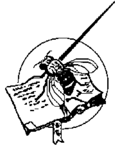
Research Notes



The clastogenic effects of Aflatoxin B_1 (AFB_1) on polytene chromosomes of *Drosophila melanogaster*.

Sisman, Turgay[1], Handan Uyasal, and Hakan Askin. [1]Atatürk Universty, Science and Art Faculty, Biology Department, 25240 Erzurum-TURKEY; Tel: +90 0 442 231 43 37; Fax: +90 0 442 236 09 48; Corresponding author; e-mail: tsisman@atauni.edu.tr, turgaysisman@hotmail.com.

Abstract

In this study the effects of AFB_1, a mold metabolite, on third instar larval salivary gland polytene chromosomes of *Drosophila melanogaster* were investigated. The various chromosomal abnormalities (= aberrations) as fragment loss from teminal, semibreak, asynapsis, regional shrinking, ectopic pairing on arms of polytene chromosome, were observed in polytene chromosome slides from third instar larvae which have developed in the medium containing AFB_1. It was found that the difference between control and test groups from the point of view of the chromosomal abnormalities is statistically significant ($P < 0.05$).

The possible mechanism of the all chromosomal abnormalities caused by AFB_1 is discussed.

Introduction

Mycotoxins are toxic metabolites which are produced by a lot of toxic fungi species, and when they have been received by animals and humans they cause chronic and acute intoxications. Approximately 300 mold species produce mycotoxins, and those of 100 mold species have high toxicity for humans and animals (Steyn, 1995). According to previous experimental studies, aflatoxins are the most dangerous of these fungal secondary metabolites.

Aflatoxins are secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*, and they have carcinogenic, mutagenic, hepatotoxic and teratogenic effects (Davis and Diener, 1978). These fungi species contaminate a lot of feeds, and the contaminated feeds seriously threaten health of human and domestic animals. AFB_1 causing approximately 75-83% of aflatoxicosis events is the most widespread metabolite of molds growing on the agricultural crops in nature. Although there are 18 different kinds of aflatoxins, the most determined of aflatoxins in feeds are AFB_1, AFB_2, AFG_1, AFG_2 (Hartley *et al.,* 1963).

Anwar *et al.* (1994) investigated the clastogenic effects of AFB_1 on various experimental animals. In that study, it was found that 0.1 μg/g AFB_1 on rat bone marrow cells and 1 μg/g AFB_1 on mouse caused abnormalities of chromosomal structure such as deletions, ring chromosomes, and breaks. In a similar study, it was found that the frequency of chromosomal aberrations increased in rat bone marrow cells as connecting to augmention of AFB_1 concentration (1-20 μg/g) (Ito *et al.,* 1989). These chromosomal aberrations especially are aneuploids and deletions. Bose and Sinha (1991) reported that various chromosomal defects occured on bone marrow cells of mouse nourished 0.05 μg/day AFB_1 during six weeks. In addition, El-Khatib *et al.* (1998) determined micronucleus on bone marrow cells of white female rats fed with foods including 8 μg/kg AFB_1.

But, unfortunately according to our literature investigations, there is not a study related to effects of AFB_1 on polytene chromosomes. What are the effects of AFB_1 on polytene chromosomes of third instar larvae of *Drosophila melanogaster*? What are the mechanisms of these effects? The main aim of this study is to find the proper answers to above questions.

Material and Methods

Cultures of Drosophila: For all of the applications, the third instar larvae of the Oregon R (OR) wild type culture *Drosophila melanogaster* was used. The imago flies were replaced as 7 males and 7 females in 150 ml vials including Standard Drosophila Medium (SDM) to get third instar larvae. After they were restrained a definite time for ovulation, the flies were taken away from culture vials and the vials were set in incubator ($25 \pm 1^{o}C$). After four days following ovulation, the third instar larvae were formed in the vial. The third instar larvae leave the medium and live on the side of the vial.

Application of AFB_1 to Larvae: For our experiments polytene chromosome slides were prepared from salivary glands of third instar larvae. To get these larvae, the application dose of AFB_1 was not to cause death in parents, and the eggs grown in the medium including AFB_1 were to complete their metamorphosis. Because of these reasons, 0.8 ppm AFB_1 was selected as the application dose, because it was understood that larval development was maintained with this dose by previous tests.

In our investigations, the culture vials containing 0.8 ppm concentration of AFB_1 + SDM were used as experimental groups and the other culture vials containing only SDM were used as control groups. Third instar larvae fed on medium containing AFB_1 and 4-6 days old cultures were used for the experiments. The polytene chromosome slides prepared from these larvae were examined under the microscope. The regions of chromosomal aberrations were identified according to Bridges' salivary gland chromosome maps.

Results

The polytene chromosomes of salivary gland of third instar larvae growing up in medium containing 0.8 ppm AFB_1 have been investigated. In the prepared slides, 1896 cells were appraised and various chromosomal abnormalities were observed in 636 of those cells. The chromosomal abnormalities are fragment loss from terminal, ectopic pairing between homologous and non-homologous arms of chromosomes, asynapsis, regional shrinking on the arms, and semibreaks.

These chromosomal abnormalities are that: in X, the fragment loss from terminal on 1AC and the regional shrinking on 7AC (Figure 1); in 2L, ectopic pairing between zones 21D and non-determined arm of chromosome (Figure 2); in 2R, ectopic pairing between zones 56A-58F and between zones of non-determined regions (Figure 3); in 3R, ectopic pairing between zones 84A-54E bant region of 2R, and asynapsis between zones 85B-86F and 87F-90D (Figure 4); in 3R, semibreak on 86BC (Figure 5). According to the chromosomal abnormalities not observed in control groups, the difference between control and application groups is statistically significant ($P < 0.05$).

Discussion

In this study, the clastogenic effects of AFB_1 on polytene chromosomes of third instar larvae of *Drosophila melanogaster* were investigated. In the polytene chromosome slides prepared from third instar larvae, various chromosomal abnormalities were observed on the different chromosome arms and bant regions. Those abnormalities are semibreak, ectopic pairing, asynapsis, and regional

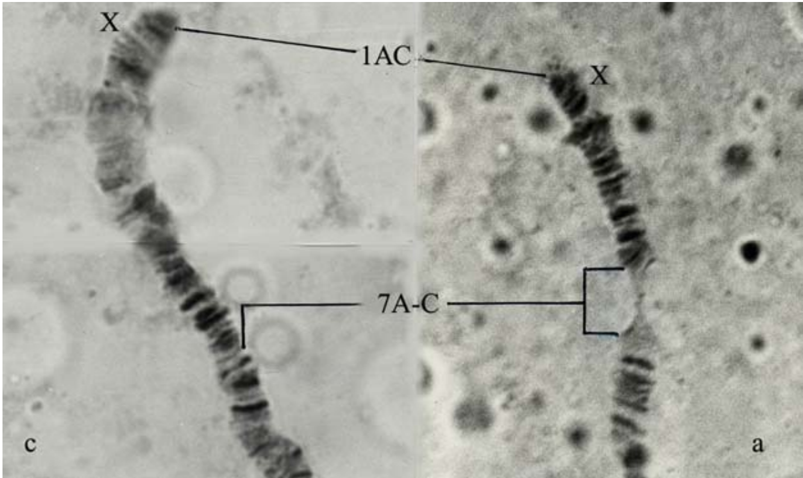


Figure 1. The fragment loss from terminal on 1AC and the regional shrinking on 7AC, X (c: control, a: application).

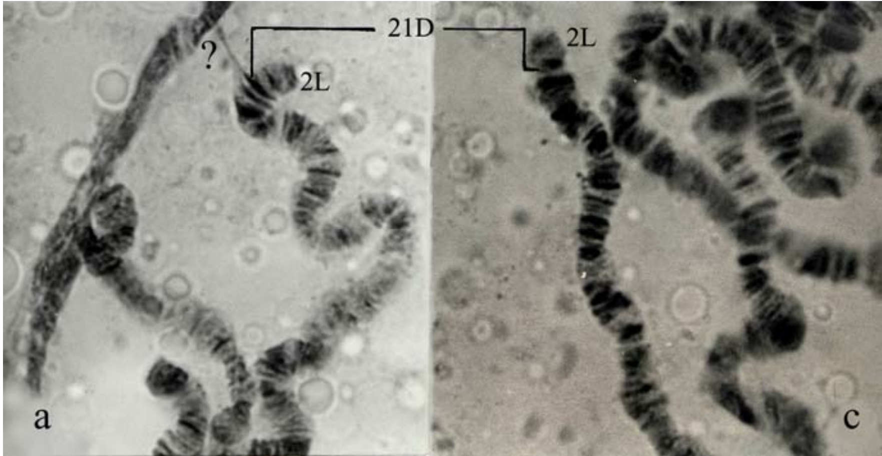


Figure 2. Ectopic pairing between zones 21D and non-determined arm of chromosome, 2L.

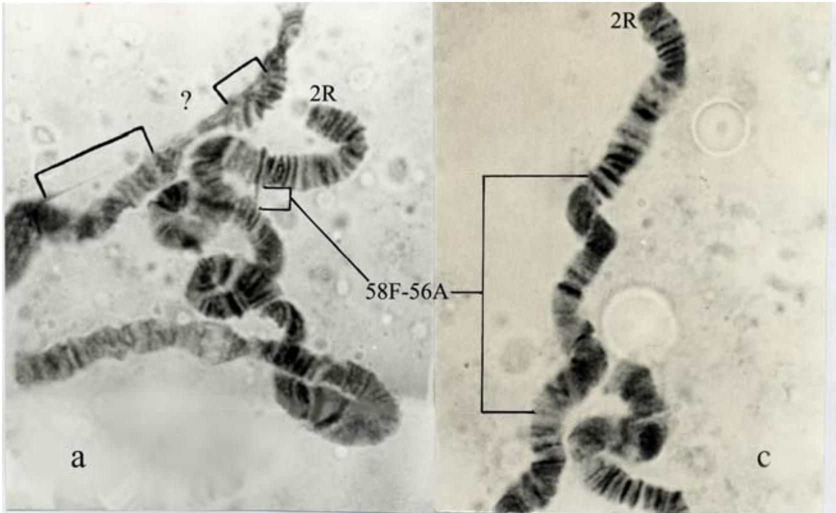


Figure 3. Ectopic pairing between zones 56A-58F and between zones non-determined regions, 2R.

