Since In(3R)Ubx balances both *tronc*[1] and *ogl*[1], these genes were placed within or near the two inversions. If we project the inverted regions on the *D. melanogaster* map, two separated intervals emerge (81F1-84E1 and 89E1-93F, bottom horizontal black bars). Further evidence in support for placing *ogl* in this region comes from the fact that no recombinants were recovered between *Dl* and *ogl*[1] in a stock where *Dl*, *ogl*[1] and In(3R) Ubx were left segregating for several generations. Since Dl is balanced by this inversion and recombinants between *Dl* and *ogl*[1] are rare, *ogl*[1] seems to be close to *Dl*. Together these results favor that *ogl* might be localized in the second interval (89E1-93F), near Dl. This region in *D. melanogaster* contains *glass*, a mutant whose description is very similar to *ogl*.

The great similarity of the *bd* phenotype to the *D. melanogaster* X-linked *yellow* (*y*), as well as our findings of the autosomal position of *bd* on the second chromosome, initially led us to test molecularly whether the *bd* strain contained insertions or deletions in one of the autosomal *y*-related genes that were computationally identified on the second chromosome. We could not detect any evident deletions or insertions in the coding regions of these four genes.

bd is the second mutation on the second chromosome with a phenotype similar to *yellow* identified in *D. simulans*. The first is *straw*, identified in both *D. simulans* (*sw*, 2-61) and *D. melanogaster* (*stw*, 2-55). The genetic mapping of *bd* is currently underway and may soon provide a more approximate position of the gene. So far, preliminary data suggest that *bd* is not close to either *net* or *pm* (38 units away from *pm* and 41 units away from *net*, n = 368). However, in the same tests, the distance between *pm* and *net* (2-108, Sturtevant,1929) appears grossly underestimated (2-44) due to the large distance between these two genes, which results in significant number of undetected

double crossovers (Figure 4). Thus, it seems that closer markers will be needed to have a more precise position of *bd*.

swg[1] and *ogl*[1] resemble mutants previously identified in *D. melanogaster*. *swg* is similar to *miniature* and *ogl*[1] resembles *glass* (*gl*). We mapped *ogl*[1] on the third chromosome inside or close to In(3R)Ubx, which includes the region that contains the *D. melanogaster gl* gene. Future allelism tests and molecular data should resolve whether *ogl* corresponds to the *D. melanogaster gl*.

Acknowledgments: We thank C.M. Mizutani for assistance in collecting the wild type strains and access to the Sacra Família site. We also thank J. Um for the deficiency tests. This work was partially funded by the Department of Biology of CWRU to the course BIOL 359/459. J.S. is a Master Student partially supported by the College of Arts and Sciences, and J.C. is an undergraduate student at CWRU.

References: Coyne, J.A., and P.D. Sniegowski 1994, Sturtevant, A.H. (1929). Publs Carnigie Instn 399: 1-62.

New mutants of *D. simulans* in Koltzov Developmental Biology Institution, Moscow.

Dmitrieva, Olga[1], Elena G. Ugnivenko[1], Kirill Kirsanov[2], Roman Sidorov[2], and Elizabeth M. Khovanova[1]. [1]N.K. Koltzov Institute of Developmental Biology RAS, Moscow, Russian Federation; [2]Inst. Carcinogenesis, N.N. Blokhin Cancer Research Center RAMS, Moscow, Russian Federation.

All the mutations listed below are of a spontaneous origin, besides those with compound X chromosomes. These last strains were obtained by E.G. Ugnivenko in 1975 by X-irradiation of the original *y w* strain. Permissive temperatures for temperature-sensitive strains are between 19° and 22°C. All the strains besides the y^2 and the *y w* derive from our laboratory.

Recessive mutants, no selection required

1. *vermilion-724.* Eyes are bright red, vermilion. This mutation is on the X chromosome. It is recessive, analogous to the correspondent mutation in *Drosophila melanogaster*. Mass culturing is allowed. Fertility and viability are very good.
2. *yellow*^{2}. The body and bristles are yellow, wings are grey. The mutation originated spontaneously in the strain *vermilion*. The y^2/y flies have the y^2 phenotype.
3. *yellow* (a revertant from y^2 to *y*) – body and bristles are yellow, wings are grey. The strain contains also *vermilion.* Culture does not require individual crosses. *See also the description of the* y^2 *allele in the strain # 7, together with* vg^X.
4. *yellow*^{bold} – The body, bristles and wings are yellow. Microchaetae are rare, especially on the central lane between the left and right dorsocentral macrochaetae. In total, only four rows of microchaetae are present instead of eight. Viability and fertility are not very good. Females *yellow*^{bold} / *yellow* have a *yellow* phenotype. Requires constant attention because of the possible reversion of *yellow*^{bold} to *yellow*.
5. *white* – white eyes; X-chromosomal, 4.5 M. Well viable and fertile.
6. *vestigial*[1]. Phenotypically corresponds to *vg* mutants in *D. melanogaster*. Dense culture is recommended. Viability is lower than in the wild type. Avoid high and low temperatures.

7. *yellow vestigial*^{X}. *y* – yellow body and bristles. *vg*^{X} – reduced, laterally griped notum and scutellum, halteres and wings are missing; some individuals may have remnants of the wing blade, frequently shapeless or bubbled. Weak viability and fertility. Mass culture is allowed.
8. *porcupine* (*pcp*) – rough, grainy eyes; recessive, autosomal. Macrochaetae are multiplied and grow in bunches from two to six macrochaetae in each. The number of microchaetae is also increased. Viability and fertility are fine. Mass culture is allowed.
9. *#65* (not received a regular name yet, the gene is not mapped). Reddish-orange eyes, getting darker with age, becoming orange-brownish in a combination with *vermilion* also floating in the strain.
10. *vestigial*^{X} *y*$^{+}$ is like the strain # 7 with no yellow.
11. *#1206* (no name assigned) is an autosomal recessive mutation. Flies have large, rough grainy eyes, wide wings, frequently with small nodes on the veins. The viability and fertility are moderate.
12. *radius_incompletus_scutellum* (*risc*) *vermilion* (*v*). Autosomal recessive mutation. Flies have a shortened scutellum of a semilunar shape. Scutellar macrochaetae grow near a scuto-scutellar suture in groups of three macrochaetae in each from both sides of the scutellum. Left and right bunches of scutellar macrochaetae are distant from each other. On the wing, the radius is absent in the upper 1/3 – ¼ of the wing. Eyes are bright red due to a *vermilion* mutation. The mutation *risc* frequently reverts to *risc*$^{+}$, so the strain requires permanent attention. Mass culture is recommended. Viability and fertility are fine.
13. *lozenge* (*lz*). Homology to a correspondent mutation in *D. melanogaster* was not shown. Eyes are almond-shaped, narrower than in *D. melanogaster*, rough and strongly granulated. The eye color is yellow-orange in the center, nearly white near edges. The mutants poorly survive cold. Otherwise well viable and fertile strain, mass culture is recommended.
14. *grooved-1* (*gv-1*) is a recessive allele of the dominant gene *Gv* found in *D. simulans* in our lab. The notum is shortened and to some extent bifid, the dorsocentral macrochaetae are approximate to each other and are not oriented parallel to the Anterior – Posterior line, like in the wild type. The posterior dorsocentral macrochaetae are more distant from this line than the anterior ones. Eyes are reduced, sometimes missing, irregular-shaped. Irregularities in the left and right eye are independent, they are not symmetrical. There are also some flies with one or both normal compound eyes. Both viability and fertility are very good. Mass culture is recommended. Sometimes the *gv-1* mutants revert to a wild type, so they must be checked from time to time.

X-chromosomal mutants with *y w* females with attached XX and *sn, f* or *y*^{bold} males

15. *singed* (*sn*) – the bristles are corkscrew-shaped, like in *D. melanogaster*. The females are *y w* in this strain. Screening for detached XX resulting in appearance of *y w* males is necessary.
16. *forked* – the mutant is not identified well; it may represent a weak variant of *singed*. Macrochaetae are hunched angularly, sometimes split at the tip. The phenotype generally fits one of the *forked* mutation in *D. melanogaster*.
17. *yellow*^{bold}, females *y w* with attached XX, males are *yellow*^{bold}, eye color is normal. *See the description of the strain # 4 for the characteristic of the y*^{bold} *mutation.*

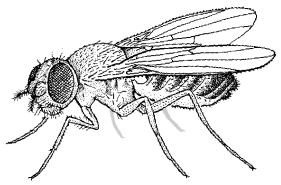
Autosomal dominant mutations

18. *Ubx.* The mutation is not finally characterized. Flies have large halteres turned down, with a row of bristle at the margin (like a costal row in *D. melanogaster*). Eyes are orange due to the presence of *v* together with an unknown mutation affecting eye color. Cross only mutant individuals, reject the normal ones. The strain is viable and fertile well.
19. *Cbx-1*. Wings are short; the proximal part of the wing is rudimentary, uplifted, and specifically orientated. The scutellum is reduced, it has a trapezoidal shape. Wings are often double-layered, bubble-shaped. Autosomal dominant mutation, mutant (homozygous and heterozygous) females are nearly sterile, mutant males are fertile. The viability of the strain is good. Cross normal virgins form the culture to the mutant males. Some mutant females are also allowed.
20. *#1379* (no name is assigned). In heterozygotes *1379/+* wings are divergent. Homozygotes mostly die; rare escapers that survived have a ventral wing hinge practically fully disrupted, a duplication of a part of the proximal structures of the dorsal wing hinge and other abnormalities in the structure of the wing and the axillary apparatus. Homozygotes are practically not viable and sterile. Sometimes they have duplications of the structures of the notum in the place of the pleura. Heterozygotes have a practically disrupted tegula. For culturing, multiple heterozygotes identified by divergent wings (with the angle from 45° to 90°), should be crossed together, the normal flies should be rejected. Heterozygotes are vigorous and fertile well.
21. *vestigial*^{Dominant} (*vg*^{D}). In heterozygotes *vg*^{D} / + wings are notched. Rare, practically nonviable homozygous survivors have no wings and halteres. Heterozygotes have good viability and fertility. Allelism with *vg* is not shown. Cross multiple *vg*^{D}/+ heterozygotes with notched wings and reject normal flies.
22. *#2110* (no name assigned). Wings are different in shape and length, narrowish, with nodes on the veins, some individuals have shortened wings, left and right wings may have a different shape. An autosomal dominant mutation, well selectable from a wild type. Cross only mutants, irrespective of sex, rejecting the normal flies. Viability and fertility are good.
23. *#1724* (no name assigned). An autosomal dominant mutation. Hetero- and homozygotes have narrow rough eyes. Cross the mutants, rejecting normal flies.

Recessive mutations, selection required

24. *rotund-1* (*rn-1*). Wings are smaller than normal due to the underdevelopment of the proximal part, round-shaped (this gave a name to the strain). Tarsi consist of 5 segments, sometimes their quantity is reduced, and they have abnormal shape. Homozygous females are sterile. Cross phenoltypically normal virgins (heterozygotes *rn*/*rn*$^{+}$) and homozygous mutant males *rn*/*rn*. Some homozygous females may be present in the culture. The viability of the strain is good.

This list represents only a small fraction of the unpublished *D. simulans* mutants we have, and we plan to expand it. The collection of *D. simulans* strains is partially supported by the RFBR grant #08-04-01596-a.



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Teaching Notes

Negative synergistic epistasis in *Drosophila melanogaster*.

Rex, Caitlin M., Sarah J. Rossiter, Amanda C. Lyons, and R.C. Woodruff. Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403.

Introduction

Spontaneous deleterious mutations are a constant part of the genomes of all organisms. These genetic changes and their synergistic interactions may in part be responsible for the maintenance and evolution of sexual reproduction and genetic recombination, inbreeding avoidance, senescence, the evolution of mate choice by the good gene mechanism, evolution of degenerate Y chromosomes, DNA repair, genetic control of DNA-element transpositions, extinctions of endangered species by mutational meltdown, and positive correlations between recombination rate and nucleotide diversity (Baer *et al*., 2007; Charlesworth and Charlesworth, 1998; Desai *et al*., 2007; Drake *et al*., 1998; Sanjuan and Elena, 2006). Yet, the role of deleterious mutations in these evolutionary processes depends on the degree of negative synergistic epistasis among the mutations, the average fitness effects of each mutation, including reduction in fitness in homozygotes and dominance in heterozygotes, and the deleterious genomic mutation rate (Garcia-Dorado *et al*., 2004; Lynch *et al*., 1999). The estimations of selection coefficients and dominance of deleterious mutations in higher organisms are diverse, and the presence of negative synergistic epistasis for these mutations is controversial. In addition, although there are estimations of the deleterious genomic mutation rate in a number of higher organisms, including nematodes, *Drosophila*, and humans, these estimations are

broad, ranging from about 0.01 to 10 per generation (Eyre-Walker and Keightley, 1999, 2006; Gong *et al*., 2005). Hence, it is important to determine whether negative synergistic interactions occur among deleterious mutations and to determine the distribution of their fitness effects (see Urwin and Nunn, 2005; Wolf *et al*., 2000).

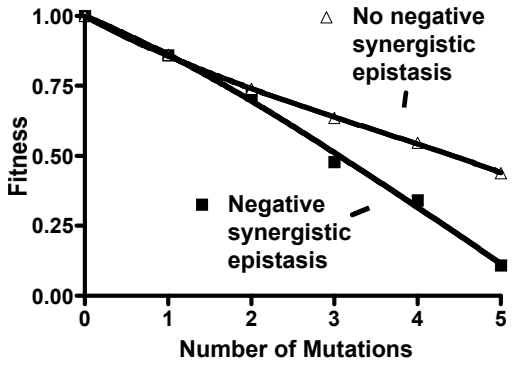
Example of Epistasis

As a model of the possible influence of negative synergistic epistasis on health and fitness in a diploid organism, let us consider two genes with wild-type alleles *A* and *B*, deleterious alleles *a* and *b*, four possible gametic haplotypes (*AB*, *Ab*, *aB* and *ab*), nine possible genotypes, and fitness values that are dependent upon whether there is or is not negative synergistic epistasis. In this model (shown below), *s* = selection coefficient against the homozygous mutant alleles, with *s* for deleterious mutations ranging from almost zero to near one; *h* = dominance coefficient for heterozygotes, with *hs* describing the extent to which heterozygotes express the harmful effects of the mutant allele and ranging from zero for completely recessive homozygous mutants to one; and synergistic epistasis = ε, with zero or negative values of ε for four genotypes (*R* = recessive genotype and *H* = heterozygous genotype).

	AB	*Ab*	*aB*	*ab*
AB	1	$1-h_b s_b$	$1-h_a s_a$	$(1-h_a s_a)(1-h_b s_b)+\varepsilon_{HH}$
Ab		$1-s_b$	$(1-h_a s_a)(1-h_b s_b)+\varepsilon_{HH}$	$(1-h_a s_a)(1-s_b)+\varepsilon_{HR}$
aB			$1-s_a$	$(1-s_a)(1-h_b s_b)+\varepsilon_{RH}$
ab				$(1-s_a)(1-s_b)+\varepsilon_{RR}$

For example, for *aabb* let the fitness of *aa* = 1- s_a = 0.9 and the fitness of *bb* = 1- s_b = 0.9. In the absence of negative synergistic epistasis (ε_{RR} = 0) the fitness of *aabb* will be multiplicative and equal to 0.81(0.9 × 0.9), which is below that of *AABB* individuals (fitness set at one). For negative synergistic epistasis let ε_{RR} = -0.1, and the fitness of *aabb* will be 0.71 [(0.9 × 0.9) -0.1]. Since a fitness value of 0.71 is less than 0.81, this is an example of negative synergistic epistasis. How does one identify such negative synergistic interactions, especially when interactions may not be strong?

A hypothetical example of the expected changes in mean fitness over time for the accumulation of new deleterious mutations in the presence and absence of negative synergistic epistasis is as follows:



With synergistic epistasis the decrease in fitness (viability) with an increase in the number of mutants is lower than expected (and non-linear) if the mutants do not interact.

Crosses Used to Measure Negative Synergistic Epistasis and Results

The following set of crosses were used to measure negative synergistic epistasis in *Drosophila melanogaster*, using easily identified visible mutants that are also homozygous recessive lethals: Curly (curly wings) that is associated with the CyO balancer second chromosome; Glazed (glazed eyes) that is associated with an inversion of the second chromosome; and Stubble (short bristles) that is associated with the TM3, third chromosome, balancer chromosome (Lindsley and Zimm, 1992). Below the crosses are the number of progeny recovered from the F1 cross and their fitness (viability) in relation to a fitness of one for the wild-type progeny.

In(2LR)Px/CyO;+/+ × +/+;Df(3R)CA/TM3, Sb & l(2)24Dc/In(2LR)Gla × CS (+/+;+/+)

↓ ↓

CyO/+; TM3, Sb/+ virgin females × Gla/+;+/+ males

↓

Possible Progeny	Expected Proportion	Phenotypes	Number Mutants	Number Recovered	Relative Fitness
CyO/Gla;TM3, Sb/+	1	Curly, Glazed, Stubble	3	60	0.02
CyO/+; TM3, Sb/+	1	Curly, Stubble	2	1096	0.38
Gla/+; TM3, Sb/+	1	Glazed, Stubble	2	1216	0.42
CyO/Gla; +/+	1	Curly, Glazed	2	2058	0.72
CyO/+; +/+	1	Curly	1	2503	0.87
Gla/+; +/+	1	Glazed	1	2459	0.86
+/+; Sb/+	1	Stubble	1	2049	0.72
+/+; +/+	1	wild type	0	2864	1

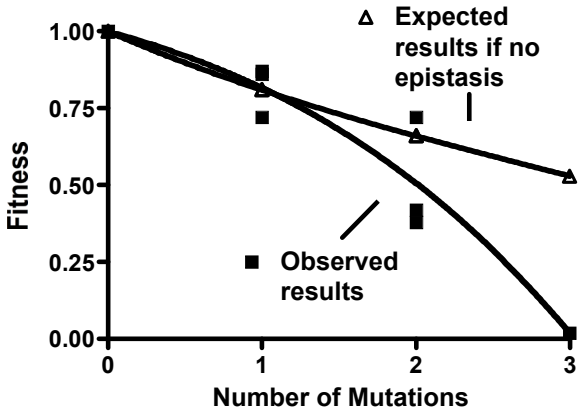
Observed Negative Synergistic Epistasis

Using the relative fitness for each progeny type, the epistasis values for different combination of mutants, using the method of Jasnos *et al*. (2008), are as follows.

Epistasis = [(fitness +)(fit Cy Gla)] - [(fit Cy)(fit Gla)]
= [(1)(0.72)] - [(0.87)(0.86)] = 0.72 - 0.75 = -0.03

Epistasis = [(fitness +)(fit Cy Sb)] - [(fit Cy)(fit Sb)]
= [(1)(0.38)] - [(0.87)(0.72)] = 0.38 - 0.63 = -0.25
Epistasis = [(fitness +)(fit Gla Sb)] - [(fit Gla)(fit Sb)]
= [(1)(0.42)] - [(0.86)(0.72)] = 0.42 - 0.62 = -0.20
Epistasis = [(fitness +)(fit Cy Gla Sb)] - [(fit Cy)(fit Gla)(fit Sb)]
= [(1)(0.02)] - [(0.87)(0.86)(0.72)] = 0.02 - 0.54 = -0.52

The relationship between fitness and the number of deleterious mutations is shown in the figure below; the expected assumes an average decrease in fitness for a single mutation of 0.82, which is the observed average fitness of the Curly, Glazed and Stubble flies. There is a clear non-linear decrease in number of progeny with the number of mutants. A paired t test, however, did not show a significant difference for the two lines (P = 0.15).



In summary, we observed negative synergistic epistasis among three dominant visible mutations in *Drosophila melanogaster*. A non-linear relationship was observed between the number of mutations and the reduction in viability.

References: Baer, C., *et al*., 2007, Nature Reviews Genetics 8: 619-631; Desai, M.M., *et al*., 2007, Genetics 177: 1001-1010; Charlesworth, B., and D. Charlesworth 1998, Genetica 102/103: 3-19; Drake, J.W., *et al*., 1998, Genetics 148: 1667-1686; Eyre-Walker, A., and P.D. Keightley 1999, Nature 397: 344-347; Eyre-Walker, A., and P.D. Keightley 2006, Nature Reviews Genetics 8: 610-618; Garcia-Dorado, A., *et al*., 2004, *Evolution: from Molecules to Ecosystems*. (Moya, A., and E. Font, eds.). pp. 20-32. Oxford University Press, Oxford; Gong, Y., *et al*., 2005, Biol. Lett. 1: 492-495; Jasnos, L., *et al*., 2008, Genetics 178: 2105-2111; Lindsley, D., and G. Zimm 1992, *The Genome of* Drosophila melanogaster, Academic Press, Inc., NY; Lynch, M., *et al*., 1999, Evolution 53: 645-663; Urwin, R.E., and K.P. Nunn 2005, European J. Hum. Genet. 13: 370-375; Sanjuan, R., and S.F. Elena 2006, Proc. Natl. Acad. Sci. USA 103: 14402-14405; Wolf, J.B., *et al*., 2000, *Epistasis and the Evolutionary Process.* Oxford University Press.

Rare male mating advantage in *Drosophila melanogaster*.

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Introduction

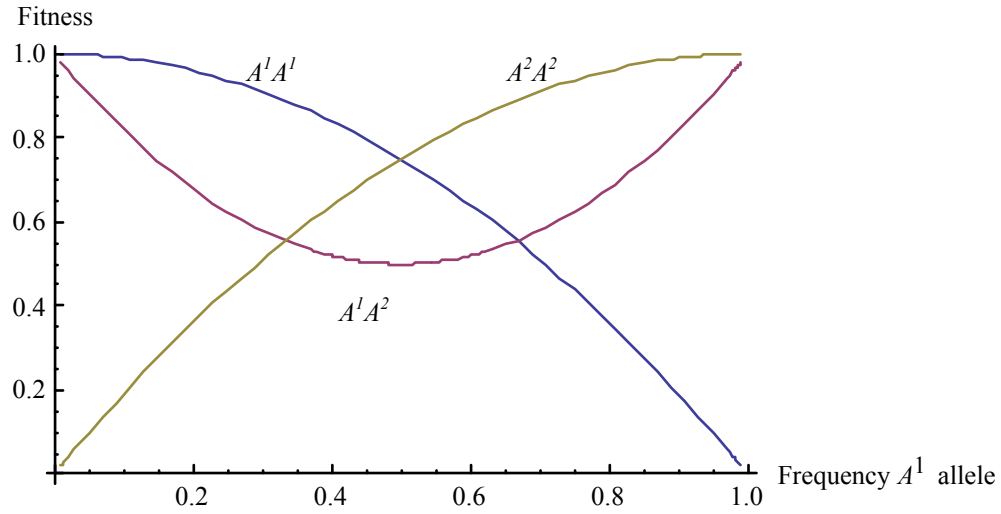
An understanding of how genetic variation is maintained in populations, including humans, is important because organisms cannot evolve to meet changes in the environment without genetic variation or new beneficial mutations. For example, organisms with low amounts of genetic variation will be less likely to evolve to maintain defenses against new parasites and microbial infections. This is especially important in endangered animals and plants, where the population size is low (Frankham *et al.*, 2002), and in humans where infections with new antibiotic resistant bacterial strains have become more common (Maree *et al.*, 2007).

One mechanism to maintain genetic variation in populations is frequency dependent selection (Hedrick, 2005; Hamilton, 2009). As the frequencies of alleles at a gene change in nature, there is selection for low frequency alleles. For example, if a gene has alleles A^1 and A^2, the A^1 allele or the A^2 allele (or both alleles) would be selected for when it is in a low frequency. This keeps both alleles in the population and helps to maintain standing genetic variation. For example, rare flower color alleles in orchids are selected by insect pollinators (Gigord *et al.,* 2001).

Frequency dependent selection can be modeled for a gene with two alleles as follows (see Hamilton, 2009). Let p be the frequency of the A^1 allele and q be the frequency of the A^2 allele. At Hardy/Weinberg equilibrium the frequency of $A^1A^1 = p^2$, the frequency of $A^1A^2 = 2pq$, and the frequency of $A^2A^2 = q^2$. Also let selection against $A^1A^1 = s_{11}$, selection against $A^1A^2 = s_{12}$, and the selection against $A^2A^2 = s_{22}$.

Fitness of A^1A^1	Fitness of A^1A^2	Fitness of A^2A^2
$1 - s_{11}p^2$	$1 - s_{12}2pq$	$1 - s_{22}q^2$

For example, as the frequency of A^1A^1 goes down, their fitness goes up, as shown below.



A mechanism for frequency dependent selection is the rare male mating advantage (Powell, 1999; Hedrick, 2005). If rare males also carry rare alleles, their increased mating ability, above the expected based on their frequency, will help to maintain a balance where more than one allele will be present in populations.

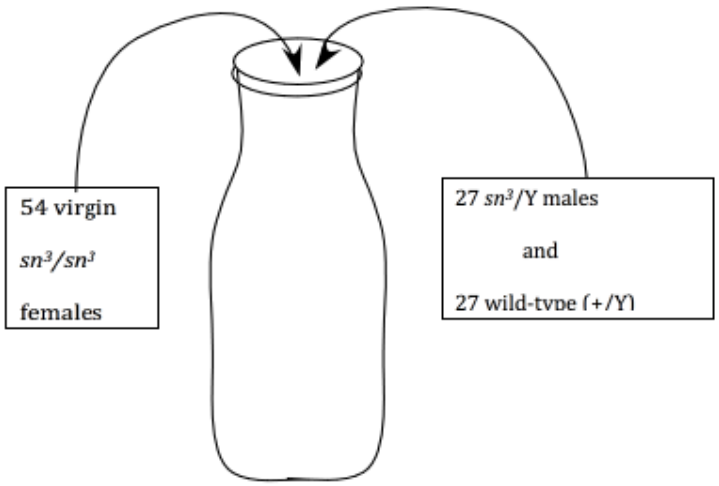
Rare male mating advantage has been reported in over 10 species of *Drosophila* and in a few vertebrates (reviewed in Spiess and Kruckeberg, 1980; Powell, 1999; Som and Singh, 2005). Yet, others have not observed such an effect (Markow *et al*., 1980), and some have concluded that rare male mating advantage has not been shown to be an important mechanism for the maintenance of genetic variation (Bryant *et al*., 1980).

It is the objective of this teaching exercise to measure rare male mating advantage in the model organism *Drosophila melanogaster* using easily recognizable visible genetic markers.

Control Crosses

As a control, we will first determine if two phenotypically distinct types of males (sn^3 males with short bristles and wild-type, Canton-S, males with long bristles) have similar mating abilities when they are at the same frequency. If their mating abilities are not equal, this will be taken into consideration when analyzing subsequent matings where each male is placed in a rare frequency.

In the following control crosses in half-pint bottles, note that the Y chromosome does not contain a gene pairing partner for the X-linked *singed* gene and that the sn^3 allele is a recessive mutation. In addition, sn^3/sn^3 females and sn^3/Y males have very short bristles, whereas sn^3/+ females and +/Y (wild-type) males have long bristles.



After two or three days, each female was placed individually, without males, into separate vials. The parental females were then cleared from each vial after seven days, and the F1 progeny of each female were scored as follows to determine if they:

1. Mated with sn^3/Y males:
   Would have sn^3/sn^3 (short bristles) female offspring and sn^3/Y (short bristle) male offspring.

2. Mated with wild-type (+/Y) males:
   Would have wild-type (sn^3/+; long bristles) female offspring and sn^3/Y (short bristles) male offspring.
3. Mated with both sn^3/Y and wild-type (+/Y) males:
   Would have a mixture of sn^3 and wild type females and sn^3/Y male progeny.

The ratio of sn^3 matings to wild-type matings in these control crosses gives an estimation of the relative mating ability of the sn^3 and wild-type parental males. If they have equal mating abilities, then ½ of the 54 females in a bottle will mate with sn^3 males and ½ with wild-type males. Double matings will be recorded, but not included in the final data analysis.

Experimental Crosses

A. 45 sn^3 / 9 + Crosses:
In each bottle we mated:
54 sn^3/sn^3 virgin females × 45 sn^3/Y males and 9 +/Y (wild-type) males

As in the control crosses, after 2-3 days single females were placed, without males, into separate vials.

If the previously observed relative mating ability of sn^3 males and wild-type males in the control crosses is the same, it is expected that 5 in 6 crosses will be with sn^3 males and 1 in 6 will be with wild-type males. A rare-male mating effect will give a significantly higher mating frequency for wild-type males than 17% (1 in 6) of the total matings.