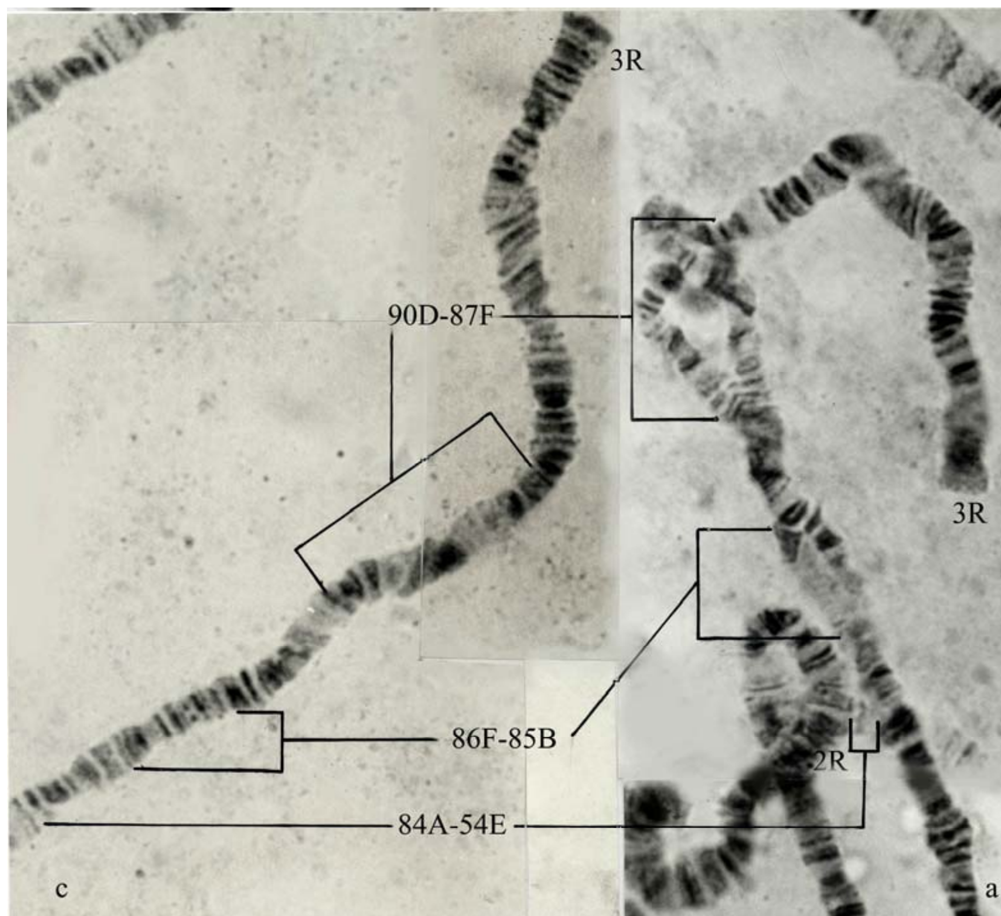


Figure 4. Ectopic pairing between zones 84A-54E bant region of 2R, and asynapsis between zones 85B-86F and 87F-90D, 3R.

shrinkings. In the previous chromosomal investigations of assorted researchers (Abdou *et al.,* 1989, Dietert *et al.,* 1985), the results resembling our findings have been reported. But according to our literature studies, there isn't a direct study of related to clastogenic effects of AFB_1 on polytene chromosomes. Because of this, our findings will be discussed with the investigation results observed in different animal groups.

According to IARC (1993), AFB_1 caused chromosomal structure defects such as gene mutations, break, SCE (Sister Chromatid Exchange) and micronucleus in mammals, plants, insects and bacteria. SCE was observed five times more than in the control group in blood cells of chicken embryos exposed to AFB_1 (Dietert *et al*., 1985). Chromatid breaks were also determined as related to AFB_1 in Chinese hamster (Barta *et al.,* 1990).

At the level of chromosomes, the effects of AFB_1 have also been reported in various plants. For example; AFB_1 caused chromosomal aberrations such as anaphase bridges, chromatid breaks, polyploid cells, micronucleus, polarisation in anaphase and telophase, ring chromosome in root tip cells of onion (Abdou *et al.,* 1989; Reis, 1971). Similar results have also been observed in root tip cells of *Vicia faba* (Lilly, 1965).

According to the findings mentioned above, AFB_1 affects chromosomes of vivid cells. Because of untidiness in genetic data, the metabolism has not fully processed, tumors have occurred

and the organism might show death. When the larvae of *Drosophila melanogaster* exposed AFB_1 were grown up, various malformations (phenotypic abnormalities) were observed in grown individuals (Uysal and Şişman, 2003). These malformations are tumor formation on legs, lack of one or two wings, atrophy of legs and lack of segment on legs. The type of malformations could be accepted as a result of which AFB_1 affected chromosomes. A similar study has also showed that 0.32 μM AFB_1 caused tumor formations on wings, abdomen and legs (Sidorov *et al.,* 2001).

Mycotoxins, especially aflatoxins, are bound to cellular macromolecules like DNA, RNA, and protein and obstruct the synthesis of the macromolecules. Activity of RNA polymerase connected to DNA is also blocked by aflatoxins (McLean and Dutton, 1995). According to Allison and Paton (1965), the secondary metabolites caused chromosomal structure defects blocking activity of lysosomal enzymes. Besides it has been reported that the metabolites that occurred with effects of cytochrome p-450 and aryl hydrocarbon hydroxylase are responsible for the inhibition of macromolecules (Cavin *et al.,* 1998). The metabolites are probably AFB_1-8,9-epoxide (Busby and Wogan, 1984) and AFB_1-2,3-epoxide (Benasutti *et al*., 1988).

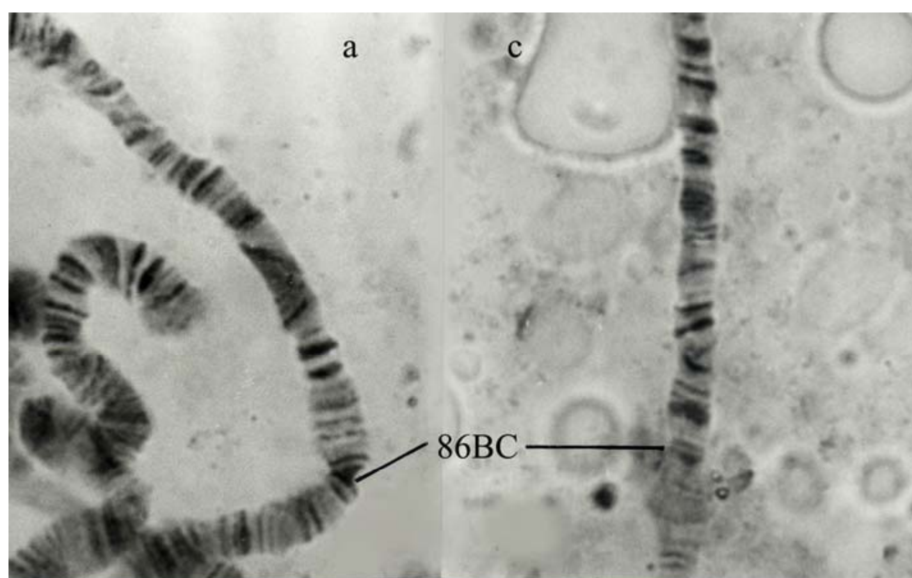


Figure 5. Semibreak on 86BC, 3R.

Studies at the molecular level have found that metabolites of AFB_1 adduct to cell DNA (Gradelet *et al.,* 1998), the target base is guanine (Lyman *et al.,* 1988), and AFB_1-8,9-epoxide constitutes formation of 8,9-dihydro-9-hydroxy (N7-guanyl) AFB_1 bounding to guanine (Benasutti *et al*., 1988). This form is 90% the complex of AFB_1-DNA and the tumor formation begins with increasing promutagenic area on DNA.

As a result of our study, AFB_1 causes clastogenic effects on polytene chromosomes of salivary glands of *Drosophila melanogaster* larvae. Because of untidiness of genetic structure, enzymes of xenobiotic metabolism are not synthesised, so detoxification mechanism can not work. This situation brings about teratogenic effects or mortality in different developing stages of *Drosophila melanogaster*.

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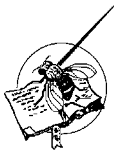

A preliminary report on *Drosophila* fauna of Islamabad (Capital, Pakistan).

Amin ud Din, Muhammad[1,2], K. Mazhar[2], S. Haque[2], and M. Ahmed[2]. [1]Department of Biology, Government College Dera Ghazi Khan, Punjab, Pakistan; [2]Department of Biology, Quid -e-Azam University, Islamabad, Pakistan; For correspondence, email: amin1158@hotmail.com

Considerable progress has been made in the field of taxonomy and systematics of the Genus *Drosophila* in the Indian subcontinent. More than 130 species belonging to its different sub-genera were reported by Fartyal and Singh (2001) from India, a neighboring country of Pakistan.

Nothing is known in the biological literature regarding *Drosophila* fauna of Pakistan, despite the prevalence of luxuriant flora and suitable climatic conditions for *Drosophila* diversification. This region includes luxuriant hilly and forest areas in Kashmir, northern areas and NWFP, vast agricultural regions in Punjab and Sindh, and varied climatic conditions in Balochistan. The survey conducted on *Drosophila* in Islamabad (Capital of Pakistan) was the first step towards a broad research program for exploration and description of the *Drosophila* fauna of Pakistan.

Islamabad is located in the North East corner of the Punjab. It lies between latitudes 33º-36' and 33º-49' North and longitudes 72º-50' and 73º-24' East rising gradually from an elevation of 503 m to 610 m above the sea level (Geological survey of Pakistan; personal communication). It is bounded on the north-east by the Margalla hills. The climate of Islamabad is sub-tropical, with two dry spells, one lasts from the end of the monsoon in September till the start of winter rains in December-January, and a second dry spell occurs during summer from May to July. In Islamabad, the

average summer and winter temperatures are 35ºC and 18ºC, respectively (SUPARCO, personal communication).

Collections were made by using ripe/fermented fruits; mainly banana and oranges, with watermelon, guava, apple, plums and grapes occasionally, as baits. Only viable flies were considered, but it is a fact that at least three different species remained unidentified due to non-viability in the laboratory. After crossing for reproductive isolation, and morphological characterization, the viable flies were identified by using keys proposed by wheeler (1952) and Okada (1956). These were *D. melanogaster, D. immigrans, D. takahashii, D. suzuki, D. nepalensis, D. hydei, D. jambulina, D. malerkotliana, D. leontia* and *D. bifasciata*.

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The maternal effect and evolutionary conservatism of *miniature* gene in *Drosophila virilis*.

Zhuk O.W., and I.A. Kozeretska. Department of General and Molecular Genetics, Biological Faculty, Taras Shevchenko Kyiv University, Ukraine; olga_zhuk@univ.kiev.ua.

The cuticle layers formation is regulated by several genes expressed in the cells-precursors of epidermal tissues. *miniature* and *dusky* genes are known by their role in the wing cuticle organization. They form so-called *m-dy* gene complex in *Drosophila melanogaster*. The two genes are closely localized in the chromosome, mutants on both genes have similar phenotype, and both the genes code proteins, which belong to the same protein family of cuticulins (Di Bartalomeis *et al.*, 2002; Akten *et al.*, 2002; Roch *et al.*, 2003). The main structural feature of these proteins is presence of *zona pellucida (ZP*) domain (Sebastiano *et al.*, 1991; Holt *et al.*, 2002).