B. 9 sn^3 / 45 + Crosses:
In each bottle we mated:
54 sn^3/sn^3 virgin females × 9 sn^3/Y males and 45 +/Y (wild-type) males

As in the control crosses, after 2-3 days single females were placed, without males, into separate vials.

If the previously observed relative mating ability of sn^3 males and wild-type males in the control crosses is the same, it is expected that 1 in 6 crosses will be with sn^3 males and 5 in 6 will be with wild-type males. A rare-male mating effect will give a significantly higher mating frequency for sn^3 males than 17% of the total matings.

Data Analysis

The frequencies of matings were analyzed using the chi-square test by comparing the expected frequencies of mating based on the control crosses with the observed frequencies in the two rare male experimental crosses using the Prism program.

Table 1. Test of rare male mating advantage.

	Control Crosses # Mating Males		45 sn^3 / 9 + Crosses		9 sn^3 / 45 + Crosses # Mating Males	
	sn^3	+	sn^3	+	sn^3	+
Observed	107	103	120	57	27	136
Expected	105	105	147	30	28	135
	p = 0.84		p = 0.001		p = 0.88	

Results

A total of 16 bottles were set up in the control crosses; 13.1 females per bottle survived and produced progeny. A total of 10 bottles were set up for the 45 sn^3 / 9 + experiment; 17.7 females per bottle survived and produced progeny. A total of 10 bottles were set up in the 9 sn^3 / 45 + experiment; 16.3 females per bottle survived and produced progeny.

The results of the control crosses (54 sn^3/sn^3 females × 27 sn^3/Y males and 27 +/Y males), the 45/9 crosses (54 sn^3/sn^3 females × 45 sn^3/Y males and 9 +/Y males), and the 9/45 crosses (54 sn^3/sn^3 females × 9 sn^3/Y males and 45 +/Y males) are shown in Table 1 and in Figures 1, 2 and 3. Since the observed frequencies of sn^3/Y and +/Y male matings were not significantly different from the expected frequency (1:1) in the control crosses (P = 0.84), the mating ability of each male type was the same when the two genotypes are of equal proportions. Hence, if there is no male mating advantage for either genotype (sn^3 or + males) when their frequencies are equal, the expected frequency of matings in the 45 sn^3 / 9 + crosses is expected to be 5:1 (five times as many expected matings of sn^3/Y males as compared to matings of +/Y males). In addition, in the 9 sn^3 / 45 + crosses the expected frequency of matings is expected to be 1:5 (five times as many expected matings of +/Y males as compared to matings of sn^3/Y males).

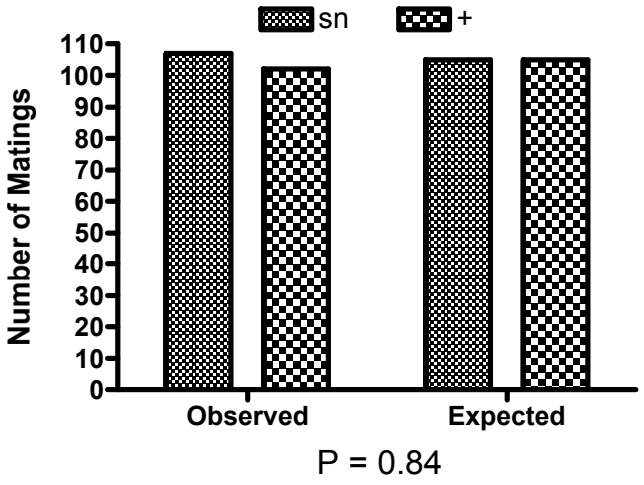


Figure 1. Control Crosses: Frequencies of male matings in crosses of 54 sn^3/sn^3 females with 27 sn^3/Y males and 27 wild-type (Canton-S) males per bottle.

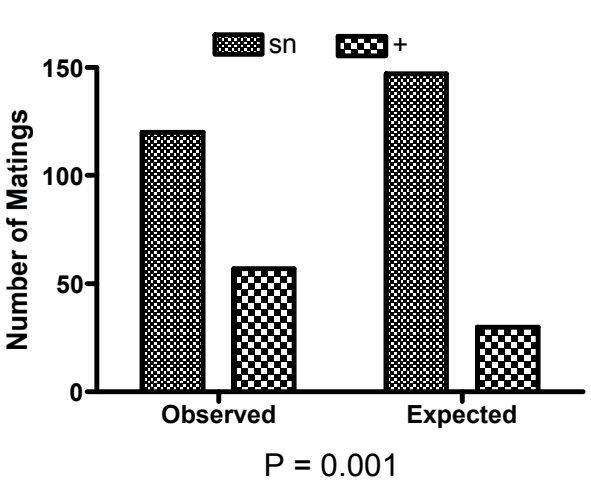


Figure 2. 45 sn^3 / 9 + Crosses: Frequencies of male matings in crosses of 54 sn^3/sn^3 females with 45 sn^3/Y males and 9 wild-type (Canton-S) males per bottle.

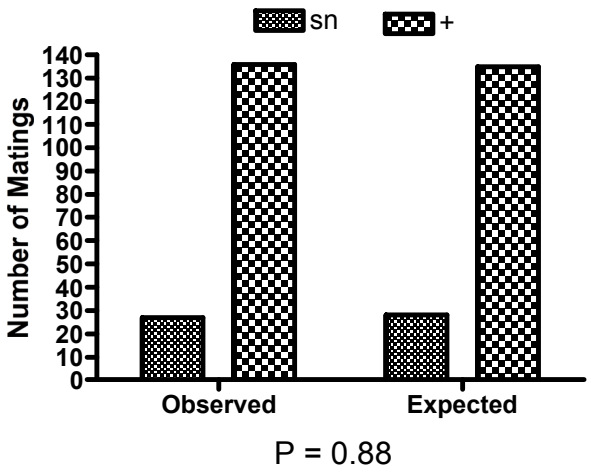


Figure 3. 9 sn^3 / 45 + Crosses: Frequencies of male matings in crosses of 54 sn^3/sn^3 females with 9 sn^3/Y males and 45 wild-type (Canton-S) males per bottle.

As shown in Table 1, the frequency of wild-type (Canton-S) male matings was significantly higher than expected (P = 0.001) in the 45 sn^3 / 9 + crosses. Hence, there is a rare male mating advantage for the wild-type males. On the other hand, in the 9 sn^3 / 45 + crosses there was not a significant mating advantage for the sn^3/Y males (P = 0.88). Hence, there was no rare male mating advantage for the sn^3/Y males; also see Figures 1, 2 and 3.

In summary, in crosses with sn^3/Y and +/Y (Canton-S) males there was a significant rare male mating advantage for the wild-type (Canton-S) males, but not for the sn^3/Y males. This rare male mating advantage for Canton-S males does not seem to be due just to higher mating activity of these males, because Canton-S males did not mate more than sn^3/Y males in the control crosses, where there were equal frequencies of the two males (P = 0.84). Others have also observed one-sided rare male mating advantage in *D. ananassae* for visible markers and for chromosomal inversions (Som and Singh, 2004, 2005).

As a follow-up of this study, we plan to determine if the Canton-S males also show a rare male mating advantage with sn^3 males in experiments where mating pairs of flies are identified during copulation instead of scoring for progeny phenotypes (Ehrman, 1970). It would also be interesting to compare the rare male mating behavior of Canton-S males with males from other stocks that contain sex-linked visible makers, such as *forked* (small bristles), *yellow* (yellow body color), and *white* (white eyes).

References: Bryant, E.H., A. Kence, and K.T. Kimball 1980, Genetics 96: 975-993; Ehrman, L., 1970, Proc. Natl. Acad. Sci. USA 65: 345-348; Frankham, R., J.D. Ballou, and D.A. Briscoe 2002, *Introduction to Conservation Genetics*: Cambridge University Press, Cambridge; Gigord, L.D.B., M.R. Macnair, and A. Smithson 2001, Proc. Natl. Acad. Sci. USA 98: 6253-6255; Hamilton, M.B., 2009, *Population Genetics*. Wiley-Blackwell, New York; Hedrick, P.W., 2005, *Genetics of Populations*. Jones and Bartlett Publishers, Sudbury, MA; Maree, C.L., R.S. Daum, S. Boyle-Vavra, K. Matayoshi, and L.G. Miller 2005, Emerging Infectious Diseases 13: 236-242; Markow, T., A.C. Richmond, L. Mueller, J. Sheer, S. Roman, C. Laetz, and L. Lorenz 1980, Genet. Res. 35: 59-64; Powell, J.R., 1999, *Progress and Prospects in Evolutionary Biology: The*

Drosophila *Model*. Oxford University Press, Oxford; Som, A., and B.N. Singh 2004, Behavior Genetics 34: 335-342; Som, A., and B.N. Singh 2005, Genet. Mol. Res. 4: 1-17; Spiess, E.B., and J.F. Kruckeberg 1980, Amer. Nat. 115: 307-327.

The identification of hidden genetic variation (recessive visible mutations) in a natural population of *Drosophila melanogaster*.

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"It is clear that descriptions of the genetic variation in populations are the fundamental observations on which evolutionary genetics depends." (Lewontin, 1974).

Dobzhansky in 1955 compared two hypotheses for the genetic structure of natural populations: the classical school, which predicted that most genes are homozygous for wild-type alleles, because most mutations are deleterious and are selected against, and the balanced school, which predicted that there are numerous heterozygotes for many genes, mainly due to overdominance (heterozygotes are more fit than homozygotes) (see discussion in Ford, 1964; Lewontin, 1974). It is now known from protein electrophoresis and DNA analyses that there is an immense amount of genetic variation in most species and populations, but most of this variation is probably not maintained by overdominance (see Hedrick, 2005, for a discussion of this topic).

Before the advent of protein electrophoresis and DNA analyses, which allowed for the differentiation of heterozygotes and homozygotes, how did Dobzhansky and others know that there was hidden genetic variation in natural populations, hidden because this variation was due to recessive mutations that were not expressed in heterozygotes? Dobzhansky (1955) pointed out that when one looks at *Drosophila* from nature, few, if any, visible mutations are observed: "…natural populations of *Drosophila* show scant variability in externally visible traits" (Dobzhansky, 1955). He then explains how this hidden genetic variation was first observed.

> "The pioneer work of Chetverikov, Timofeeff-Ressovsky, and Dubinin and his collaborators during the late twenties and the early thirties demonstrated that the paucity of overt phenotypic variability does not mean genotypic uniformity. When the *Drosophila* flies collected in nature are inbred in laboratory cultures, a fair proportion of them prove to be heterozygous for recessive mutants affecting the visible external morphology of the fly. Many of the classical mutants which grace the pages of genetics manuals were thus shown to exist concealed in natural populations." (Dobzhansky, 1955; see a detailed discussion of these studies in Dobzhansky, 1937; Spencer, 1947; Lewontin, 1974).

Others, including scientists from the USA, also identified recessive visible mutations in wild *Drosophila* (see Mickey, 1954; Spencer, 1947, 1957).

The objective of this study is to attempt to identify hidden, recessive visible mutations in a natural population of *D. melanogaster*. Are these mutations in nature now as they were in the earlier experiments of the Russian and American scientists? The answer is yes.

We collected *D. melanogaster* by sweeping bananas in Perrysburg (Wood County), Ohio on October 10, 2008. Eighty-two presumably mated females were placed the next day singly in vials of

Drosophila food, and 65 (79%) of the females gave progeny. Three of these females were *D. simulans*, a sibling species of *D. melanogaster*, that can be identified by differences in the structure of the male genitalia (Ashburner, 1989).

No visible phenotypic changes were observed in 1,436 F1 male progeny from these wild *D. melanogaster* females. This was expected, since any recessive X-linked visible mutations that are deleterious would be quickly selected against in nature in hemizygous males. Hence, none of the 62 fertile, wild females were heterozygous for recessive sex-linked visible mutations.

In an attempt to make homozygous any autosomal recessive visible mutations that were heterozygous in the original wild females, or their mates, four vials of single F1 females were mated with single F1 sibling males from 40 of the 62 wild lines, for a total of 160 F1 sibling crosses. One hundred and fifty nine of the 160 crosses produced F2 progeny. In some of these F2 progeny, recessive visible mutations, if present, should become homozygous and be expressed because of inbreeding. We screened at least 20 F2 progeny from each F1 cross for altered adult morphologies. Seventeen of the 159 F1 crosses had one or more F2 flies with altered phenotypes, suggesting uncovered recessive visible mutations. We next determined if these 17 presumptive visible mutations bred true by mating F2 sibling males and females and scoring F3 progeny for visible mutant phenotypes.

Of the 17 possible visible mutations, 10 bred true in subsequent F3 progeny. These 10 mutations were present in nine of the parental, wild females from nature; *i.e.*, 11% (9/80) of the fertile parental females from nature, or their mates, carried one or two hidden recessive visible mutations as autosomal heterozygotes. This is probably an underestimation of the true frequency of recessive visible mutations in the Perrysburg, Ohio, population, because not all mutations in the wild flies will be made homozygous by the mating scheme of this study. Using four F1 sibling matings for each parental line in this study allows for only about 68% of visible mutations in nature as heterozygotes to become homozygous and expressed in F2 flies (Spencer, 1947).

Descriptions of the visible mutants recovered in this study are given in Table 1, and photographs of three of these mutants (dark eyes, outstretched wings, and halteres that developed wing tissues) are shown in Figures 1, 2 and 3.

Table 1. Descriptions of recovered visible mutations from a natural population of *D. melanogaster*.

F1 Cross	Visible Mutant Recovered in F2 and Confirmed in F3 flies
A4*	Dark trident-like structure on thorax
C2	Dark trident-like structure on thorax
C3	Dark trident-like structure on thorax
I1	Black thorax
Q2	Wings out (see Figure 1)
5D	Wings flattened at ends
8D	Wings out
13D	Black eyes (see Figure 2)
16B	Bright red eyes
21B	Halters as small wings (see Figure 3)

*A4 = the F1 vial four from parental, wild-type female A.