Characteristics of the $miniature^{G1}$ (m^{G1}) mutant allele are close to those of the $miniature^{42}$ (m^{42}) described earlier (Kozeretska *et al.*, 2004). These features include a reduced wing size; an increased density of hairs on the wing surface; the cell outlines clearly visible in an optical microscope; a significantly perturbed, in comparison with the normal homogeneous, orientation of the wing hairs; a wavy shape of the wing edge. About a half of the mutants are characterized by diverged wings with an angle about 45º with respect to bilateral axis, the wings are slightly raised at the same time. Dark rounded, oval, or irregular structures (2 to 8 μm in diameter) were found in the mutant wing veins and called neomorphic vein structures (nvs). In the mutant wings the cuticles of the dorsal and ventral parts are not attached together; a rather thick lumen, filled with a fluid, remains between the dorsal and ventral plates. Inner surface of the cuticle looks corrugated because of the inner cuticle outgrowths. The mutant alleles m^{G1} and m^{42} are complemented by each other.

Cytological analysis of chromosomes in salivary glands did not reveal obvious chromosome aberrations in heterozygous $m^{G1}/+$ females.

The main difference between the mutant alleles m^{42} and m^{G1} is the female sterility in the mutant strain m^{G1}. We have analyzed the level of gonadal reduction in the females of the new allele.

All the females studied had well-developed ovaries; we did not detect any changes in their structure. Such a picture when a mutation does not affect the maternal gonad formation, but influences on embryo development, is characteristic of maternal effect genes. We have studied the development of eggs laid by m^{G1} females. There are two peculiarities: (1) these eggs do not plunge into medium as normal eggs do, they lay horizontally on the surface; (2) The most part of the m^{G1} eggs are unfertilized, some of the eggs quit developing in early, pre-cellular stages. These facts can be considered as evidence for the role the *miniature* gene plays in the fertilization or polarity establishment in the developing egg.

To test the sequence homology of the *miniature* and *dusky* genes between two distinct species we have applied an *in situ* hybridization of biotin-labeled fragments of these genes of *D. melanogaster* with polytene chromosomes of *D. virilis.* The polytene chromosomes were isolated from salivary glands of *D. virilis* third instar larvae and hybridized as described by Lim *et al.* (1993). The hybridization probes were made using 875 bp and 390 bp RT-PCR products containing, respectively, the *miniature* and *dusky* gene fragments of *D. melanogaster*. The fragments were cloned in EcoRI/HindIII-pBS and sequenced using PE Applied Biosystems Prism 310 Genetic Analyzer. The probes were labeled by nick-translation with biotinylated dUTP and detected with avidin-conjugated horseradish peroxidase, by using the ABC Vectastain kit (Vector Lab), and then stained with diaminobenzidine. Chromosomes were hybridized with each probe individually.

Figure 1. *In situ* hybridization of the salivary gland chromosomes of *Drosophila virilis* with biotinylated fragment of *Drosophila melanogaster* gene.

The fragment of the *miniature* gene of *D. melanogaster* used for the hybridization corresponds to the most conservative ZP domain. This probe hybridized strongly at unique site 12A of X-chromosome of *D. virilis* (Figure 1) on salivary glands chromosomes map (Gubenko and Evgen`ev, 1982). This localization of the *miniature* gene in *D. virilis* is in a good agreement with the recombination data (Gubenko *et al.*, 2004). The fact of such hybridization implies a high level of the *miniature* gene conservatism, at least in the region of the functionally significant ZP domain.

The attempt to localize *dusky* was not so successful. The fragment used as the probe is not so unique, and we have observed sites of hybridization on the X-chromosome. It is interesting to note that the hybridization of this probe with polytene chromosomes of *D. melanogaster* results in the same number of sites. One of them, 10E, corresponds to the locus of cytogenetic localization of *dusky* in *D. melanogaster*. The BLAST analysis of the fragment used for the hybridization also shows eight homology items, in accordance with our hybridization sites. The locus that is the closest to the *miniature* localization in *D. virilis* was the site 12B. This site, however, was not the strongest among the eight hybridization sites. Thus, the question about the maintenance of the *m-dy* complex in *D. virilis* remains open.

In addition, it is necessary to say a few words about genetic symbols that are used in *D. melanogaster* and *D. virilis* genetics. The investigations of these species have been carried out independently for a long time; that is why some genes-homologs are designated by different symbols.

For instance, *m* is a symbol of the *miniature* locus in *D. melanogaster*, but in *D. virilis* this is a symbol for the another gene - *magenta*. We propose to unify the genetic symbols of these two species and to symbolize, in particular, the *miniature* locus in *Drosophila virilis* as *m*.

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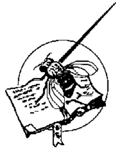


Suppressing apoptosis fails to cure "extra-joint syndrome" or to stop sex-comb rotation.

Held, Lewis I., Jr. Department of Biological Sciences, Texas Tech University, Lubbock, Texas 79409.

One of the strangest fly phenotypes ever described is an "extra-joint syndrome," where the leg has an extra, inverted joint in all but one of its five tarsal segments (Held *et al.*, 1986). This defect is extreme for the *spiny legs 1* allele at the *prickle* locus (*pk*^{sple1}) and milder for other mutations in the planar cell polarity (PCP) pathway that also cause disoriented bristles (*e.g.*, *frizzled*). Its resemblance to the segment-polarity syndrome of the embryo is intriguing, but its etiology remains a mystery.

A possible clue comes from a similar phenotype obtained with surgical experiments on abdominal segments of the milkweed bug. When Wright and Lawrence (1981) excised more than half the length of a tergite, the cut edges healed together, and the next instar had an extra, inverted segment boundary in the new tissue that grew between the edges.

If leg and body segmentation are homologous processes, as some authors have proposed (Minelli, 2003), then defects in the PCP pathway might evoke extra leg joints by a similar route. To wit, a mutation might cause more than half of the cells along each affected tarsal segment to die, and healing might elicit intercalation of reversed-polarity tissue containing an extra joint.

To test whether cell death is an essential part of the *pk*^{sple1} syndrome's etiology, I used the baculovirus *p35* gene to suppress apoptosis, as has been done for the eye (Hay *et al.*, 1994). To express *p35* in the tarsal region and incidentally in the arista, I used *Distal-less Gal4* (*Dll-Gal4*) to drive a *UAS-p35* construct in flies that were also homozygous for *pk*^{sple1}.

The desired genotype was created as follows. *Dll-Gal4*/*CyO* males were mated with *pk*^{sple1} females, and non-*Curly* (*Dll-Gal4*/*pk*^{sple1}) F_1 virgins were collected, wherein crossing over between *Dll* and *pk* should yield recombinant *Dll-Gal4 pk*^{sple1} 2nd chromosomes (25% total). These virgins were mated to F_1 males from a cross between females carrying *UAS-p35* on the 3rd chromosome and *pk*^{sple1} males. F_2 flies carrying both *Dll-Gal4* and *UAS-p35* were distinguished by short or missing claws, though rarely an extra (third) claw was present. Their aristae were swollen at the base, with extra posterior lateral branches near the tip, as is typical for loss-of-function *hid* alleles that suppress apoptosis (Cullen and McCall, 2004). Such flies comprised 28% of the total eclosed F_2 (52/187), and 21% of them (11/52) had disoriented bristles (as per *pk*^{sple1}) — thus evincing no lethality for the

combined genotype (5.9% actual ≈ 6.3% expected based on 100% viability). Flies were cultured at 25˚C, and pupae were transferred to humid, empty petri dishes to prevent adults from getting mired in the food. The legs of 10 desired flies were mounted between cover slips in Faure's solution and examined at 200×.

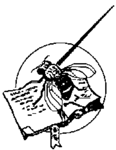
All 20 second legs of these *Dll-Gal4 pk* ^{sple1} / *pk* ^{sple1}; *UAS-p35*/+ flies displayed at least one extra, inverted joint, as assessed by a ball-and-socket articulation (without regard to the presence of an intersegmental membrane). Six of them had the full complement of four extra, inverted joints. The overall average was 3.0 extra, inverted joints per leg. In contrast, 20 control second legs of *pk* ^{sple1} F_2 siblings (disoriented bristles but normal claws and aristae) had 4.0 extra joints, while 20 control second legs of *Dll-Gal4* (*pk* ^{sple1} / +?); *UAS-p35*/+ F_2 siblings (normal bristles but defective claws and aristae) had no extra joints.

These results argue that apoptosis is not needed for the extra-joint syndrome. The lower expressivity of extra, inverted joints in *Dll-Gal4 pk* ^{sple1} /*pk* ^{sple1}; *UAS-p35*/+ flies (3.0 *vs.* 4.0) may be due to synergism between polarity disruption and apoptosis suppression. In about half the legs (9/20) the T5 segment was deformed (swollen with vesicles, invaginations, or blebs), and the normal joint between T4 and T5 was missing, in contrast to the relatively normal controls.

I also studied the sex combs of 10 *Dll-Gal4* (*pk* ^{sple1} / +?); *UAS-p35*/+ F_2 males to see whether suppressing apoptosis impedes rotation of the sex comb. Among their 20 first legs, only one comb had an odd angle. It was split, and each half had rotated ~ 60˚ (*vs.* the normal ~ 90˚). There was no effect on tooth number (mean = 10.25, N = 20), though 13/20 combs had 1-3 teeth apart from the main row. These results imply that apoptosis is not needed to "make room" for rotation of the sex comb into proximal territory on the basitarsus during normal development (Held *et al.*, 2004), nor to refine the number of teeth (Held, 2002). How the sex comb rotates remains unclear. Apoptosis had seemed a plausible guess, since flies do use it to adjust patterns (Meier *et al.*, 2000), but the present data rule it out.

Acknowledgments: Fly stocks were obtained from Konrad Basler (*Dll-Gal4*/*CyO*), Ross Cagan (*UAS-p35*), and the Bloomington Stock Center (*pk* ^{sple1}). The idea for this experiment came from a discussion with Erika Neeley, an undergraduate HHMI fellow at TTU.

References: Cullen, K., and K. McCall 2004, Dev. Biol. 275: 82-92; Hay, B.A., T. Wolff, and G.M. Rubin 1994, Development 120: 2121-2129; Held, L.I., Jr., 2002, *Imaginal Discs: The Genetic and Cellular Logic of Pattern Formation*, Cambridge Univ. Press, New York; Held, L.I., Jr., C.M. Duarte, and K. Derakhshanian 1986, Roux's Arch. Dev. Biol. 195: 145-157; Held, L.I., Jr., M.J. Grimson, and Z. Du 2004, Dros. Inf. Serv. 87; 76-78; Meier, P., A. Finch, and G. Evan 2000, Nature 407: 796-801; Minelli, A., 2003, *The Development of Animal Form: Ontogeny, Morphology, and Evolution*, Cambridge Univ. Press, New York; Wright, D.A., and P.A. Lawrence 1981, Dev. Biol. 85: 317-327.



A new paracentric inversion in the left arm of the third chromosome of *Drosophila ananassae.*

Singh, P., and B.N. Singh*. Genetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi-221005, India. *E- mail: bnsingh@bhu.ac.in.