The frequency of visible mutations per tested wild female and their mates is too high (10 mutations in 40 lines) to be due to new mutations. Visible mutations occur at a rate of about 1 to 3 per 100,000 gametes in *D. melanogaster* (Woodruff, Slatko, and Thompson, 1983). The identified visible mutants are more likely due to preexisting recessive mutations carried as heterozygotes. Evidence in support of this hypothesis comes from a separate study that is currently in progress in our laboratory to measure the influence of new deleterious mutations on viability. In this study, two females and two of their brothers were mated each generation for 16 generations from an original yellow-body stock that had been maintained by single brother-sister crosses for 159 generations. Hence, this inbred stock was homozygous for all, or almost all, genes. Any recessive visible mutants would, therefore, have to arise by new genetic changes, because the original inbred stock was homozygous and did not carry any visible mutations as heterozygotes. We observed no new visible mutations among 14,257

progeny from these inbred yellow flies. This highlights the rare rate of new visible mutations in *D. melanogaster* and gives support to the hypothesis that the 10 visible mutants observed in this study were due to preexisting genetic variation in the 40 Perrysburg, Ohio, natural population lines. Up to 10%, or more, of previous collections of *D. melanogaster* in Russia and the USA carried recessive visible mutations as heterozygotes (Dobzhansky, 1937; Spencer, 1947; Lewontin, 1974). After reviewing a number of published studies, Lewontin (1974) concluded that every female *D. melanogaster* in nature has on average one recessive visible mutation carried as a heterozygote.

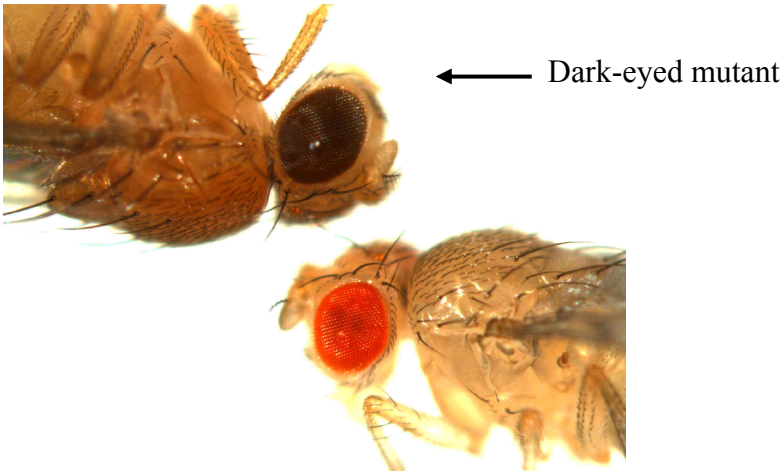

Figure 1. Recessive autosomal dark-eyed mutant from nature compared to a fly with wild-type eyes.

Figure 2. A recessive autosomal outstretched-wing mutant from nature.

Figure 3. Two flies with a recessive autosomal visible mutation causing halteres to develop into wing-like tissue (a homeotic mutant).

The seven possible visible mutants that did not breed true (folded wings, dark thorax, wings curved up, three with wings held out, and altered eye color) were probably caused by interactions of multiple genes that were uncoupled in F2 flies, environmental influences (not likely, since the environment was similar in all crosses), or developmental differences that were not inherited.

A class discussion of the results of this study could include the following topics:

1) Do humans also carry recessive visible mutations that are hidden as heterozygotes? The answer is yes, because recessive visible mutations are expressed in homozygotes in a higher frequency in human pedigrees that include matings between closely related individuals (Hedrick, 2005).

2) Do humans also carry other recessive deleterious mutations as heterozygotes? It has been estimated that each human carries a number of such deleterious mutations. For example, the proportion of infant deaths in the progeny of first-cousin matings is higher than in progeny of unrelated humans, due to inbreeding and homozygosis of recessive mutations (Hedrick, 2005).

3) How are recessive deleterious mutations, such as those identified in this study, maintained in natural populations? If they are deleterious, one might expect them to be eliminated over time. One possible answer is that the deleterious effects of these mutations are hidden when they are in heterozygotes, *i.e.*, the mutations are completely recessive. If *A* is the wild-type allele and *a* is the mutant allele, a population genetics model of a recessive deleterious mutation with no dominance is (*s* is the selection coefficient against the *aa* homozygotes, and the *AA* and *Aa* individuals have on average the largest number of offspring):

	AA	*Aa*	*aa*
Fitness =	1	1	1-*s*

Even if the deleterious mutations do reduce the fitness of heterozygotes a small amount, *i.e.*, the deleterious mutations have some dominance, there will be a balance between mutations that produce new deleterious alleles and selection that removes deleterious alleles. This can be shown in the following model (*h* is the dominance coefficient; if *h* is greater than zero but less than one, the heterozygotes will be less fit than the *AA* homozygotes, but more fit than the *aa* homozygotes):

	AA	*Aa*	*aa*
Fitness =	1	1-*hs*	1-*s*

At equilibrium the recessive mutation allele (*a*) will have a frequency ($\hat{q}$) equal to (Hedrick, 2005):

$$\hat{q} = \frac{u}{hs}$$

It has been estimated that in *D. melanogaster*, *u* per gene is about 1×10^{-5}, and for slightly deleterious mutations *h* is about 0.33 and *s* is about 0.03 (Simmons and Crow, 1977), giving:

$$\hat{q} = \frac{0.00001}{(0.33)(0.03)}$$
$$\hat{q} = 0.001$$

Hence, if the sampled Perrysburg, Ohio population were at Hardy/Weinberg equilibrium, the expected frequencies of flies with the *A* and *a* alleles would be:

$$AA = p^2 = (0.999)^2 = 0.99800$$
$$Aa = 2pq = 2(0.999)(0.001) = 0.00199$$
$$aa = q^2 = (0.001)^2 = 0.000001$$

Only about one fly in 1,000,000 would, therefore, be expected to express the recessive visible mutation (*aa*). On the other hand, about one fly in 500 would be expected to be heterozygous (*Aa*). In this study we observed that about one to three flies in 80 carried a specific recessive visible mutation as a heterozygote. The reason(s) for this difference in frequencies of heterozygotes is unknown, although changes in *u* or *hs* could bring the two values closer.

References: Ashburner, M., 1989, Drosophila: *A Laboratory Handbook*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; Ford, E.B., 1964, *Ecological Genetics*. Methuen and Co. Ltd., London; Dobzhansky, T., 1937, *Genetics and the Origin of Species*. Columbia University Press, New York; Hedrick, P.W., 2005, *Genetics of Populations*. Jones and Bartlett Publishers, Sudbury, MA; Lewontin, R.C., 1974, *The Genetic Basis of Evolutionary Change*. Columbia University Press, New York; Mickey, G.H., 1954, Am. Nat. 88: 241-255; Simmons, M.J., and J.F. Crow 1977, Ann. Rev. Genet. 11: 49-78; Spencer, W.P., 1947, Advances in Genetics 1: 359-402; Spencer, W.P., 1957, Texas Univ. Publ. 5721: 186-205; Woodruff, R.C., B.E. Slatko, and J.N. Thompson, jr. 1983, *The Genetics and Biology of* Drosophila. Vol. 3C, pp. 37-124. (Ashburner, M., H.L. Carson, and J.N. Thompson, jr., eds.). Academic Press, New York.

The *BLOG*: A new electronic resource for teaching in the XXIST century.

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Abstract

This paper describes the potential of a *blog* as a tool for curriculum innovation in the context of university attendance. It describes an experimental implementation of this service on the subject of Genetics in the career of Biology at the Faculty of Sciences, UNAM. The results indicate the contribution of this tool to improve the quality of teaching-learning process.

Introduction

Students all over the world today have access to the world wide web and thus many things are competing for their attention. As our students' world changes, so too must the methods by which we teach and engage our students (Lara, 2005; García-Manzano, 2006). In this article, we propose one way that teaching can be significantly enhanced by using one of these web services, the blogs.

Blogs, a contraction of the words *web* and *log*, is a type of website usually maintained by an individual with regular entries of commentaries or news of a particular subject based on the idea of a newspaper that collects texts and files in chronological order. They are regularly updated by its author or authors. Blogs provide the functionality that enables people to publish their thoughts and ideas easily online. One of the primary reasons for the rapid adoption of blogs is that they are quick to setup and easy to use, requiring absolutely no programming knowledge or HTML skills (Gallego-Torres, 2006).

Presenting that basic and supplementary content of a course in a blog, the information can be constantly updated in the same way the materials used in the classroom are available any time in a blog. Thus the documents, notes, presentations, images, videos, comments, among others represent the following advantages:

1. Synthesize, disseminate and update the contents of the course that is taught.
2. Encourage communication between teacher and students through comments and participation.
3. It's possible to know the interest of students on certain issues.
4. The analysis and discussion of certain subjects not covered in the syllabus.
5. Use the tools of Web 2.0 for spreading: collection of data, projects and papers of interest. It also is possible the access to specialized, certified and updated information complementary to that revised in the classroom.
6. Use strategies and cutting-edge electronic tools for teaching.

We teach in the Biology career, a course on Genetics in which we wanted to prove the effectiveness of a blog for teaching. We use the blog to emphasize the use of *Drosophila*. We have included a link in the blog to a laboratory manual of genetics which included twelve different interactive practices in which *Drosophila melanogaster* is used (Rodriguez-Arnaiz -coordinator- 2005).

Objectives

General

Try to know if a blog represents for both teachers and students a new way to transmit actualized information that complements, strengthens, and increases the classroom sessions from the collection of accumulated knowledge on the web in a dynamic, updated, efficient, and reliable form.

Particular

- Make use of available resources on the Web to attract the student's interest in a subject.
- Use attractive and original materials (animations, videos and pictures, among others) to cover all the issues seen in class.
- Promote in students the integrity of the subject with other related sciences.
- Encourage communication between teacher and students from discussions and comments generated by the information that is added to the blog.
- Strengthen communication, share and how to do skills.

Description of the Experience

We have investigated the relationship between the use of a blog and the student motivation over one semester, July-December 2009. Students were asked to try the blog, read about issues related to new literacy, and then make a proposal for potential uses or limitations of weblogging in education. We teach Genetics at the career of Biology, in the Faculty of Sciences of the Universidad Nacional Autónoma de México (UNAM). The group consists of 25 students.

Material and Methods

Create a Blog

For the development of the blog it is recommended the Google's Blogger service, which requires a Gmail account and facilitates the use of other resources offered by Google™. For its creation it must be considered the design by choosing a template that is visually appealing.

Moreover, the content is another aspect in which it should be considered the type of items to attach, such as links to websites and/or other blogs, RSS, gadgets[1], etc. (Gallego-Torres, 2006). Also, choose the information presented to the public, through the use of inputs, which can be pictures, articles, videos, and news coming from certified sites that are recognized for their veracity.

Updating the Blog

This can be done at least every five days, according to the frequency of sessions of the course.

Dissemination of the Blog

We spent quite a bit of time offering guidelines, tips and examples, including an explicit definition of blog, and the way of use it. Spread between students the blog URL web address and explain their characteristics and performance.

For example, when students enter to the blog (http://ciber-genetica.blogspot.com/), they can see in one of the inputs the link to the laboratory manual of Genetics. In this link there are practices in which there is a description of how to make the *Drosophila*'s medium, an anesthizer or flynap, and the crosses, among others. The screen in Insert 1 is seen once you enter to the blog.

Once in the laboratory manual link the student can choose from the index the practice of interest. For example, if the student goes to the link of practice #3, the student can obtain the information needed to construct an anesthizer for *Drosophila*.

There are also links to other laboratory manuals (in English), web sites about Genetics, news, journals, and images, among others.

Results

The blog "Ciber-genética" has been developed and evaluated. The survey had 16 questions that pertain answering several questions about which weblog motivate students for studying. The set of questions relevant to this paper are included in an appendix.

We asked open-ended follow-up questions to a number of the closed, discrete questions, and the responses to those questions were coded by the principal investigator into categorical responses. An example will be explained below. While the coding is obviously interpretive and not validated by outside readers, the open-ended questions did generate responses that we would not have anticipated, and, therefore, this particular strategy was appropriate for this exploratory study.

Assessment of the Blog

Number of individuals in the test (evaluators).

The analysis of the blog was done by 22 undergraduate students of genetics.

Conditions under which the test was conducted

Each evaluator was provided with an evaluation sheet which included the following items

A link to:

a) They were asked to carefully review the blog and record their answers. An evaluation sheet was provided.
b) During two hours the students reviewed and filled the evaluation.

Observed results

We have done two evaluations of the blog during the semester, one at the middle and the other at the end. The following results are shown: *Blog's objective* (Figure 1), *Visual materials and complementary* (Figure 2), and *Integration with the classes of the course* (Figure 3).

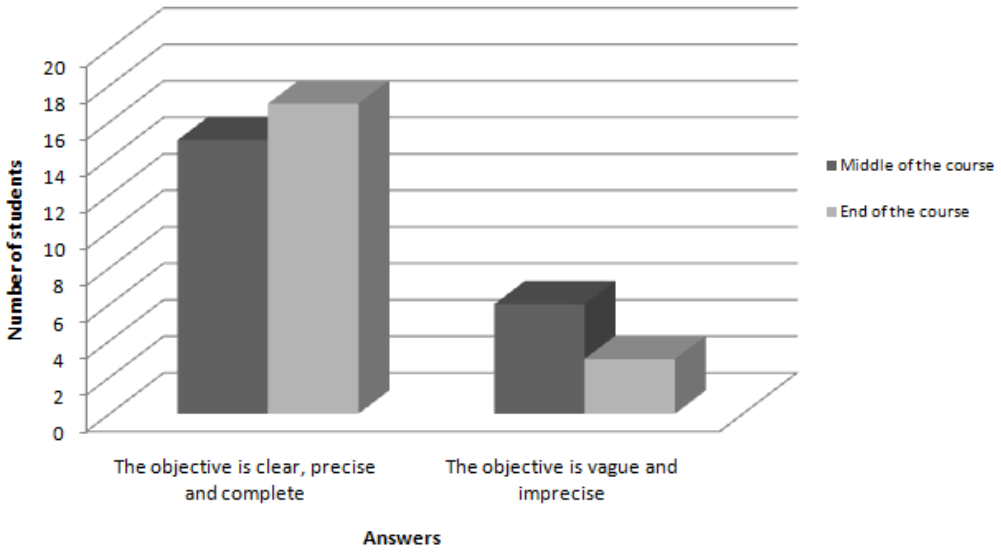

Figure 1. The Blog's objective.

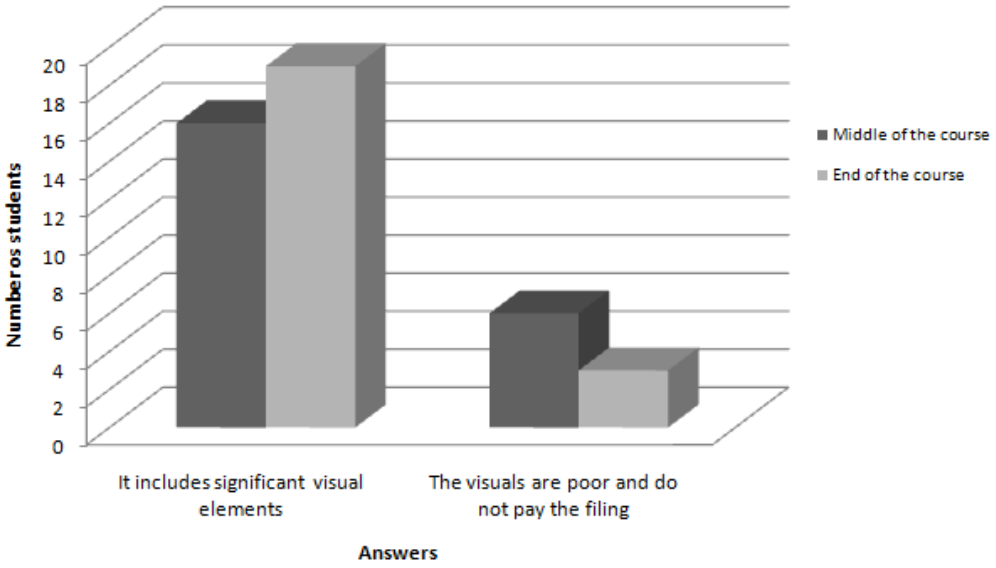

Figure 2. Visual materials and complementary.

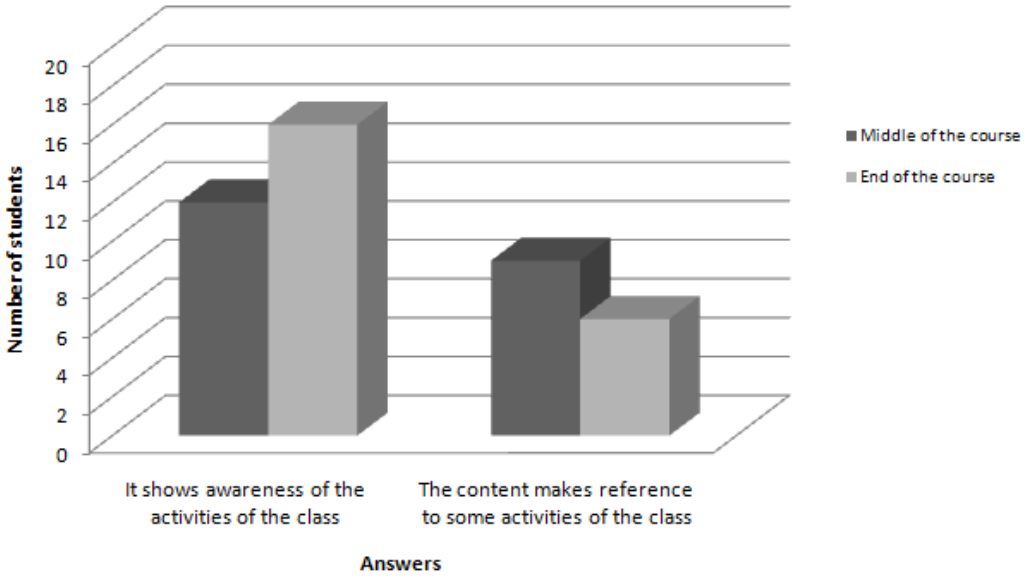

Figure 3. Content integration with the classes of the course.

In the figures it can be seen that the students showed more interest and satisfaction in each item as the course advanced. Also it was mentioned that the blog is a resource that they would like to use in other courses. About the written comments we gathered, the students notice that the blog resulted to be very useful and significant in the study of Genetics.

Discussion

Based on the results, we conclude that, overall, the blog proved to be a useful and valuable tool that helped to enrich and enhance the education offered, as well as an excellent opportunity to obtain a kind of information on *Drosophila melanogaster*, a model species used in many current and classic genetic studies. According to the results, we can say that the students felt that the blog allows them to reinforce the knowledge presented in class through the extension of this knowledge based on

the information in the blog. Furthermore, the expansion of communication facilities and the extension of spatial and temporal boundaries allowed the students to focus their learning on an individual basis. Finally, students particularly appreciated the blog for the novel contributions with respect to the visual material that was available in an organized, easy and permanent shape.

Footnote: [1]They are small programs that are tasked to submit certain information and can be attached to any page on the network.

Acknowledgments: This project is conducted with funding from DGAPA, UNAM. Project PAPIME PE 201509.

References: Gallego-Torres, A., 2006, Guías fáciles de las TIC-Blogs. Official Association of Telecommunication Engineers. Madrid. Available at: http://www.coit.es/pub/ficheros/blogs_425672d7.pdf; García-Manzano, A., 2006, Blogs y Wikis en tareas educativas. Available at: http://observatorio.cnice.mec.es/modules.php?op=modload&name=News&file=article&sid=378; Lara, T., 2005, Blogs para educar. Available at: http://www.tiscar.com/?page_id=337; Rodríguez-Arnaiz, R., A. Becerra, L. Castañeda-Partida, A.N. Castañeda-Sortibrán, L.J. Delaye, M.G. Ordaz-Téllez, V. Valadéz-Graham, and M.J. Vázquez-Cuevas 2005, Manual de Prácticas de Genética y Cuaderno de Trabajo. Las Prensas de Ciencias (U.N.A.M.). México. 296 p.

APPENDIX: Evaluation Form

Evaluation of the Genetics Group's Blog
Title: Ciber-Genética

Design: Dra. Rosario Rodríguez Arnaiz
Dra. América Nitxin Castañeda Sortibrán
Maintenance: Marco Antonio Carballo Ontiveros
Advice: Dra. Laylla Michán Aguirre

Evaluator's name:

_____

Age:_____ **Gender: W / M** **Date of application:**_____

This evaluation is important to provide a better service and to update the students, so we ask you to respond in the most authentic possible way.

No.	Question	Answer				
1 $^{\Omega}$	Does the title match the content?	In its entirety	Mostly	Partially	Almost no	Not applicable, because: _____ _____ _____ _____ _____ _____
2 $^{\pi}$	The blog's colours are suitable?	In its entirety	Mostly	Partially	Almost no	No

3 π	The organization of the content (tags, links, images, etc) is suitable?	Excellent	Very good	Good	Deficient	Very deficient
4 π	How do you perceive the grammar and the spelling of the blog?	They are almost always correct.		Includes some grammatical errors, also with the spelling.	It has grammatical errors and also errors with the spelling.	
5 Ω	Blog's Objective	It offers clear, accurate and complete information about the purpose of the blog.		Provides information about the objective but it is vague and imprecise.	There is no information about the objective.	
6 Ω	How often the blog is updated?	The information is published frequently.		It publishes information 1 or 2 times a week.	Few information is published.	
7 π	Number of links	Includes a significant number of links.		Includes an average number of links.	Includes very few links.	
8 π	Quality of the links	All links are from reliable and relevant resources.		Over 50% of the links are from reliable and relevant resources.	The links are not from reliable and relevant resources.	
9 π	Visual materials and complementary	It includes significant visual elements (charts, illustrations, graphics and multimedia). They are relevant to the topic of the blog.		The visual elements are por. The images are randomly selected, are of poor quality and distract the reader.	Does not include visual elements.	
10 π	Copyright	Sources are cited appropriately. It includes elements of public domain or with permission.		Sources are cited but elements are used without permission.	The blog does not include the sources from which information is retrieved.	
11 Ω	Blog's degree of integration with the material discussed in class.	The elements of the blog are aware of the activities of the class (with the concepts, themes, etc).		The elements of the blog make some reference to the matters mentioned in the activities of the class.	The elements of the blog do not refer to the matters covered in the activities of the class.	
12 Ω	Quality of the articles cited (comprehension)	Adequate information is presented and enjoyable to understand.		It hinders the comprehension of the information presented.	The information presented is not understandable.	
13 Ω	The presence of articles enriches the themes seen in class.	Most of them enriche the information viewed in class.		In half of the cases yes.	Not at all	
14 Ω	Does the blog's content help in understanding the subject?	Okay, because: _____ _____ _____		Indifferent, because: _____ _____ _____	Disagree, because: _____ _____ _____	
15 Ω	Is the blog an element that improves the quality of the course?	Okay, because: _____ _____ _____ _____		Indifferent, because: _____ _____ _____ _____	Disagree, because: _____ _____ _____ _____	
16 Ω	Is it convenient to use blogs on other subjects?	Yes, because: _____ _____ _____		No, because: _____ _____ _____	Not know, because: _____ _____ _____	

Ω : Evaluation of background, π: Evaluation of form.

Comments and observations

_____
_____
_____
_____
_____
_____
_____

Thanks for your help!

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)-325-4821; 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. 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 behavioral genetics using *Drosophila* larval phototaxis.

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Introduction

This exercise will teach students about behavior, the genetic basis of behavior, and neuronal development. The exercise involves a distinctive *Drosophila melanogaster* larval behavior, negative phototaxis, quantified in a simple Petri dish arena assay. Variation in this behavior is demonstrated in a simple assay, easily accomplished during a 3-hour lab session, in which students collect larvae to be tested, construct the assay arenas, and collect data.

Larval *D. melanogaster* generally display negative phototaxis – that is they are repelled by light (Lilly and Carlson, 1990). In third instar larvae, this behavior gradually diminishes as the larvae leave the food medium in search of a pupation site and immediately prior to pupation, larvae respond indifferently to light (Godoy-Herrera *et al*., 1992). Larvae detect light using Bolwig's organ, consisting of an anteriorly located cluster of 12 photoreceptors (Hassan *et al*., 2000; reviewed in Friedrich, 2008). Four of the 12 photoreceptors become the adult eyelet, these future adult receptors express the rhodopsin 6 gene that is also active in 8 photoreceptors of Bolwig's organ (Sprecher and Desplan, 2008). A characteristic head swinging behavior may mediate the negative phototaxis behavior. If so, this behavior suggests that the larvae detect differences in light intensity through unequal stimulation of photoreceptor cell clusters and use this stimulation to orient away from the light stimulus (Busto, Iyengar and Campos, 1999; Scantlebury, Sajic and Campos, 2007).

There are a number of mutations in *D. melanogaster* that have been shown to affect larval phototaxis behavior. β3-tubulin is expressed in *Drosophila* embryos and functions in development of Bolwig's organ and nerve; larvae with mutations of the β3-tubulin gene show defects in phototaxis (Dettman *et al*., 2001). Busto, Iyengar, and Campos (1999) presented evidence that mutants for either *neither inactivation nor afterpotential C* (*nina c*) and *no-receptor potential A* (*norp A*) display defects in phototaxis. *Nina C* mutant adults have reduced rhabdomeres due to retinal degeneration and defects in phototransduction (Porter *et al*., 1992), and *norp A* protein is expressed in all adult photoreceptors and encodes phospholipase C (Schneuwly *et al*., 1991). Stocks of these three mutations are available through the Bloomington Stock Center (http://flystocks.bio.indiana.edu/). Alternatively, for a more involved experiment, students could be charged with their own crosses and stocks to create mutants via *P*-element excision (*e.g*., of the e03267 line in the Harvard Exelixis Collection with a *P*-element inserted near the *norp-A* gene), making this specific teaching lab the culmination of a semester long project.

Materials and Methods

Behavioral assay procedures and protocols are similar to those described by Lilly and Carlson (1990). Details are given below.

Fly Preparation

Approximately 10 days before the lab session, adult flies should be transferred to new food to lay eggs. Approximately an hour before the lab, adults should be removed from stock vials, leaving

only larvae. This timing ensures that very few larvae will be late 3rd instar or entering the pupal stage (*i.e.*, relatively light-insensitive), decreasing error associated with the assay, and maximizing the number of larvae collected from each vial. Four or five vials of each genotype per lab group should be sufficient to collect 3 sets of approximately 50 larvae of the genotypes to be assayed. The number of larvae per test plate could be lowered to 10 or 20 larvae to save time during the lab.

Assay Plate Preparation

Behavioral assays are conducted using agarose plate "arenas". Before students begin collecting their larvae they should start to boil 2 beakers of water for their agarose. Alternatively, to save time, plates can be prepared prior to the lab by the instructor. Figure 1 is a schematic diagram showing how the test plates are constructed. To make the test plates, equal numbers of dyed and clear agarose plates are made; about 30mL of 1% agarose is needed for each plate. To make the darkened agarose approximately 10 drops each of red, blue, and green food colouring are added to 200 ml 1% molten agarose. Once the agarose is hardened plates are cut into four equal sections, removing two diametrically opposed sections and replacing them with the opposite coloration of agarose. After switching out the sections approximately 10 ml of clear 1% agarose is poured on top of the test plates so as to ensure that no crevices or bumps exist, otherwise larvae will burrow into spaces in the agarose. Allow the test plates to cool to room temperature before testing.

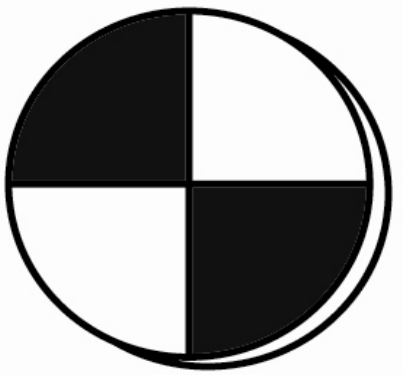

Figure 1. The Larval Phototaxis Assay is constructed from darkened and regular clear agarose. For each assay, approximately 50 larvae are placed onto each plate. Plates are then placed on a light box and larvae are allowed to migrate for 5 minutes.

Collection of Larvae

Early third instar larvae are removed from the culture by adding 1-2 mL of water to the medium and lightly agitating the surface with a paintbrush; this water and culture mixture is then poured into a Petri dish or weigh boat and third instar larvae can be carefully collected using tweezers or a paintbrush. Dissection microscopes with black stages are very helpful for this step. Collected larvae are placed into a pool of clean water to rinse off any remaining food particles. After being rinsed they are placed into another pool of water in a covered Petri dish where they remain until testing.