Drosophila ananassae exhibits a high degree of chromosomal polymorphism. A total of 70 paracentric and 17 pericentric inversions and 13 translocations have been described in *D. ananassae*

(for references see the review by Singh, 1998a). However, only three paracentric inversions, namely, AL in 2L, DE in 3L and ET in 3R, are coextensive with the species. Chromosomal polymorphism has also been studied in Indian populations of *D. ananassae*, and there is evidence for geographic differentiation of inversion polymorphism (Singh, 1998b).

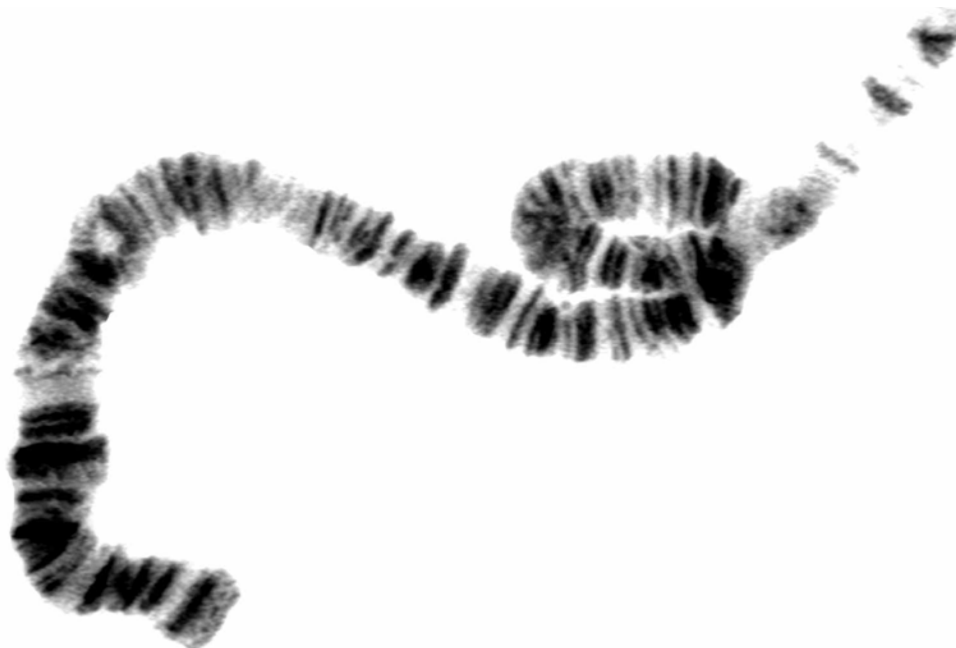


Figure 1. Microphotograph of a new inversion (heterozygous) in 3L of *Drosophila ananassae.*

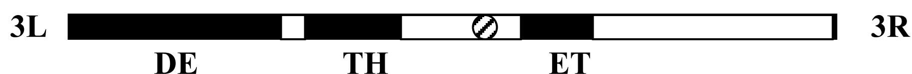
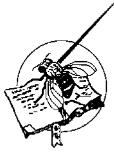


Figure 2. Location of DE, TH (new) and ET inversions in the third chromosome of *Drosophila ananassae.*

In this note we report a new paracentric inversion in the left arm of the third chromosome (3L), detected in F1 larvae resulting from a cross between wild caught male from Bhubneswar, Orissa, India in May, 2005 and standard homozygous female from GH-ST stock being maintained in our laboratory. According to the reference map of polytene chromosomes of *D. ananassae* constructed by Ray-Chaudhuri and Jha (1966), the inversion extends from 9A to 10E in the left arm of the third chromosome. The microphotograph of the new inversion (heterozygous) which has been named as theta (TH) is shown in Figure 1. Figure 2 depicts the location of delta (DE), and theta (TH) in 3L and eta (ET) in 3R. The theta (TH) inversion occupies nearly 24 percent region of 3L. The delta (DE) inversion occupies nearly 60 percent of 3L. The chromosome distance between delta and theta inversions is nearly 6 percent of the total length of 3L.

Acknowledgments: Financial support from CSIR, New Delhi in the form of Junior Research Fellowship (JRF) to PS is gratefully acknowledged.

References: Ray-Chaudhuri, S.P., and A.P. Jha 1966, Proc. Int. Cell Biol. Meet. Bombay: 352-383; Singh, B.N., 1998a, J. Expt. Zool. India 1: 3-13; Singh, B.N, 1998b, Ind. J. Exp. Biol. 36: 739-748.



Characterization of UDP-glycosyltransferase genes in *Drosophila pseudoobscura*.

Bourgeois, Gregory, Laurie S. Stevison, Daniel Ortiz-Barrientos, and Mohamed A.F. Noor. Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA; Corresponding author: E-mail: NOOR@DUKE.EDU.

Abstract

UDP-glycosyltransferases (UGTs) are enzymes used in olfaction that may play a role in mating discrimination in *Drosophila pseudoobscura*. Here, we investigate regions containing putative UGTs on the fourth chromosome of *D. pseudoobscura* by testing for expression and analyzing the sequences. We were able to confirm expression in two of three putative UGT regions, and we identified an intron and localized the end of transcription in one putative UGT.

Introduction

UDP-glycosyltransferases (UGT's) are biotransformation enzymes that have been implicated in olfaction and detoxification in vertebrates and invertebrates (Radominska-Pandya *et al*., 1999; Wang *et al*., 1999). UGTs transfer UDP-glucose, a sugar molecule, to hydrophobic substrates. This process makes them hydrophilic so that they can be eliminated by excretion. They are primarily expressed within olfactory organs in *Drosophila* (Wang *et al*., 1999).

Recently, Ortiz-Barrientos *et al*. (2004) mapped the genetic basis of a behavioral discrimination polymorphism in *D. pseudoobscura* and found putative UGT sequences on the fourth chromosome that may contribute to this phenotype. The study suggested that female mating discrimination may involve traits that respond to olfactory signals. One of the regions identified by Ortiz-Barrientos *et al*. (2004), *Coy-4*, is the region of interest in the present study. A sequence within this region bore similarity to five UGTs from *D. melanogaster*. We looked for all long open reading frames within this region and found three distinct putative protein-coding regions bearing amino acid sequence similarity to UGTs; we have dubbed these UGT region 1, UGT region 2, UGT region 3 (see Figure 1). Regions 2 and 3 were oriented in one direction while region 1 is oriented in the opposite direction. This study was designed to characterize each of these regions by first identifying RNA expression then sequencing cDNA to determine the location of transcript ends and possible introns in *D. pseudoobscura*.

Materials and Methods

The fly strains used were *D. pseudoobscura* Mather, CA 17 (collected 1997) and Flagstaff, AZ (collected 1993). We extracted DNA using the protocol of Gloor and Engels (1992) and RNA using the RNeasy Protect Mini and QIAshredder Kit (Qiagen).

The primers for each region were designed using the *D. pseudoobscura* genome sequence (Richards *et al*., 2005). The primers were used for reverse transcription of RNA, followed by PCR amplification of the resulting cDNA. The sizes of the cDNA were checked via agarose gel electrophoresis and compared with genomic DNA PCRs using the same primers. We selected products of the expected sizes and purified them using Qiaquick Gel Extraction Kit (Qiagen). We

then sequenced the products in both directions using ABI Big Dye version 3.1 Dye Terminator on an ABI 3100 DNA sequencer (Perkin-Elmer).

To identify the 3' end of one of the UGT transcripts, we used 3' RACE using the Ambion RLM-RACE Kit. A subset of these products was cloned using the TOPO TA Cloning Kit (Invitrogen). These cloned products were also sequenced and analyzed.

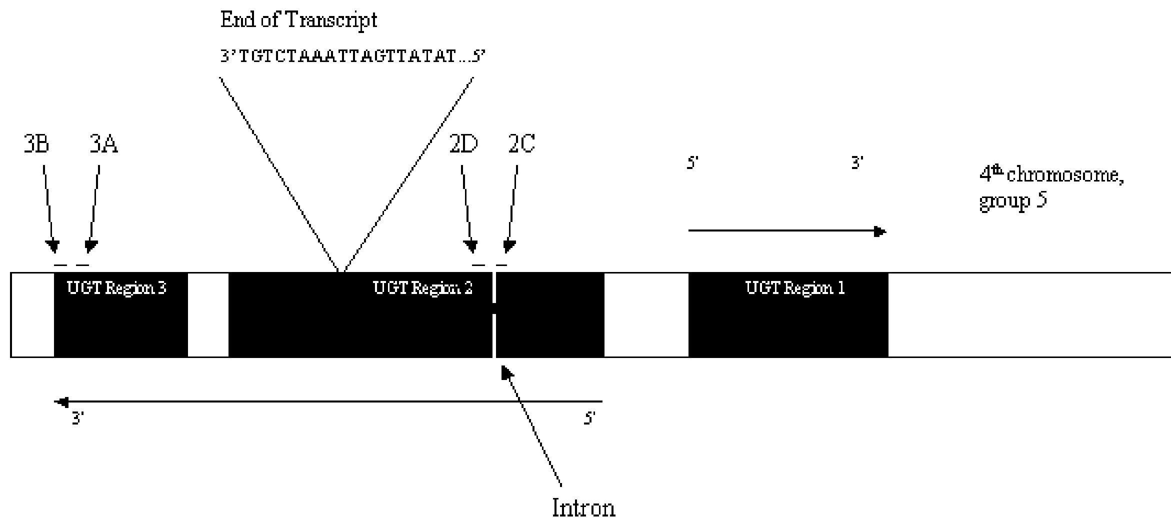


Figure 1: Three putative UGT regions are on the fourth chromosome. The primers used successfully for genomic DNA and cDNA analysis are shown in their approximate annealing areas. The 3' end of the UGT region 2 transcript is shown 38 bp after the putative polyadenylation signal. The putative direction of transcription of each region is also noted. UGT regions 2 and 3 are transcribed in the opposite direction of UGT region 1.

Results

UGT region 1

We successfully amplified and sequenced genomic DNA from within the UGT region 1 sequence. Primer combinations that successfully amplified the correct sequence (as defined by the closest BLAST match to the corresponding region of the *D. pseudoobscura* genome sequence) were 1A + 1B and 1C + 1D (see Table 1). However, despite repeated attempts using the same primers, no RT-PCRs successfully recovered the sequence of this region. The 3' RACE was also unsuccessful.

UGT region 2

We amplified and sequenced both genomic DNA and cDNA from region 2 using one primer pair, 2C + 2D. Using this pair, we were able to determine the presence of an intron in this region. The cDNA sequence bearing the spliced intron was submitted as partial CDS to GenBank (Accession AY880679). There were a couple of other primer pairs used that worked with genomic DNA only (2A + 2B and 2E + 2F). We also successfully performed a 3' RACE on this region to determine the end of the transcript using the primer RACE. The transcript ends with the sequence …TATATTGATTAAATCTGT. A putative polyadenylation signal (ACUAAA) was noted 38 bp before the 3' end of the transcript (see GenBank accession DQ058143).

UGT region 3

We were able to amplify and sequence both genomic DNA and mRNA from region 3 using one primer pair.

Table 1. List of primers used in study that were successful in amplifying *D. pseudoobscura* genome or cDNA.

UGT	Primer Abbreviation	Primer Sequence
1	1A	GGATACGGAGCACATGAGCAGAG
1	1B	GATGCTAGGGACACAAGACGCC
1	1C	ACGGTCATCTACTGGTCCGAATATGTT
1	1D	GACTTGTTGAATCACTTCTCTGTTTCAGGTT
2	2A	CGGCAAGCATGAGTGCCA
2	2B	ATTAATGCTGGGCACATAGGCGATT
2	2C	TGGAGTACCGCAACAAAAACTCCTAC
2	2D	CATTCCCCAAGAACTCCGCCATAT
2	2E	CCACGGATGGAGCTATACTCCT
2	2F	CGATCAAACCCAATGCTCTAAGGC
2	RACE	ATATGGCGGAGTTCTTGGGGAATG
3	3A	CATTGGTCACCGAGGGCTTTG
3	3B	GTGCGGCACCATGGTGG

Discussion

Our aim in this study was to confirm and characterize the three putative UGT regions from the *Coy-4* region. We were able to confirm transcription of UGT regions 2 and 3 through RT-PCR and further characterize UGT region 2 using 3'RACE. Despite several attempts using different primer pairs, we were unsuccessful in finding conclusive evidence for the transcription of UGT region 1.

UGT region 2 yielded the most conclusive evidence of formation of a UGT protein. We tried five different primer pairs, confirming genomic DNA with three of these and confirming expression through RT-PCR with one of these pairs and with 3' RACE. This was the only region that yielded successful 3' RACE results. Using sequences from the 3' RACE, we identified the end of the transcript. The putative polyadenylation signal at the end of the UGT region 2 was ACUAAA, a single-base variant of the typical polyadenylation signal AAUAAA, and has been found in the human genome (Beaudoing *et al.*, 2000).

UGT region 3 was less characterized than region 2, yet we accumulated data that confirmed its transcription. Through RT-PCR, we confirmed expression from this region and obtained sequence. We were also able to conclude that this region expresses a separate transcript from region 2 despite their close proximity. We initially hypothesized that UGT regions 2 and 3 could have been a hybrid protein. This was shown to be incorrect based on the 3' RACE sequences from region 2 and the sequence from region 3 which was outside of the end of the region 2 transcript. Further investigation in this region should attempt to characterize this transcript more using expression data from RT-PCR and 3' RACE.

We were able to confirm the sequence of UGT region 1 genomic DNA through PCR. All attempts at finding expression, including 3' RACE, were unsuccessful. More primers could be designed for this region to determine expression.

Some of the difficulty involved in this study involved the design of the primers because UGT sequences are very conserved. This was certainly a problem that was encountered in all regions, especially region 1. Through the use of more methods of looking at expression, like RT-PCR, 3' and 5' RACE, these regions may be able to be characterized further and perhaps eventually lend support of their use in mating discrimination by female *D. pseudoobscura.*

Acknowledgments: This work was funded by a Howard Hughes Medical Institute grant through the Undergraduate Biological Sciences Education Program to LSU (to G.P.B.) as well as National Science Foundation grants 0211007 and 0314552 (to M.A.F.N.). National Institutes of Health grant P20 RR16456 from the Biomedical Research Infrastructure Network Program of the

National Center for Research Resources provided support for L.S.S. as an undergraduate fellow. The authors thank L. Burk and A. Chang for technical support.

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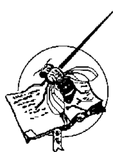


Study of *Drosophila* association with certain plant species in Islamabad, Pakistan.

Amin ud Din M[1,3,4], S.M.N. Khan[2], A. Bakhsh[1], R. Aleem[3], S. Haque[3], and A. Salam[4]. [1]Department of Biology, G. C. DG Khan, Pakistan; [2]Department of Zoology, AJK Univ., Muzafarabad; [3]Department of Biology, QAU, Islamabad, Pakistan; [4]Inst. of P&A Bio., BZU, Multan, Pakistan; For correspondence, email: amin1158@hotmail.com.

The contributions of *Drosophila* as a model system for understanding basic biological mechanisms are even more evident today than in the previous years. It's why a number of workers have been busy exploring the various ecological aspects of *Drosophila* fauna like association with different plant species, because understanding of the pattern in which different species of *Drosophila* are distributed across and within different vegetation types is necessary for accurate interpretation of their local ecology and biodiversity (Van Klinken and Walter, 2001).

According to various studies, the restricted geographical distributions of many native and some cosmopolitan species of *Drosophila* have suggested that they may have specific habitat preferences, such as traps placed among oak trees, which attract five times as many *Drosophila pseudoobscura* as do those in a meadow or shady ravine (Dobzhansky and Epling, 1944) and *D. occidentalis* comes in greater numbers to traps placed near a stream than to those in drier areas (Cooper and Dobzhansky, 1956). Similarly, Montgomery (1975) found 77% of the pictured-winged *Drosophila* species to be specific to a single host family of plants. Similarly *D. repleta* has found to be associated with Cactaceae and *D. subobscura* restricted to fruit bearing plants (Monclus, 1978). Van Klinken and Walter (2001) and O'Grady *et al*. (2003) have also studied the ecological association of various *Drosophila* species and discussed the possible reasons of such associations with plants.

In Pakistan, a similar study was conducted during the favorable season (September to April) to know the association of *Drosophila* species with plants in Islamabad. Collections of *Drosophila* species like *D. immigrans* (D1), *D. hydei* (D2), *D. takahashii* (D3), *D. leontia* (D4), *D. melanogaster* (D5), *D. malerkotliana* (D6), *D. Suzuki* (D7), and *D. nepalensis* (D8) were made by using ripe fermented fruits from the plants *Cassia fistula, Callistemon citrinus, Dodonaea viscose, Thevetia peruvirana, Eucalyptus lanceolatus, Bougainvalia spectabelis, Sambucus nigra, Punica grantum, Ficus carica, Psidium guava, Carissa carandas,* and *Lantana camara,* and information with respect to the plant species from which the flies were collected was recorded that is presented in Table 1.

The results indicated that the traps from two plant species (*Thevetia pervirana* and *Sambucus nigra*) remained without flies. Actually these plants are poisonous and insecticidal. So these are not suitable for *Drosophila* collection. Three plant species (*Bongainvilla spectabelis, Ficus carica,*

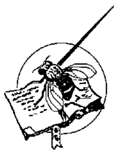
Lantana camara) attracted all the *Drosophila* species, because these are bushes and their fruits are sweet laxative. Among *Drosophila* species, *D. immigrans* and *D. hydei* are found associated with maximum plant species and proved as generalists, while all the six remaining *Drosophila* species are restricted with certain plants like most of the Hawaiian Drosophilidae species group as studied by O'Grady *et al.* (2003). The possible reasons for their specific association may be the chemical nature of plant parts or differences in mouth parts of *Drosophila* species.

Table 1. Association of *Drosophila* species with different plant species.

Plants		D1	D2	D3	D4	D5	D6	D7	D8
Botanica Names	Family								
Cassia fistula	Caesalpiniaceae	+	+	-	-	-	-	-	-
Callistemon citrinus	Myrtaceae	+	+	-	-	-	-	-	-
Dodonaea viscosa	Sanatha	+	+	-	-	-	-	-	-
Thevetia pervirana	Apocynaceae	-	-	-	-	-	-	-	-
Eucalyptus lanceolatus	Myrtaceae	+	-	-	+	-	-	-	-
Bongainvilla spectabelis	Nyctaginaceae	++	+	++	+++	+++	++	+	+
Sambucus nigra	Sambucaceae	-	-	-	-	-	-	-	-
Punica granatum	Puniaceae	+	+	-	-	+	+	+	+
Ficus carica	Moraceae	++	++	+	++	+++	++	+++	+++
Psiduim guava	Myrtaceae	+	+	-	-	-	-	++	++
Carissa carandas	Apocynaceae	+	+	-	+	+	-	-	-
Lantana camara	Verbenaceae	++	++	++	+++	++	++	++	++

(Symbol - stands for absence; + for 1-5 flies; ++ for 6-10 flies and +++ for more than 10 flies per trap in proper season of *Drosophila* species)

References: Van Klinken R.D., and G.H .Walter 2001, J. Trop. Eco. 17: 705-718; Cooper, D.M., and Th. Dobzhansky 1956, Ecology 37: 526-533; Dobzhansky, Th., and C. Epling 1944, Carnegie Inst. Wash. Publ. 554: 1-183. O'Grady, P.M., M.W.Y. Kam., F.C. Val, and W.D. Perreira 2003, Ann. Entomol. Soc. Am. 96(1): 12-38; Montgomery, S.L., 1975, Proc. Hawa. II Entomol. Soc. 22(1): 65-101; Monclus, M., 1978, Bol. R. Soc. Esp. Hist. Nat. Sect. Biol. 74: 197-214.



A new inversion in *Drosophila ananassae* population from Allahabad, Uttar Pradesh.

Singh, P., and B.N. Singh*. Genetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi-221005, India. * E-mail: bnsingh@bhu.ac.in.

Chromosomal polymorphism is very common in the genus *Drosophila*. There are intra- and interspecific variations with respect to the degree of chromosomal polymorphism. The commonest type of chromosomal variability is due to paracentric inversions. Chromosomal polymorphism has also been studied in certain *Drosophila* species found in India. The most noteworthy is *D. ananassae,* which is a cosmopolitan and domestic species. Previous studies on chromosomal polymorphism in *D. ananassae* from different places showed 71 paracentric and 17 pericentric inversions and 13 translocations (Singh, 1998; Singh and Singh, 2005). However, the three cosmopolitan inversions namely AL in 2L, DE in 3L and ET in 3R are of common occurrence in natural populations and have become coextensive with the species.

In the present communication, we report a new paracentric inversion named 'Iota' (IT) in the left arm of the third chromosome of *D. ananassae*. This new inversion was detected from a single F1

Figure 1. Microphotograph of a new inversion (heterozygous) in 3L of *D. ananassae.*

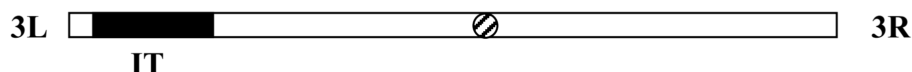


Figure 2. Location of IT inversion (new) in the third chromosome of *D. ananassae.*

larva from a naturally impregnated female collected from Mumfordganj, Allahabad (Uttar Pradesh) in September, 2005. The break points of the new inversion were established following the reference map of polytene chromosomes of *D. ananassae* constructed by Ray-Chaudhuri and Jha (1966). This inversion extends from 1C to 4A in 3L and occupies nearly 32 percent region of 3L, while DE and TH inversions cover nearly 60 percent and 24 percent region of 3L, respectively, (Singh and Singh, 2005). Microphotograph of the new inversion (heterozygous), which has been named IT is shown in Figure 1 and its location in the third chromosome is depicted in Figure 2. With this new inversion the total number of paracentric inversions in *D. ananassae* becomes 72.

Acknowledgments: Financial support from CSIR, New Delhi in the form of a Junior Research Fellowship (JRF) to PS is gratefully acknowledged.