Table 1. Data table with typical results for wildtype and mutant larva with defects in larval phototaxis.

Trial 1	Wildtype	Mutant
Dark	48	28
Clear	2	22
Total	50	50
D-C	46	6
RI=(D-C)/ (D+C)	46/50 = 0.92	6/50 = 0.12

Testing Larval Phototaxis

Approximately 50 larvae of a given genotype are placed in the centre of the test plate that is then placed, without its cover, on a light box in a dark room for 5 minutes. Plates are then scored for the number of larvae on the dark and clear portions of the plate and a response index (RI) is calculated. The RI is the number of larvae on the dark portions (D) minus the number on the clear portions (C) with this quantity divided by the total (D+C), i.e., RI = (D-C)/(D+C). See Table 1 for a typical results table that students would fill in during lab.

Each genotype should be assayed in triplicate so students can determine the mean RI and standard deviation. In more advanced courses, students can perform a simple one-way ANOVA and

subsequent Tukey's HSD to determine significant differences for the data they have collected during this lab.

Conclusion

The above protocol offers instructors a dynamic teaching exercise that can be altered to accommodate students at a variety of levels, from an introductory genetics course, to an advanced behavioral or developmental genetics course. This teaching protocol gives students an opportunity to gain experience working with *Drosophila*, a model species widely used in the study of behavior, development, and genetics. The protocol provides students with hands-on experience in the acquisition of scientific data, from the basic level of sample collection and preparation, to the more advanced data analysis and presentation. The protocol also provides the opportunity for direct experience assaying for mutant phenotypes and in more involved versions the possibility of incorporating actual mutagenesis experiments with this behavioral assay.

References: Busto, M., B. Iyengar, and A.R. Campos 1999, J. Neurosci. 19: 3337-3344; Dettman, R.W., F.R. Turner, D. Hoyle and E.C. Raff 2001, Genet. 158: 253-263; Friedrich, M., 2008, BioEssays 30: 980-993; Godoy-Herrera, R. Alarcon, M. Caceres, H. Loyola, I.I. Navarrete, and J.L. Vega 1992, Revista Chilena de Historia Natural 65: 91-101; Hassan, J., M. Busto, B. Iyengar, and A.R. Campos 2000, Behav. Genet. 30: 59-69; Lilly, M., and J. Carlson 1990, Genet. 124: 293-302; Scantlebury, N., R. Sajic, and A.R. Campos 2007, Behav. Genet. 37: 513-524; Schneuwly, S., M.G. Burg, C. Lending, M.H. Perdew, and W.L. Pak 1991, J. Biochem. 266: 24314-24319; Sprecher, S.G., and C. Desplan 2008, Nature 454: 533-537; Porter, J.A., J.L. Hicks, D.S. Williams, and C. Montell 1992, J. Cell Biol. 116: 683-693.

Drosophila adult eye model to teach Scanning Electron Microscopy in an undergraduate cell biology laboratory.

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Abstract

We have devised an undergraduate laboratory exercise to study tissue morphology using fruit fly, *Drosophila melanogaster*, as the model organism. *Drosophila* can be reared in a cost effective manner in a short period of time. This experiment was a part of the undergraduate curriculum of the cell biology laboratory course aimed to demonstrate the use of **S**canning **E**lectron **M**icroscopy (**SEM**) technique to study the morphology of adult eye of *Drosophila*. The adult eye of *Drosophila* is a compound eye, which comprises of 800 unit eyes, and serves as an excellent model for SEM studies. We used flies that were mutant for *Lobe* (*L*), *eyeless* (*ey*), and *pannier* (*pnr*) for our studies. The mutant flies exhibit different morphologies of the adult eye. We employed a modified protocol, which reduces sample preparation steps and makes it practically feasible to complete the protocol in

assigned time for the cell biology laboratory. The idea of this laboratory exercise is to: (a) familiarize students with the underlying principles of scanning electron microscopy and its application to diverse areas of research, (b) to enable students to sharpen their observation and quantitative microscopy skills, and (c) minimize the preparation time for the instructor.

Keywords: *Drosophila melanogaster*, eye, tissue morphology, Scanning Electron Microscopy (SEM), cell biology, undergraduate education.

Introduction

Research is an important component of habits of inquiry and learning in the undergraduate curriculum. A large array of laboratory courses has been developed for undergraduate students in order to expose them to techniques used in biomedical research. Interestingly, many new text books and accompanying supplementary materials provide exhaustive and detailed information through images and movies on diverse subject material studied using the **S**canning **E**lectron **M**icroscopy (**SEM**) technique. Although animations and videos can provide an overall idea, it is important that students get a "hands-on" exposure to learn the techniques like how to use SEM to capture high resolution images. We devised a laboratory to introduce students to the SEM technique, its principle and applications, which will allow them to get a hands-on experience on the scanning electron microscope. Furthermore, this exercise can be finished in a single laboratory session with some preparation done prior to the demonstration to the students.

For this laboratory exercise, we chose to study the morphology of the well studied adult eye model of *Drosophila*. This model is highly versatile as in addition to studying the pattern and morphology of the normal flies, the variations in eye development can be easily demonstrated. There are several molecularly characterized mutants that directly or indirectly affect the morphology of the adult eye. Using the normal and mutant flies, we can demonstrate the limitations of conventional light microscopy in terms of resolution and magnification. This may help the students to appreciate (i) SEM has a much greater resolving power than light microscopes, (ii) SEM uses electromagnetic radiation instead of light, and (iii) SEM can obtain much higher magnifications of up to a million times.

SEM produces images by probing the specimen with a focused electron beam that is scanned across a rectangular area of the specimen (raster scanning). The electron beam of SEM is generated from a filament, which may be made up of various types of materials. The most common filament is made up of a loop of tungsten which functions as the cathode. A beam of electrons is produced at the top of the microscope by heating this metallic filament. The electron beam follows a vertical path through the column of the microscope and makes its way through electromagnetic lenses that focus and direct the beam on the sample. Electrons in the beam interact with the atoms constituting the sample material and are scattered back, producing the back scattered electrons or the secondary electrons. A detector collects the secondary or backscattered electrons, and converts them to a signal that is sent to a viewing screen similar to the one in an ordinary television, producing an image (Figure1). These signals contain detailed information about the sample's surface topography, composition and other properties such as electrical conductivity.

Vacuum is an essential requisite for SEM. If the sample is in a gas filled environment, the beam is unstable as gases could react with the electron source, causing it to burn out or result in ionization of beam. Alternatively, other molecules, which come from the sample or the microscope itself, may form compounds and condense on the sample and thereby reduce contrast and obscure details in the image.

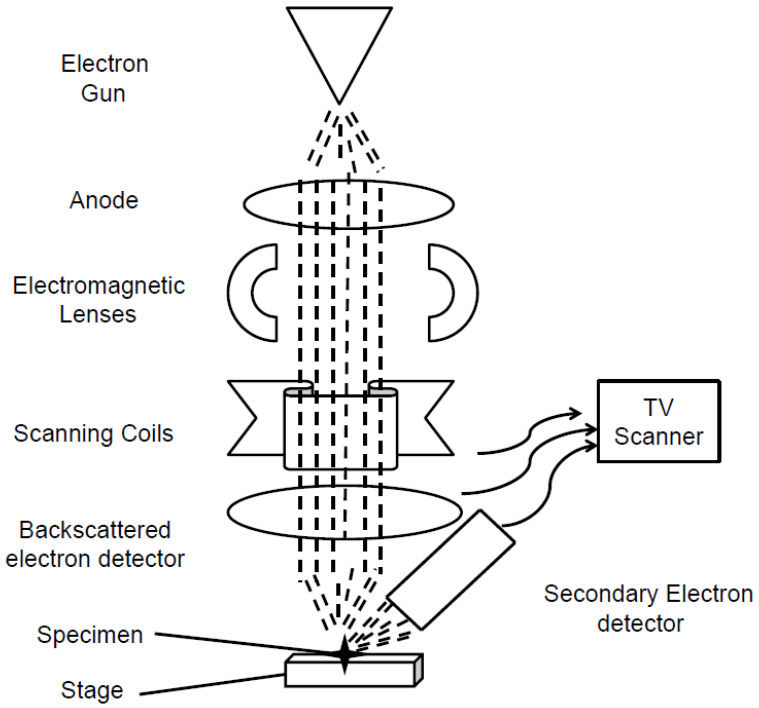

Figure 1. Schematic flow diagram of a Scanning Electron Microscope. Electron microscopes use a particle beam of electrons to illuminate a specimen and create a highly-magnified image. The electrons from electron gun pass through anode, electromagnetic lenses, coils, detectors and strike the gold coated specimen placed on the stage. The electromagnetic lenses focus electron beam to a specific plane relative to the specimen and thereby forming the image. The secondary electrons generated because of electrons striking specimen surface are detected by secondary electron detector and converted into signal that is sent to a TV scanner.

Protocol

We have employed *Drosophila melanogaster* to study the morphology of the adult eye. *Drosophila* eye is a compound eye made up of 750-800 unit eyes referred to as ommatidia. We selected the flies that were mutant for genes involved in eye development (obtained from the Bloomington Stock Center, Indiana; http://flystocks.bio.indiana.edu/). The Bloomington Stock center is a repository of various fly strains and mutations, which are available upon request to the scientific community. We selected fly mutant strains for genes *eyeless* (*ey*) [ey^2 (BL 648), which shows complete loss of the eye field], *Lobe* (*L*) [L_2/CyO (BL 319), a mutant which shows selective loss of the ventral eye], *pannier* (*pnr*) [$pnr^{vx6}/TM6B$, (BL 6334)], which is an embryonic lethal mutation that can generate dorsal eye enlargement in genetic mosaics where *pnr* function is eliminated in patches of cells (Xue and Rubin, 1993; for review see, Blair, 2003). These three different mutants exhibit a range of phenotypes of eye size from enlarged eye to half eye, and to no-eye in comparison to the wild-type eye (Figure 2). However, each of these mutants show a range of phenotypes due to penetrance. Therefore, for our lab exercise, we selected flies that showed distinct eye phenotypes from a large population of each mutant stock.

This exercise helped students to learn two basic experimental operations: (a) sample preparation, and (b) basic operation of the scanning electron microscope. Students also learned some background information on the development and morphology of the normal eye.

The entire methodology of the SEM can be divided into three major steps: (1) sample preparation, (2) sample mounting and sputter coating, and (3) imaging.

1. Sample Preparation:

This step includes preparation of sample and is carried out prior to the research laboratory. For SEM, biological samples need to be dehydrated and dried. Dehydration is carried out to

gradually reduce the water content of the tissue to the point that the tissue is completely into a non-aqueous solvent. Dehydration is done using ascending concentration series of ethanol or acetone. The flies of different eye mutants were passed through a series of ascending concentrations of acetone to dehydrate the sample. The adult fly samples were dehydrated by incubating for 24 hours each in 30%, 50%, 70%, 90%, 95%, and 100% concentrations of acetone. Thus at the end of the seventh day, the sample is completely dehydrated and is present in 100% acetone. To achieve best results, sample was dehydrated in 100% acetone twice.

In earlier protocols, dehydrated samples were subjected to critical point drying. The presence of surface tension during drying is disruptive to tissues and causes visible distortions. Therefore, the critical point drying is carried out in vacuum where fluid and gaseous phases exist together and there is no surface tension. The critical point drying is achieved using liquid carbon dioxide (CO_2). However, drying can also be achieved using commercially available chemicals. Here, we employed **H**exa**m**ethyl **D**i **S**ilazane (**HMDS**), which is a chemical of choice used for drying SEM samples that mainly include insect tissues, large fleshy tissues or soft invertebrates (Braet *et al*., 1997). Furthermore, it does not require vacuum. Drying with HMDS prevents the tissue morphology from getting damaged in freeze drying or liquid CO_2 drying procedures. Following 100% acetone, samples were incubated overnight in 1:1 mixture of 100% acetone: HMDS (Electron Microscopy Sciences Cat# 16700). Samples were then incubated in 100% HMDS solution for 24 hours and they were allowed to air dry at room temperature in a fume hood. The lids of the tubes were left open to allow the HMDS to evaporate.

2. Sample Mounting and Sputter Coating:

Each HMDS treated dehydrated sample was mounted on a metallic stub (a sample holder for electron microscope, available from Electron Microscopy Sciences Cat# 75944). Sample was held onto the stub by a conductive carbon tape (Electron Microscopy Sciences Cat# 77825-12). Maximum contact of the sample with the tape was ensured so that sputter coating is good. Sample was arranged on the stub in such a way that the area of interest (in this exercise the eye tissue) in the sample is perpendicular to the plane of the observation in the microscope. Mounted tissue on the stub was then sputter coated in vacuum with an electrically conductive layer of gold (or some other inert heavy metal). This step is important since it makes the sample conductive, enhances the secondary and backscattered electron emission and increases the mechanical stability of the tissues. Coating is an essential step to prevent accumulation of static electric charge on the specimen during electromagnetic irradiation. Improper coating on the tissue results in charging, which may result in deflection of electron beam, deflection of secondary electrons and periodic burst of secondary electrons. Gold is the preferred metal for coating the samples because of its high atomic number. Further, sputter coating with gold produces high topographic contrast and resolution. Depending on the type of sample, there are several other coating materials like Gold Palladium alloy, Platinum, Iridium, Tungsten, Osmium, Graphite and Carbon. The sample stub was subjected to sputter coating at pressure of 100 psi for a period of 35 seconds and a current of 45 milli amps under vacuum using sputter coater (DV 502) from Denton Vacuum Company.

We have described a protocol for sample preparation for SEM. However, there have been continuous improvements in the processes of sample fixing, drying and coating methods. A variety of new adaptations to SEM have also emerged that enables a large spectrum of samples to be analyzed using SEM technique. There are alternative methods for fixation, dehydration and coating depending on the nature of sample and approach used summarized in Table 1.

Table 1. Alternative materials used in SEM for fixation, dehydration, drying and sputter coating in different model systems.