References: Ray-Chaudhuri, S.P., and A.P. Jha, 1966, Proc. Int. Cell Biol. Meet. Bombay 352-383; Singh, B.N., 1998, J. Expt. Zool. India 1: 3-13; Singh, P., and B.N. Singh 2005, Dros. Inf. Serv. 88: 10-11.

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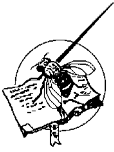

Drosophila desertorum in Big Bend National Park, Texas: The search for females.

Etges, William J. Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701. wetges@uark.edu.

Eight *Drosophila desertorum* males were unexpectedly collected in March, 2004, in the Chisos Basin at ca 5100 ft in Big Bend National Park, Texas. The significance of this collection was discussed (Etges and Heed, 2005) given the paucity of information for this species and because the only previous collection records were from almost 50 years ago in Hidalgo, Mexico, some 1100 km south of Big Bend National Park. The purpose of this report is to present collecting information from another attempt to recover both sexes of this species from Big Bend NP and provide a more comprehensive record of the drosophilid fauna of this area.

Table 1. *Drosophila* species collected in Big Bend National Park, March 19-25, 2005.

Locality	Number of individuals	Species
Chisos Basin Campground (3/20-3/23)		
	625	*D. longicornis* (est. number) *
	512	*D. hamatofila* (est. number)
	463	*D. pseudoobscura*
	9	*D. desertorum males*
	9	*D. ritae*
	1	*D. hydei*
Chisos Basin Hillside (3/20-3/23).		
	1201	*D. longicornis* (est. number)
	605	*D. hamatofila* (est. number)
	300	*D. pseudoobscura*
	76	*D. desertorum males*
Rio Grande Village Campground (3/23-3/25). A Mesquite Forest next to the Rio.		
	290	*D. longicornis* (est. number)
	145	*D. hamatofila* (est. number)
	981	*D. pseudoobscura*
	9	*D. hydei*
	1	*D. pegasa*
	2	*D. repleta* dark abdominal bands (1 female laid many eggs, none hatched)
	46	*D. mel/sim*
	15	*D. carbonaria*
	2	*D. cardini* group
	2	*D. funebris*
	1	*D. macrospina limpiensis* (?)
	23	*Notogramma* sp.

* est. number – Upon return to the lab, *D. longicornis* and *D. hamatofila* were initially counted and sorted into one group, and then separated by species. Some had subsequently died, so the original numbers were estimated by using the ratio of *longicornis/hamatofila* in the survivors.

+ I did not check for the presence of *D. azteca*

Flies were baited in the Chisos Basin camping area and on the hillside east of the entrance road into the Chisos Basin visitor's area, as well as a site adjacent to the campground in Rio Grande Village (Table 1). Several species of *Opuntia* were abundant in these areas, but no fermenting pads were found. The *Notogramma* sp. were also collected in 2004 from the same site and were identified

as *N. purpuratum* Cole (Diptera: Otitidae), but they were reared from a fermenting Turk's head cactus, *Echinocactus horizonthalonius* (Evans, 1998).

The three most common species in the Chisos Basin were *D. longicornis*, *D. hamatofila*, and *D. pseudoobscura* (Table 1). Many more *D. desertorum* were recovered from the Chisos Basin than in 2004, but no females were identified after several weeks of intensive searching in the laboratory. Male *D. desertorum* are somewhat distinctive, being larger than *D. longicornis* and *D. hamatofila* with lemon yellow testes. The distinctive shape of the aedeagus (Vilelam 1983) allowed easy identification of males (Etges and Heed, 2005), but females are either completely cryptic or were absent. Male *D. desertorum* were stored in food vials with other repleta group females from the Chisos collection in the hopes that *D. desertorum* females were present and morphologically identical to female *D. longicornis* or *D. hamatofila*. In some vials, small amounts of crushed *Opuntia* cactus tissue were added, a requirement for oviposition by some species (Etges, unpubl. data). All F_1 progeny from these "crosses" were checked for the presence of *D. desertorum* males. None were observed. All surviving wild-caught *D. desertorum* males were stored in ethanol at -20º C; these samples are available upon request.

It is unlikely that no *D. desertorum* females were collected, so perhaps conditions for oviposition were unsuitable. Females may not be attracted to banana bait. The assumption that this species is cactophilic may also be incorrect. Given the large numbers of collections made of repleta group species in the last 50 years (Patterson and Stone, 1952; Oliveira *et al*., 2005), the number of rarely collected species known only from one or a few collections (Wasserman, 1992), and the difficulty with which some species can be cultured in the lab (like *D. desertorum*), it is very likely that there are more *D. repleta* group species waiting to be discovered.

Acknowledgments: I thank the Department of Biological Sciences at the University of Arkansas, Fayetteville, for providing transportation, students in BIOL 5853 Field Ecology for discussion, and the National Park Service for providing a collecting permit.

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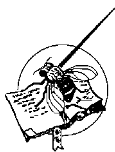


Incidence of ovarian abnormalities in *Drosophila melanogaster*.

Bussel, Irvin, Nazgol Afsarpour, Mahtab Jafari, and Michael R. Rose. Department of Ecology and Evolutionary Biology, University of California, Irvine.

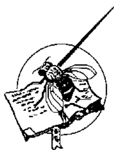
The fruit fly *Drosophila melanogaster* has been used in the study of cancer, especially those mutants in which neoplastic tumors occur at a high rate. More than 60 tumor suppressor genes have been found to elicit tumor growth *in vivo* in *Drosophila* tissues, when inactivated. Although cancer develops in these mutants, cancer has not been reported to occur during normal *Drosophila* aging. Consequently, it has been assumed that cancer is not a normal cause of death, or a normal phenotype of aging, in fruit flies. This is strikingly different from what we find in aging mammals. Mammals show an exponential increase in the incidence of cancer with age. In this project, we studied aging

Drosophila ovaries for the incidence of cancer without the effects of carcinogens. Although oocyte production can continue throughout life, daily egg production declines starting in mid-life. The mechanistic reasons for this decline are largely unknown. However, age-related diseases, like ovarian cancer, could be the cause. *Drosophila* ovaries have some of the requirements for age-related incidence of cancer, most importantly cell proliferation during adulthood, unlike most adult tissues in *Drosophila*. We evaluated two cohorts of laboratory fruit flies. Samples of females at specific intervals were dissected and examined for excess cell proliferation as well as any ovarian abnormalities.

Table 1. Represents incidences of ovarian abnormalities in IV Stock. Data is broken down into age-specific occurrences of finding flies with one ovary or no ovaries. Also, each occurrence is established according to the percentage of incidences for that day.

	Incidence			Percentage of Incidence	
Age	1 Ovary	No Ovaries	Total	1 Ovary	No Ovaries
6	4	0	50	0.08	0
12	4	1	48	0.083333333	0.020833333
18	4	1	49	0.081632653	0.020408163
24	3	3	47	0.063829787	0.063829787
30	3	1	48	0.0625	0.020833333
36	6	0	50	0.12	0
42	1	1	50	0.02	0.02
48	1	1	35	0.028571429	0.028571429
54	0	1	20	0	0.05
Total	26 = 6.55%	9 = 2.27%	397		

In an explorative assay of a derived postponed senescence population (CO stock), various ovarian pathologies were discovered. There was a single occurrence (0.33%) of tumorous ovaries out of 300 sets of ovaries successfully dissected. This indicates that cancer does in fact affect normal aging *Drosophila,* albeit only rarely. Ovarian cysts were also found in 2 other sets (0.66%) of ovaries. Another cohort of standard lab flies (IV stock) was also assayed by dissection. Out of 397 sets of dissected ovaries, there were 26 instances (6.55%) of females lacking an ovary as well as 9 instances (2.27%) of females entirely lacking ovaries. The results from this limited sample shed light on ovarian biology in aging *Drosophila*. Since incidences of ovarian cancer and other gross abnormalities did not increase with age, these ovarian pathologies cannot be used as an explanation of decreased fecundity during fruit fly aging.



Identification of the molecular lesions in alleles of the *Drosophila* Abelson tyrosine kinase.

Smith, Jessica A., and Eric C. Liebl. Department of Biology, Denison University, Granville, OH 43023, USA

Introduction

First isolated in 1987 (Henkemeyer *et al.,* 1987), *Drosophila* geneticists have been using the Abl^1, Abl^2 and Abl^4 alleles in a wide variety of genetic studies for over eighteen years. However, the

molecular lesions responsible for these alleles have never been, to our knowledge, identified. We have therefore sequenced all exons and intron/exon boundaries in each of these *Abl* alleles.

Materials and Methods

The *Abl*^{1}, *Abl*^{2} and *Abl*^{4} alleles, balanced over the *TM6,B, Tb* balancer chromosome, were crossed to *Df(3L)st100.62* (which removes all *Abl* exons; Belote *et al.,* 1990) also balanced over *TM6,B, Tb.* *Tb*$^+$ pupae from these crosses were collected, frozen and genomic DNA was isolated. Using a series of eleven forward and reverse primer pairs, all eight of the *Abl* coding exons, along with their respective exon/intron boundaries were amplified using standard Taq polymerase (Promega, Madison, WI). Primer sequences are available upon request. Amplified fragments were isolated over spin columns (Qiagen, Valencia, CA), and both strands were sequenced at the Ohio State University Plant-Microbe Genomics Sequencing Facility (Columbus, OH). Lesions identified were confirmed by independently amplifying and sequencing those specific fragments at least twice. Sequences were compared to the wild-type *Abl* sequence (CG4032) using the Sequencher 4.2.2 alignment program (Gene Codes, Ann Arbor, MI).

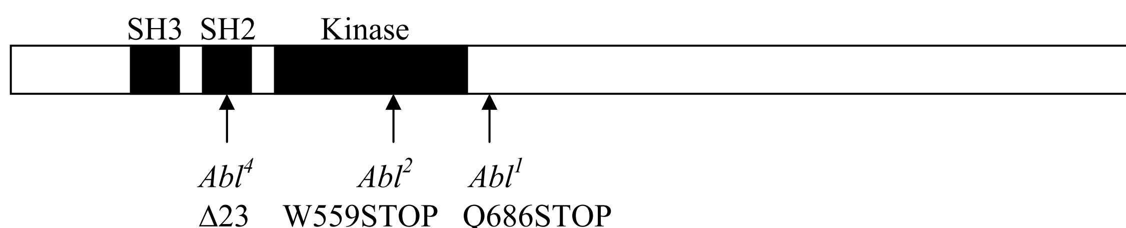


Figure 1. The Abl tyrosine kinase is represented schematically with the N-terminus to the left. The location and identity of the mutations specifically responsible for the *Abl*^{1}, *Abl*^{2} and *Abl*^{4} alleles are shown.

Results and Discussion

The Abl tyrosine kinase has several conserved domains, including an SH3 domain, an SH2 domain and a kinase domain (Figure 1; Henkemeyer *et al.,* 1988; Hoffmann, 1991).