Sample Type	References	Fixative	Dehydrating agent	Drying process	Mounting/ Sputter coating
Bacteria, virus on surfaces, as parasites	Sangetha et al., 2009; Robinson et al., 1984	Glutaraldehyde, Osmium tetra Oxide (OsO_4)	Ethanol	Critical point drying in amyl acetate	Gold, Gold-Palladium alloy
Plant tissues	Pathan et al., 2008	Glutaraldehyde, Osmium tetra Oxide (OsO_4)	Ethanol/ Acetone	Critical point drying	Mounting using Aq. Silver, Coating with chromium
Insect tissues	Braet et al., 1977, (modified by Naoto Ito)	Fixing is usually not required	Acetone	HMDS (Chemical drying)	Gold
Mammalian tissue	Wierzchos et al., 2008; Lehman et al., 1983	Aldehyde/ Formalin/ Osmium tetra Oxide (OsO_4)	Ethanol	Critical point drying in acetone	Gold, Gold-palladium alloy, Carbon coating

3. Imaging:

The final step in this exercise is to image the samples using SEM. Sputter coated sample stub was then imaged using the Hitachi S-4800 High Resolution Scanning Electron Microscope (HRSEM) available in the Nanoscale Engineering Science and Technology (NEST) facility at University of Dayton. The samples on the stub were placed in vacuum and subjected to electron beam. A voltage of 5kV was applied. The electrons from the gun strike the surface coating of gold, electrons are reflected back off the specimen to a detector, this is transmitted to a TV screen where the image is viewed and photographed. The images were taken at 130×. At magnification of 130×, the entire *Drosophila* head fits in an image plane and is a suitable resolution to study morphology of head and the compund eye. As shown in the Figure 2, each unit eye or the ommatidium is clearly visible, and this would not have been possible using a compound light microscope of 10× magnification. The high resolution SEM images provides detailed information about different kinds of bristles present among the ommatidia. Depending on the model, SEM allows the magnification of a sample up to 500,000- 1000000 times.

Advantages

1. The greatest challenge to teaching a Cell Biology laboratory is the capital investment/ commitment that a university/college must make to laboratory. The use of cost effective exercises can facilitate the execution and implementation of these laboratory programs in an undergraduate academic institution setup.
2. The students get general overview of SEM and hands-on experience of the technique starting from sample preparation to visualizing the sample on the monitor attached to SEM.
3. The sample preparation in conventional method for SEM is a little time consuming procedure. It requires critical point drying in vacuum. In our protocol, we eliminated the critical point drying method which requires vacuum.

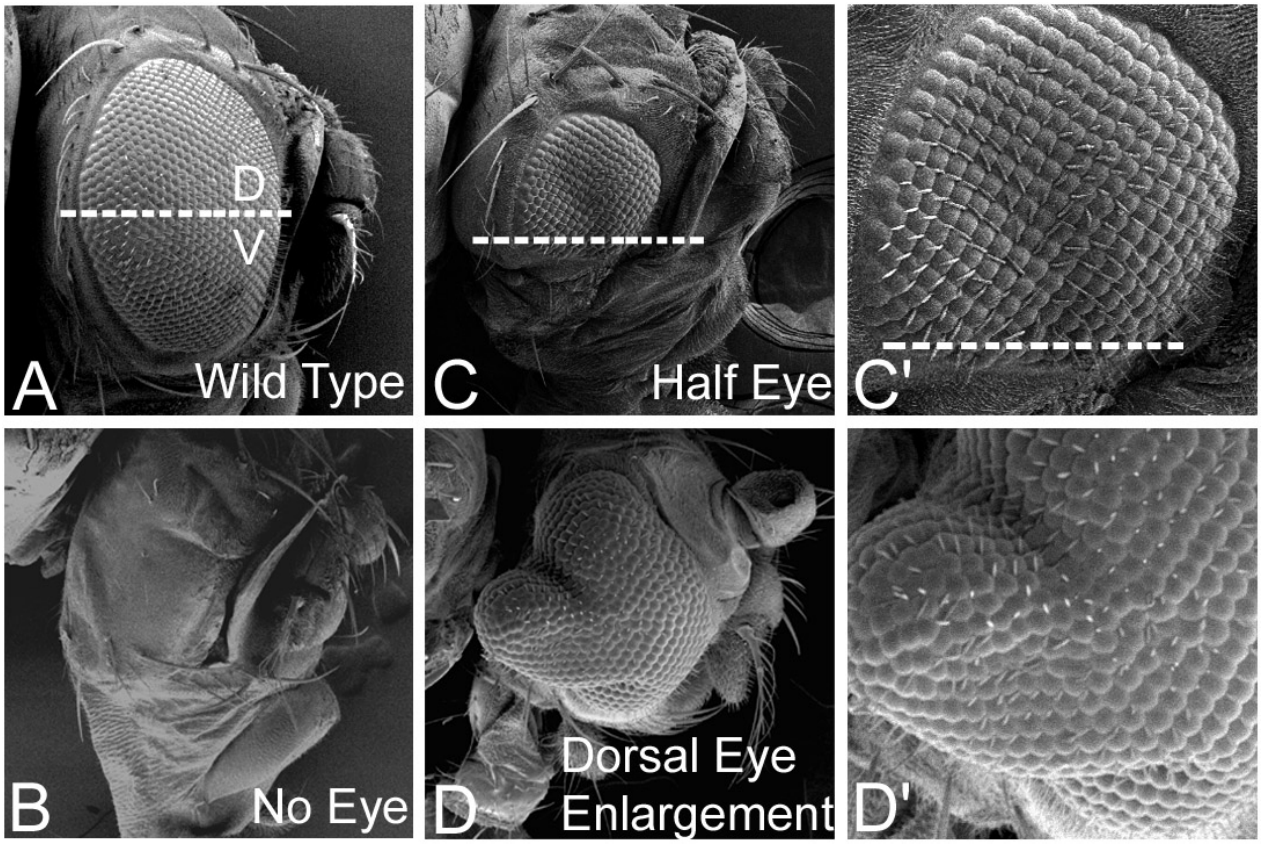

Figure 2. Scanning Electron Micrographs showing morphology of *Drosophila* eye mutants. (A) Wild type eye of *Drosophila* showing 600-800 unit eyes. The dotted line marks the equator where dorsal (D) half of the eye is above the equator and ventral (V) half is below the equator. (B) *eyeless* mutant (ey^2), showing complete loss of eye field. (C) *Lobe* mutant (L_2) shows selective loss of the ventral half of the eye. (C') Magnified view of the L_2 mutant eye. (D) Ectopic dorsal eye generated when *pnr*, a GATA 1 transcription factor, function is eliminated in patches of cells in genetic mosaics (Xu and Rubin, 1993; for review see, Blair, 2003). (D') Magnified view of the dorsal eye enlargement generated due to genetic mosaics of *pnr*.

4. Our protocol does not require post fixation treatment with Osmium tetra Oxide (OsO_4), which is highly carcinogenic and may not be an ideal chemical to use in an undergraduate laboratory. Instead, we use HMDS for final processing after dehydration in acetone series.

5. These exercises does not require educational demonstration kits that minimize the exposure of experimental details and reagents to the students.

6. This experience adds to their skill-set and helps generate a core of trained individuals who can function in academics as well as corporate settings.

High magnification images are powerful sources of communication which are preferred to words. Most laboratory science courses do not actively teach students skills to communicate effectively through images (Riemeier and Gropengießer, 2007). Our laboratory exercise meets this requirement by teaching students to (a) develop basic laboratory skills and learn tissue handling,

sample preparation and scanning electron microscopy, (b) capture digital images using the software, (c) process the image using the Photoshop or imaging software, and (d) develop a series of image portfolios to present their results.

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References: Blair, S.S., 2003, Development 130 (21): 5065-5072; Braet, F., R. De Zanger, and E. Wisse 1997, J. Microsc. 186: 84-87; Lehmann, P., P. Zheng, R.M. Lavker, and A.M. Kligman 1983, J. Inves. Dermatol. 81: 169-176; Pathan, A.K., J. Bond, and R.E. Gaskin 2008, Micron. 39(8): 1049-61; Riemeier, T., and H. Gropengießer 2007, Int. J. Sci. Educ. 1–17; (http://www.informaworld.com/smpp/content~content=a781884932~db=all); Robinson, R.W., D.E. Akin, R.A. Nordstedt, M.V. Thomas, and H.C. Aldrich 1984, Appl. Environ. Microbio. 48(1): 127-136; Sangetha, S., Z. Zuraini, S. Suryani, and S. Sasidharan 2009, Micron 40(4): 439-43; Wierzchos, J., T. Falcioni, A. Kiciak, J. Woliński, R. Koczorowski, P. Chomicki, M. Porembska, and C. Ascaso 2008, Micron. 39(8): 1363-70; Xu, T., and G.M. Rubin 1993, Development 117: 1223-1237.

Heat shock effects upon cell death in *Bar* eye quantified by scanning electron microscopy.

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Bar (*B*) is a well-known sex-linked dominant mutation that arose spontaneously in *Drosophila melanogaster* as a tandem duplication in cytological location 16A1-2 (Tice, 1914; Lindsley and Zimm, 1992). The vertical bar-eye phenotype is due to cell death, especially in the anterior region of the eye disc (Fristrom, 1969), or disruptions in the pattern of mitosis. But the extent of cell death can be influenced genetically (*e.g.*, variegated position effect; Brosseau, 1960) and by environmental conditions like temperature (*e.g.*, developmental temperature and log facet number are inversely proportional; Hersh, 1930) and chemical treatments (*e.g.*, being raised on media supplemented with acetamide, lactamide, cytosine, and other chemicals; Fristrom, 1972; and references in Lindsley and Zimm, 1992). Given its sensitivity to modifying factors, the severity of *Bar* eye cell death can be a model for quantifying experimental influences on development. But for this model system to be sensitive enough to detect comparatively small effects, eye facet (ommatidium) number must be measured very accurately. In spring 2009, the Experimental Genetics and Cell Biology Lab course taught in the Department of Zoology at the University of Oklahoma undertook to test experimental design options and the feasibility of using scanning electron microscopy of *Drosophila Bar* eyes to evaluate the effect on cell death by an experimental treatment, exposure to heat shock that activates chaperone proteins of the stress response. Additional data were

collected and analyzed later in the year. The system worked very well and can be modified to allow quantification of an almost unlimited array of physical and chemical treatments that might influence cell death or mitotic cycles during development.

The choice of a brief exposure to high temperature to activate heat shock proteins fitted this experiment into our on-going interest in genotype × stress interactions and allowed the class to design an original experiment after gaining basic information about *Drosophila* breeding programs and cellular stress responses. The initial hypothesis was that a brief heat shock exposure of third instar *Drosophila* larvae would reduce cell death in *Bar* eyes by elevating the protection of precursor cells during eye formation. The heat shock response increases the activity of heat shock proteins, which include chaperone proteins that help repair protein damage as part of the organism's stress response pathways. Chaperone proteins help refold proteins damaged by environmental stress conditions to improve cellular function, although they have also been linked to cell death (Gething, 1997).

Experiments were done with the *Basc* strain of *Drosophila melanogaster* (In(1)sc^{S1L} sc^{8R} + S, a balancer chromosome with multiple inversions carrying *white-apricot*, w^a, and *Bar*, *B*; Lindsley and Grell, 1992). Third instar larvae were collected from culture bottles raised on standard cornmeal medium at 25 ± 1°C. Groups of ten larvae were placed in 2 ml microfuge tubes containing about 0.5 ml of medium to maintain humidity. Tubes for heat shock were then submerged in the wells of a tempblock heater at 37°C for 40 minutes (Ashburner, 1989). Tubes for control samples remained on the lab bench at room temperature for the same period. Caps were then removed from each microfuge tube, and open tubes were inserted into 8 dram shell vials containing standard cornmeal medium. The third instar larvae could emerge from the small microfuge tube and pupate in the larger space from which adults were collected the following week.

Specimen preparation was simplified by the fact that *Drosophila* compound eyes will desiccate for scanning electron microscope (SEM) examination without the need for alcohol dehydration or critical point drying. Although minor deformations sometimes occur, their severity is generally small enough to counter the cost of the added time required for more sophisticated preparation when the data to be gathered are as simple as in this experiment. First, a head is dissected from the body with a single-edge razor blade, and the head is bisected near the midline between the compound eyes. Each half is then transferred with sharp forceps onto double-sided adhesive surface cut to fit an aluminum specimen plug (the size of the plug is determined by the specific SEM microscope that will be used). The two head halves are placed very near each other to facilitate identification of pairs. But no attempt was made to keep track of which was the left and which was the right hand side, since we did not plan to explore sidedness biases, if any. A small spot of silver paste helps ground each specimen to the plug surface. After drying in a desiccator for several days, plugs were sputter-coated with gold-palladium in a Hummer 6 sputter coater. Specimens were viewed with the Zeiss DSM-960A SEM at 10 kV, and digital images were taken at 350×. Sample images are shown in Figure 1. Eye facet counts were made directly from the digital images.

As expected with images of this quality, the variation due to repeatability error of counts among students was very low (average standard deviation among eight replicate counts of a representative sample of 33 images = 0.79; average number of facets for these 33 eyes = 73.96; repeatability factor from ANOVA is not significant). Some sources of variation are illustrated in Figure 2, which shows two eyes that have minor distortions due to processing or eye development. If an eye was damaged during dissection or mounting so that counts of facets were ambiguous, it was omitted from the data set. The remaining single eyes contributed to the data about average expression in treated *versus* control conditions, but they were not used in measures of symmetry (FA, fluctuating asymmetry).