The *Abl*^{1} allele is the result of a C to T transition, changing a CAG codon to TAG and resulting in the Q686STOP nonsense mutation (Figure 1). Conceptual translation of the *Abl*^{1} open reading frame results in a protein of 73.3 kD molecular weight. This is in good agreement with the reported molecular weight of 65-70 kD for the Abl^1 protein detected by immuno-blotting with polyclonal antibodies raised against the Abl SH3/SH2/kinase domains (Bennett *et al.,* 1992). This is also in agreement with the Abl^1 protein having kinase activity (Henkemeyer *et al*., 1990) as the Q686STOP lesion occurs C-terminal to the kinase domain (Figure 1).

The *Abl*^{2} allele is the result of a G to A transition, changing a TGG codon to TGA and resulting in the W559STOP nonsense mutation (Figure 1). Conceptual translation of the *Abl*^{2} open reading frame results in a protein of 59.8 kD molecular weight. This is in good agreement with the reported molecular weight of 51-53 kD for the Abl^2 protein detected by immuno-blotting with polyclonal antibodies raised against the Abl SH3/SH2/kinase domains (Bennett *et al.,* 1992). While

the Abl[2] protein does not contain an intact kinase domain, it does contain intact SH3 and SH2 domains (Figure 1).

The *Abl*[4] allele is the result of a 23 base-pair deletion at the 3' end of exon 2 that extends into the intron between exons 2 and 3. Specifically the sequence 5'TTTGAGGTGCGTAGATGG GATCT3' is deleted, where the underlined sequence compromises part or all of codons 291-293 at the 3' end of exon 2. While we have not undertaken any RNA analysis to investigate whether or how the *Abl*[4] allele may be spliced (due to the loss of the splice donor at the 5' end of the intron), conceptual translation of Abl residues 1-290 results in a protein of 29.4 kD molecular weight. This is in good agreement with the reported molecular weight of 25-35 kD for the Abl[4] protein detected by immuno-blotting with polyclonal antibodies raised against the Abl SH3/SH2/kinase domains (Bennett *et al.,* 1992). Thus the Abl[4] protein likely contains an intact SH3 domain (Figure 1).

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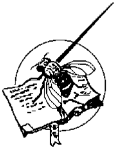

Some aspects of molting and metamorphosis in *Drosophila pavani* larvae.

Muñoz, Héctor, Daniel Frías[1], and Raúl Godoy-Herrera. Programa de Genética Humana, ICBM, Facultad de Medicina, Universidad de Chile. [1]Instituto de Entomología, Universidad Metropolitana de Ciencias de la Educación. *Correspondence*: R. Godoy-Herrera, Programa de Genética Humana, ICBM, Fac. Medicina, Universidad de Chile, Independencia 1027, Casilla 70061, Santiago – 7, Chile; e-mail: rgodoy@med.uchile.cl.

Development in multicellular animals involves regulation of size and cell number (Nijhout, 2003). In holometabolos insects pupariation and adult size depend on growth of the larva and the progression in size from one larval stage to next. On the other hand, species may differ in body size for environmental and genetic reasons. It is of interest to know how far intra- and interspecific differences cause variation in developmental patterns that produce differences in duration of larval period and adult body size. Thus, comparative investigations on molting and metamorphosis in species of *Drosophila* that differ in duration of larval period may contribute to understanding evolutionary history of the genus and knowing how in animals adult body size and the sizes of body parts are regulated. Here we report on larval molting and metamorphosis of *Drosophila pavani*, a neotropical Chilean endemic species belonging to the *Mesophragmatica* group (Brncic and Koref-Santibañez, 1957). We related molting and puparium formation with larval age, and larval body weight and size of *D. pavani*.

Subjects: Adult flies of *D. pavani* were collected in Chillán, 420 Km south of Santiago. The collections were made in a humid site in an orchard surrounded by fruit plantations and with native shrubs and grasses. About ten flies originated the Chillán strain we used.

Larval collections: Inseminated females of the Chillán strain aged 12 days were allowed to deposit eggs for 3 h on a layer of live baker's yeast. Batches of eggs of the strain were incubated at

18 ºC. Larvae eclose around 48 h after the eggs are laid. Larvae were collected at successive 24 h intervals after emergence up to 192 h of development (see Table 1).

Larval body size: Fifty larvae of each age (Table 1) were random collected and deposited in hot water (70 ºC). After that, they were individually collected and the length of each larva was measured.

Larval body weight: Four independent random samples, each sample of 50 larvae of each age (Table 1), were collected and the weight of each sample was recorded.

Pupariation age: Four independent random samples, each sample of 100 larvae of 120 h of age, were deposited in a Petri dish filled with 1 % agar without food. The Petri dishes were incubated at 18 ºC. Every day, early morning and late afternoon, during 10 successive days the Petri dishes were examined searched for pupae. The procedure was repeated, but now with larvae of 144, 168 and 192 h of development.

Table 1. Larval body size, weight and age at which larvae of *D. pavani* (the Chillán strain), form puparium. For comparison adult mean weight of each sex is also shown.

Larval age (h)	Larval body size (mm)	Larval weightl (mg)	Pupariation larval age (h)	Adult weight (mg)	
				Female	Male
24	0.89 ± 0.57	0.34 ± 0.10			
48	1.26 ± 0.72	0.28 ± 0.08			
72	1.40 ± 0.51	0.82 ± 0.03			
96	1.76 ± 0.06	2.06 ± 0.14			
120	2.84 ± 0.07	2.94 ± 0.06	0/400		
144	3.05 ± 0.83	9.61 ± 0.73	0/400		
168	2.94 ± 0.37	10.30 ± 1.03	6/400[1]		
192	3.82 ± 0.21	39.90 ± 1.07	61/400[2]	26.09 ± 0.15	18.88 ± 0.56

(1) 6 pupae found three days after began starvation
(2) 61 pupae found three days after began starvation

Results

Table 1 shows that larval body size of the Chillán strain of *D. pavani* tends to increase lineally between 24 to 192 h of development. In contrast, larval body weight tends to grow exponentially between those same larval ages. Table 1 also shows that at 168 h of development when some larvae have reached a body size of 2.94 ± 0.37 mm and a body weight of 10.30 ± 1.03 mg may form puparium.

Discussion

Larvae of the Chillán strain of *D. pavani* weigh about 1 mg at the beginning of the period. After 8 – 9 days of continuous feeding at 19 ºC, when larval period is finishing, they attain live

weights of 40 mg. However, the first indication that pupation is about to begin occurs when the larvae having 168 h of development reach a body size of 3.0 mm and a weight of 10 mg. Thus, for *D. pavani* larvae, this size and this weight should be considered the critical morphological and physiological measures at which the decision to pupate is made.

In *D. melanogaster* larvae, ecdysone and 20 hydroxyecdysone control the molting process, while juvenile hormone allows growth of the larva until it attains a proper size for metamorphosis (Caldwell *et al.*, 2005). A number of different loci control the production of all these hormones (Hao *et al.*, 2001). On the other hand, duration of larval period of *D. pavani* is about twice that of *D. melanogaster*. This raises the problem of timing of release of the hormones in species of *Drosophila* which differ in duration of the larval period. Our data suggest that at between 96 and 120 h of larval age there is an important increase in body size and weight (Table 1). A new increase in larval size and weight is produced after 120 h. It is possible that these changes in body size and weight reflect changes in the balance between the hormones responsible of molting and metamorphosis in *D. pavani*. Comparisons between *Drosophila* species that differ in the timing of developmental events could be of importance to understand the role of heterochrony in the evolutionary history of animals species that belong to the same phyletic unit.

Acknowledgment: Supported by FONDECYT 1020130.

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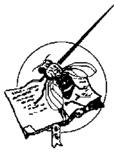

cDNA clones and expressed sequenced tag (EST) analysis of *D. ananassae.*

Oguma, Y.,[1] K. Sawamura,[1] H. Sato,[2] and M. Matsuda[2]. [1]Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan; [2]School of Medicine, Kyorin University, Mitaka, Tokyo 181-8611, Japan.

Introduction

D. ananassae has been an important model organism since classic studies in the 1930s (Moriwaki, 1934; Kaufmann, 1936; Kikkawa, 1938). In addition to the accumulation of decades of genetic information (Tobari, 1993), the sequencing of the whole genome of *D. ananassae* has been determined recently by the FlyBase and Genome Sequencing Centers. Here we report ESTs of this species. All the sequences reported here will be deposited to the DDBJ/EMBL/GenBank data libraries. cDNA clones sequenced in the present analysis will be available at the Kyoto *Drosophila* Genetic Resource Center in the near future.

Materials and Methods

mRNAs were extracted from adult female and male whole bodies of the AABBg1 strain of *D. ananassae*. This is an inbred strain derived from a strain donated to D. Moriwaki from the University of Texas in 1951. The flies were originally caught in Hawaii in 1945. Although inversion chromosomes are highly polymorphic in wild populations and also in the original strain, the chromosome constitution of the AABBg1 strain is currently fixed as *(2L+2R) ST/ (2L+2R) ST; In(3L)A/ In(3L)A*. The procedure of establishing this inbred strain is described in Tobari and Kojima

(1967). After maintenance as a small mass culture for 35 years, it was inbred by single pair matings for two generations in 2003. The AABBg1 strain has also been used for constructing the BAC library and in its end sequencing at RIKEN and in the sequencing of the whole genome at Agencourt. This strain is available from Kyorin University (matsudam@kyorin-u.ac.jp or http://kyotofly.kit.jp/stocks/).

The λ ZAP II library of *D. ananassae* cDNAs was converted to a plasmid library by *in vivo* excision and the transformants were spread on the agar plates. Colonies were randomly selected, cultivated over night, and template DNAs for the sequencing prepared by Rolling Cycle Amplification (RCA). They were sequenced by using an RV-M primer. After trimming the vector sequences and low quality bases from the DNA sequences, clustering analysis was performed using Paracel Transcript Assembler (Paracel Inc.).

Results

In total we obtained 3,452 reads (sequence data) in which the mean of good quality (Phred score ≥ 15) base length per read was 473.8. 2,957 of those reads had more than 300 bases with a Phred score of ≥ 15. After the filtering process, 3,136 valid reads were obtained with a mean read length of 380.6 bases. By clustering analysis of the 3,136 reads, we obtained 368 clusters consisting of 1,482 reads (the remaining 1,654 reads are single-read contigs). By assembly analysis of the reads included in the same clusters, we obtained 364 contigs consisting of 1,397 reads (the remaining 85 reads are single-read contigs of the same cluster). The cDNA clones of *D. ananassae* and the EST analysis information will be important resources for the *Drosophila* community.

Acknowledgments: The present research was supported by a Grant-in-Aid for Scientific Research on Priority Areas to Y. Oguma from the Ministry of Education, Science, Sports, Culture and Technology of Japan (MEXT). M. Matsuda also thanks the National Bio-Resource Project of MEXT for supporting him.

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Drosophilidae of the Brazilian Savanna, the forgotten ecosystem.