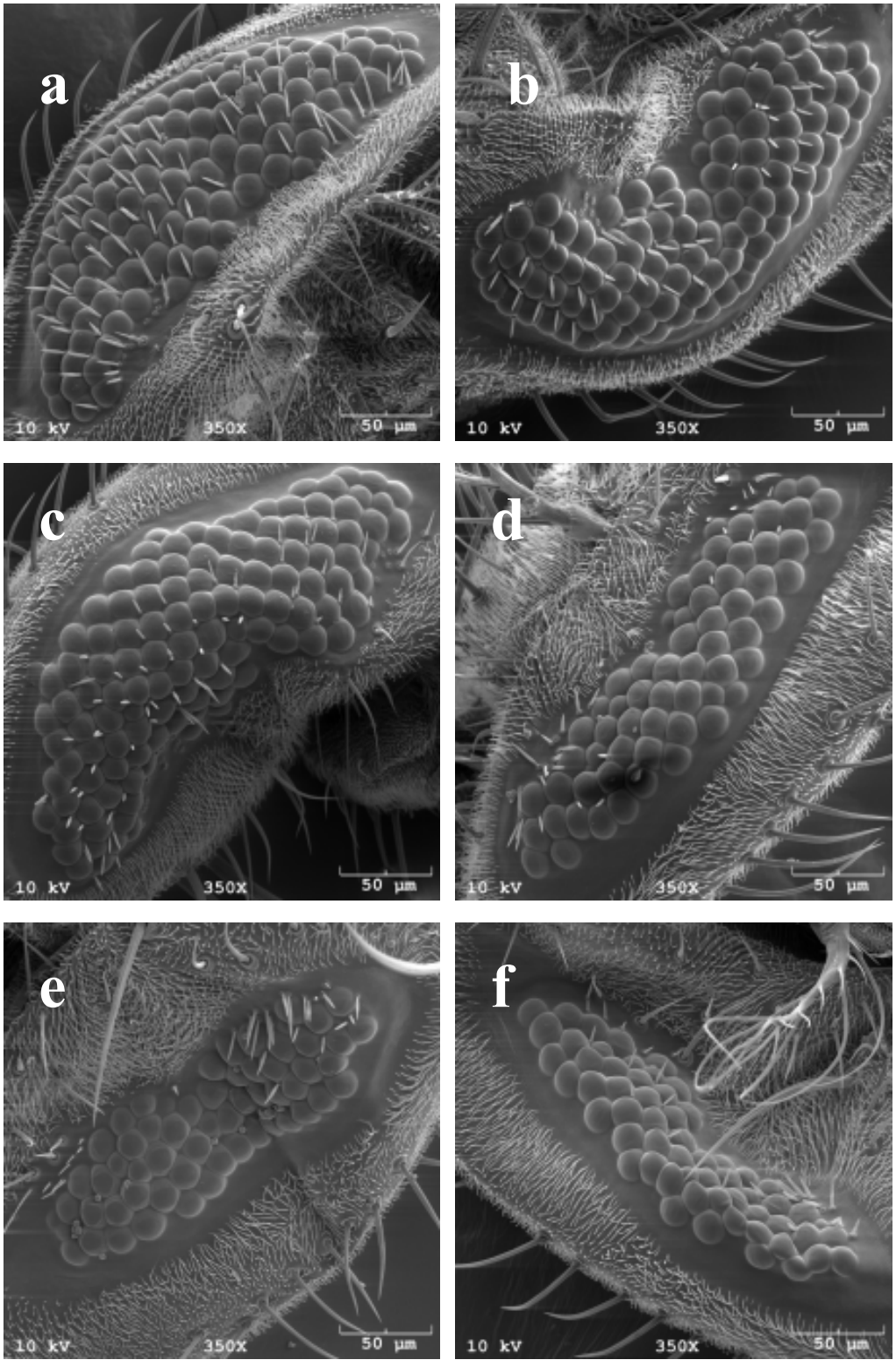

Figure 1. SEM images of *Bar* eye. a, b, Control left and right of the same fly showing deviations from symmetry; c, Control, compared to d, Heat Shock, to illustrate differences in facet number; f, g, Heat Shock to illustrate variation in facet number and appearance. All images are 350×.

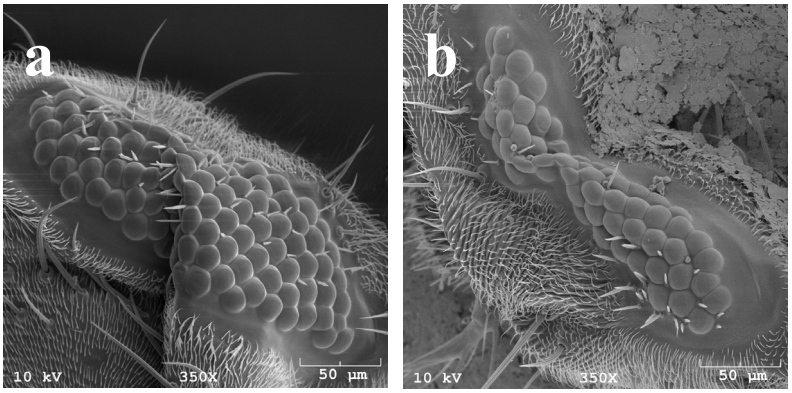

Figure 2. *Bar* eye images to illustrate some of the complexities in counting facet numbers accurately. a, slight fold in eye after processing; b, folding of tissue due to developmental variation or processing of sample.

Data from 24 control and 45 heat-shock treated eyes show that a 40-minute heat shock causes a significant reduction in the number of facets (control, mean = 101.79 ± 18.88; heat shock, mean = 77.78 ± 21.29; from t-test, $P < 0.001$). This is the opposite of the prediction from our initial hypothesis. Although we know that increased temperature during development is correlated with a reduction in eye facet number in *Bar* (Hersh, 1930), we were surprised that this effect can be traced to just a 40-minute exposure to high temperature during an approximately 10 day developmental life cycle.

Fluctuating asymmetry (FA), a measure of symmetrical development, can be interpreted as reflecting developmental stability (Markow, 1994; Møller and Swaddle, 1997; Polak, 2003). Unregulated responses to environmental perturbations are expected to increase asymmetry. In our experiment, we predicted that activating an elevated stress response should improve developmental regulation and make expression, *i.e.*, eye facet number in this case, more similar within matched pairs of compound eyes. One way to quantify symmetrical expression is:

$$FA = |L - R| / [(L + R) \times 0.5],$$

which scales the difference between the two sides of the trait by their average. Although there is a lot of variation in facet number among eyes, the degree of symmetry is surprisingly high (Figure 3; slope = 0.75). The pooled data from control and heat-shock samples yielded a correlation coefficient between eyes of 0.84 ($r^2 = 0.71$).

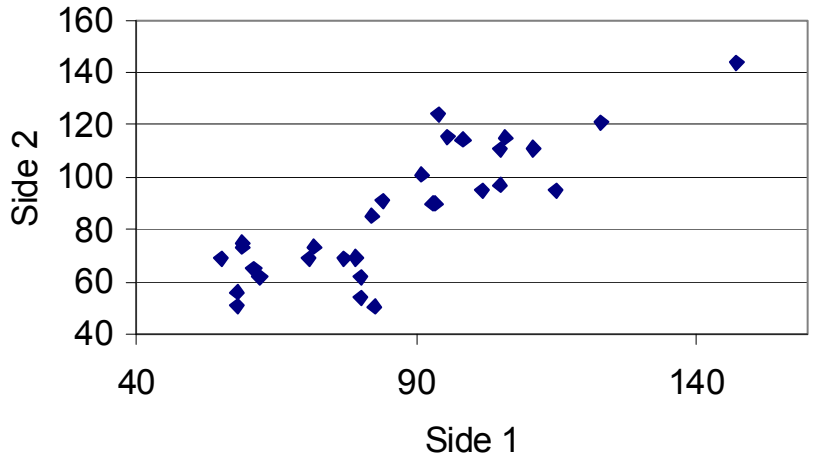

Figure 3. Symmetry of facet number of the two sides of *Bar* eye, combined control and heat shock.

But when two treatments are considered separately, the average FA is slightly higher after heat shock ($FA_{heat\ shock} = 0.1490 \pm 0.1375$, n = 19 pairs; $FA_{control} = 0.0833 \pm 0.0544$, n = 11 pairs), but the difference is not significant ($t = 2.20$, $P = 0.14$).

Heat shock proteins influence cell death (apoptotic) pathways and can either prevent or increase cell death (Fristrom, 1972). Heat shock protein 60D, for example, is involved in ommatidial apoptosis in *Drosophila* (Arya *et al*., 2007). Our data show a significant effect of heat shock on eye facet number that seems hard to account for as a simple developmental temperature influence. A more reasonable hypothesis is that the heat-shock-induced chaperone proteins assist in the maintenance of proteins involved in cell death promotion cascades. Alternatively, Vazquez *et al*. (1993) reported an overall reduction in transcription in *Drosophila* cells following heat shock for 15 minutes at 37ºC. Core histone protein transcription was suppressed at severe temperature exposure, and H1 histone and the 5C actin gene transcription was suppressed at all heat shock temperatures. RNA polymerase II, which accounts for up to 75% of all transcriptional activity in these cells, showed a five-fold reduction in activity after 37ºC heat shock. This suggests that there is priority given to proper packaging and protection of the cell's DNA. Furthermore, Westwood and Steinhardt (1989) showed that protein degradation rates increase in heat shocked *Drosophila* cells, primarily through the ubiquitone signaling pathway. Thus, the heat shock environment coincides with a number of cellularly debilitating activities that can retard overall cell proliferation and growth while the chaperone proteins and other elements of the stress response work to repair and protect protein function and keep the cell alive. In that light, the trend observed in our data encourages further exploration that can form the basis of future student-designed experiments using the *Bar* eye experimental model.

Acknowledgments: We thank Greg Strout and Preston Larson, Noble Electron Microscopy Lab, for their instruction and supervision of scanning electron microscopy and image capture.

References: Arya, R., M. Mallik, and S.C. Lakhotia 2007, J. Biosci., Bangalore 32(3): 595-610; Ashburner, M., 1989, Drosophila: *A Laboratory Handbook*, Cold Spring Harbor Press; Brosseau, G.E., 1960, Genetics 45: 979; Fristrom, D., 1969, Mol. Gen. Genet. 103: 363-379; Fristrom, D., 1972, Mol. Gen. Genet. 115: 10-18; Gething, M.-J., 1997, *Guidebook to Molecular Chaperones and Protein-Folding Catalysts.* Oxford University Press, Oxford; Hersh, A.H., 1930, J. Exp. Zool. 57: 283-306; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster. Academic Press, NY; Markow, T.A., 1994, *Developmental Instability: Its Origins and Evolutionary Implications*. Kluwer Academic Publishers, Dordrecht; Møller, A.P., and J.P. Swaddle 1997, *Asymmetry, Developmental Stability, and Evolution*. Oxford University Press, Oxford; Polak, M., 2003, *Developmental Instability: Causes and Consequences*. Oxford University Press, Oxford; Tice, S.C., 1914, Biol. Bull. 26: 221-230; Vazquez, J., D. Pauli, and A. Tissieres 1993, Chromosoma 102: 233-248; Westwood, J.T., and R.A. Steinhardt 1989, J. Cell. Physiol. 139: 196-209.

50th Annual *Drosophila* Research Conference

The 50th Annual *Drosophila* Research Conference was held on 4-8 March 2009 at the Sheraton Chicago Hotel & Towers. This golden anniversary of the conference was celebrated with a special tee shirt and a roundtable discussion involving several "industry legends," some of the early influential *Drosophila* geneticists moderated by Scott Hawley. The 2009 Organizing Committee was John Carlson (Yale School of Medicine), Lynn Cooley (Yale School of Medicine), and Rick Fehon (University of Chicago). 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 869 presentations, including 156 platform session talks and 713 posters (www.drosophila-conf.org).

Historical Address Roundtable

Mel Green (University of California, Davis)
Dan Lindsley (University of California, San Diego)
Tony Mahowald (University of Chicago)
Thom Kaufman (Indiana University)
Ruth Lehmann (Skirball Institute and Howard Hughes Medical Instititute, New York)

Plenary Lectures

Michael Dickinson (California Institute of Technology, Pasadena). Ethomics: Progress toward an automated analysis of behavior.

Jennifer Zallen (Sloan-Kettering Institute, New York). Shaping the embryo: Cellular dynamics in development.

Daniel Barbash (Cornell University, Ithaca, New York). Heterochromatin divergence: A hidden cause of speciation.

Nick Brown (University of Cambridge, United Kingdom). Integrin adhesion and morphogenesis.

John Reinitz (University at Stony Brook, New York). Canalization of gene expression in the *Drosophila* blastoderm.

Wu-Min Deng (Florida State University, Tallahassee). Cell-cell communication and proliferation control.

David Schneider (Stanford University, California). Balancing tolerance and resistance during infections of the fly.

Barry Ganetzky (University of Wisconsin, Madison). Autophagy regulates synaptic growth at the larval neuromuscular junction.

Mariana Wolfner (Cornell University, Ithaca, New York). Battle and ballet: Interactions between male seminal proteins and mated female *Drosophila*.

Tadashi Uemura (Kyoto University, Japan). Roles of organelle dynamics in shaping cells.

Daniela Drummond-Barbosa (Vanderbilt University Medical Center, Nashville, Tennessee). Stem cells, insulin, and the control of oogenesis by diet in the *Drosophila*.

Steve Henikoff (Fred Hutchison Cancer Research Center, Seattle, Washington). Structure and evolution of centromeric chromatin.

Workshops

Ecdysone Workshop

Organizers: Randall Hewes (University of Oklahoma, Norman) and Deborah Hoshizaki (National Institutes of Health, Bethesda, Maryland).

Everything You Ever Wanted to Know About Sex

Organizers: Michelle Arbeitman (University of Southern California, Los Angeles), Mark Van Doren (Johns Hopkins University, Baltimore, Maryland), and Artyom Kopp (University of California, Davis).

Evolution and Causes of Codon Usage in the Genus *Drosophila*

Organizer: Jeff Powell (Yale University, New Haven, Connecticut).

Proteomics in *Drosophila*

Organizers: Alexey Veraksa (University of Massachusetts, Boston) and Ernst Hafen (ETH Zurich, Switzerland).

The Maternal to Zygote Transition: Deciphering the Ultimate Genetic Switch

Organizers: Howard Lipshitz (The University of Toronto, Ontario, Canada) and John C. Sisson (The University of Texas, Austin).

Quantitative Modeling in Gene Expression, Development, and Cellular Processes

Organizers: Stas Shvartsman (Princeton University, Princeton, New Jersey) and David Arnosti (Michigan State University, East Lansing).

Modencode

Organizer: Susan Celniker (Lawrence Berkeley National Laboratory, California)

Drosophila Research and Pedagogy at Primarily Undergraduate Institutions (PUI)

Organizers: Mark Hiller (Goucher College, Baltimore, Maryland), Alexis Nagengast (Widener University, Chester, Pennsylvania), Janet Rollins (Mount Saint Vincent College, Riverdale, New York), and Don Paetkau (Saint Mary's College, Notre Dame, Indiana).

Gases in *Drosophila* Physiology and Development

Organizers: Gret J. Beitel (Northwestern University, Evanston, Illinois) and Dan Zhou (Albert Einstein College of Medicine, Bronx, New York).

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: Fly Board under the News menu at the FlyBase web site: flybase.bio.indiana.edu.

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Kevin Cook (Bloomington Stock Center & Nomenclature Committee; Indiana University)
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