Chaves, Natalia Barbi, and Rosana Tidon. Instituto de Biologia, Universidade de Brasília. Address: CP 04457 CEP 70904-970, Brasília, DF – BRAZIL. Corresponding author: rotidon@unb.br

The Brazilian Savanna, locally known as Cerrado, is a complex of seasonal savannas that covers most of inland Brazil and a few small contiguous areas in Bolivia and Paraguay. Containing approximately 2 million km^2, it is the second largest South American biome, exceeded only by the Amazon rainforest. This vegetation typically consists of a savanna of extremely variable structure on the well-drained interfluves, with gallery forests or other moist vegetations following the watercourses (Ratter *et al*., 1997). Climate is tropical dry winter Aw in the Koeppen system in 95%

of the biome, changing to cooler Cw at higher altitudes, and precipitation is highly seasonal, characterized by a well-defined dry season from May to September (Franco, 2002). The Cerrado is one of the 25 hotspots of the world, which are defined as areas of great endemism and less than 30% of remaining natural vegetation (Myers *et al.*, 2000). As this region is arguably under great threat, it was labeled in an article in Nature as "the forgotten ecosystem" (Marris, 2005).

Although tropical natural populations of drosophilid flies have been the object of ecological studies, the diversity and distribution of most of the neotropical drosophilid species is still poorly known when compared to the knowledge about the paleartic species of this group. In a review of Neotropical *Drosophila*, Val *et al.* (1981) concluded that "*there is a surprising lack of information on the environmental situation in which the species are found*." Medeiros and Klaczko (2004) argue that the majority of the 53 unidentified drosophilid species that they recently found in one of the best sampled regions of Brazil are in fact undescribed, suggesting that the statement of Val still holds true for tropical South America.

Here we present the list of drosophilids currently identified in the Brazilian savanna, based on an extensive review of published records of drosophilids, and added unpublished data referring to collections made by one of us (R. Tidon) between 1997 and 2004.

We recorded a total of 81 species of drosophilids (Table 1). Twelve of them are introduced in South America (*Drosophila ananassae*, *D. busckii*, *D. hydei, D. immigrans*, *D. kikkawai*, *D. malerkotliana*, *D. melanogaster*, *D. repleta, D. simulans*, *D. virilis*, *Scaptodrosophila latifasciaeformis* and *Zaprionus indianus*). Most of these exotic species are sinantropic, and have colonized natural and urban habitats (Ferreira and Tidon, 2005), modifying the original drosophilid fauna of the region. In terms of the native fauna, they were found

Table 1. Drosophilids recorded in the Brazilian Savanna. Names preceded by an asterisk (*) refer to exotic species in the South America.

Genus *Drosophila*
- Subgenus *Dorsilopha*
  - * *D. busckii* Coquillet
- Subgenus Drosophila
  - *annulimana* group
    - *D. annulimana* Duda
    - *D. aragua* Vilela & Pereira
    - *D. arapuan* Cunha & Pavan
    - *D. ararama* Pavan & Cunha
    - *D. arauna* Pavan & Nacrur
  - *aureata* group
    - *D. aureata* Wheeler
  - *bromeliae* group
    - *D. bromelioides* Pavan & Cunha
  - *calloptera* group
    - *D. atrata* Burla & Pavan
    - *D. schildi* Malloch
  - *canalinea* group
    - *D. canalinea* Patterson & Mainland
  - *cardini* group
    - *D. cardini* Sturtevant
    - *D. cardinoides* Dobzanhsky & Pavan
    - *D. neocardini* Streisinger
    - *D. polymorpha* Dobzanhsky & Pavan
  - *coffeata* group
    - *D. fuscolineata* Duda
  - *dreyfusi* group
    - *D. dreyfusi* Dobzanhsky & Pavan
  - *guarani* group.
    - *D. griseolineata* Duda
    - *D. guaraja* King
    - *D. guaru* Dobzanhsky & Pavan
    - *D. maculifrons* Duda
    - *D. ornatifrons* Duda
  - *immigrans group*
    - * *D. immigrans* Sturtevant
  - *pallidipennis* group
    - *D. pallidipennis* Dobzanhsky & Pavan
  - *repleta* group
    - *D. antonietae* Tidon-Sklorz & Sene
    - *D. borborema* Vilela & Sene
    - *D. buzzatii* Patterson & Wheeler
    - *D. coroica* Wasserman
    - *D. gouveai* Tidon-Sklorz & Sene
    - * *D. hydei* Sturtevant
    - *D. mercatorum* Patterson & Wheeler
    - *D. meridionalis* Wasserman
    - *D. nigricruria* Patterson & Mainland
    - *D. onca* Dobzanhsky & Pavan
    - *D. paranaensis* Barros
    - *D. pseudorepleta* Vilela & Bachli
    - * *D. repleta* Wollaston
    - *D. rosinae* Vilela

Table 1. Continued

D. serido Vilela & Sene
D. seriema Tidon-Sklorz & Sene
D. zotti Vilela
tripunctata group
D. albirostris Sturtevant
D. bandeirantorum Dobzhansky & Pavan
D. bifilum Frota-Pessoa
D. medioimpressa Frota-Pessoa
D. mediopicta Frota-Pessoa
D. mediopunctata Dobzhansky & Pavan
D. mediostriata Duda
D. mesostigma Frota-Pessoa
D. nappae Vilela *et al.*
D. neoelliptica Pavan & Magalhães
D. neoguaramuru Frydenberg
D. paraguayensis Duda
D. paramediostriata Townsend & Wheeler
D. trapeza Heed & Wheeler
D. unipunctata Patterson & Mainland
virilis group
* *D. virilis* Sturtevant
ungrouped species
D. caponei Pavan & Cunha
D. impudica Duda
Subgenus *Sophophora*
melanogaster group
* *D. ananassae* Doleschall
* *D. kikkawai* Burla
* *D. malerkotliana* Parshad & Paika
* *D. melanogaster* Meigen
* *D. simulans* Sturtevant
saltans group
D. austrosaltans Spassky
D. neocordata Magalhães
D. prosaltans Duda
D. sturtevanti Duda
willistoni group
D. bocainensis Pavan & Cunha
D. bocainoides Carson
D. capricorni Dobzhansky & Pavan
D. equinoxialis Dobzhansky
D. fumipennis Duda
D. nebulosa Sturtevant
D. parabocainensis Carson
D. paulistorum Dobzhansky & Pavan
D. tropicalis Burla & Cunha
D. willistoni Sturtevant
Subgenus *Siphlodora*
D. flexa Loew
Genus *Scaptodrosophila*
* *S. latifasciaeformis* (Duda)
Genus *Zaprionus*
* *Z. indianus* Gupta

in all vegetation types of the biome. However, most of the 69 neotropical species presented a narrow distribution, being recorded in only a few different habitats. Details about their geographic distribution and occurrence in the different types of vegetation of the Brazilian savanna will be presented elsewhere.

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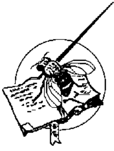

First evidence of *Drosophila malerkotliana* in the extreme South of Brazil (Porto Alegre, Rio Grande do Sul, Brazil).

Garcia, Ana C.L.,[1] Marco S. Gottschalk[2], Grazia F. Audino[1], Cláudia Rohde[1], Victor H. Valiati[3], and Vera L.S. Valente[1,2]. [1]Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Caixa Postal 15053, Porto Alegre- RS, 91501-970, Brazil; [2]Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências, Universidade Federal do Rio Grande do Sul; [3]Ciências da Saúde, Universidade do Vale do Rio dos Sinos, Caixa Postal 275, São Leopoldo- RS, 93022-000, Brazil.

One of the most important investigations in ecology is the study of invader species. Research into such phenomenon leads to an understanding of why invasions succeed or fail and provides an opportunity to investigate the interaction mechanisms between the new invader species and the native populations. Biological invasions frequently cause substantial disturbance to ecosystems as well as serious socio-economic impacts (Liebhold *et al*., 1995).

The introduction of the exotic *Drosophila* species in the New World is not a rare event. Some drosophilids have made successful invasions - a good example is the *Drosophila subobscura* invasions in South and North America. This originally Paleartic species was found for the first time in the New World in Chile in 1978 (Brncic and Budnik, 1980) and later in Argentina (López, 1985) and Uruguay (Goñi and Martínez, 1995). Twelve years after its first appearance it has spread all along the Chilean coast and has displaced most of the native *D. pseudoobscura* along the entire North American Pacific Coast, evolving at an alarmingly rapid rate (Brncic, 1995). Another example is the *Zaprionus indianus* which was first detected in Brazil in 1999 (Vilela, 1999; Tidon *et al*., 2003). Since then, this Afrotropical drosophilid has expanded its area of distribution very quickly and has now reached pest status in fig crops in the Brazilian State of São Paulo (Vilela *et al*., 2001). *Zaprionus indianus* represents one of the most successful colonizing species of this genus (Chassagnard and Tsacas, 1993) and has been found very frequently in many Brazilian States (Castro and Valente, 2001; De Toni *et al*., 2001; Tidon *et al*., 2003) and in Uruguay (Goñi *et al*., 2001).

The *Drosophila malerkotliana* fly is a relatively recent arrival in South America. It was first registered in Brazil in 1976 (Val and Sene, 1980) and nowadays is very common in that area (Sene *et al*., 1980; De Toni and Hofmann, 1995; Tidon *et al*., 2003). This species has now expanded to most South American countries probably due to more favorable climatic conditions. It also seems to be expanding in the North American continent as recent observations suggest that this fly is now established in the suburbs of Athens (Georgia) in Southern USA, at a latitude 33°57′N (Medeiros *et al*., 2004). Previously it had not been detected beyond Tallahassee (Florida) 272 miles (443 km) farther south (Birdsley, 2003).

In Brazil, *D. malerkotliana* is found in gallery forests, in forests enclaves in the *caatingas* (semi-desert) regions, in *restingas* (sea coastal areas with creeper vegetation), *cerrados* (scrub forest lands), dunes and grasslands (strand vegetation) (Val and Sene, 1980; Bizzo and Sene, 1982; Tidon *et al*., 2003). Its distribution pattern is similar to that observed for other introduced species (Val and Sene, 1980; Tidon *et al*., 2003). It should be pointed out that *D. malerkotliana* could be a good biological indicator, because this species is found more frequently in disturbed forest habitats than in undisturbed environments such as native forests (Martins, 1989, 2001; M. S. Gottschalk, unpublished data).

In the autumn of 2004, during various seasonal collections of drosophilids in different parts of the city of Porto Alegre, Rio Grande do Sul, Brazil (30°03′S), *D. malerkotliana* was captured for the

first time in this latitude. Only one male specimen emerged in a *Passiflora sp.* fruit among many fermented fruits that had been collected. This male was identified as of the subspecies *malerkotliana* from observations of the internal and external genitalia and abdominal pattern pigmentation as described by Bock (1971) and Val and Sene (1980). Observations that *D. malerkotliana* is exploring one natural resource in the urban environment could be evidence that this fly is still seeking to extend its geographical distribution throughout the south region. This new register of its presence here represents a southern record for this species, since the previous geographical limit was *Lagoa da Conceição,* Santa Catarina Island (27°42`S) (De Toni and Hofmann, 1995).

It is worth mentioning that *D. malerkotliana* is known to be generalist, (*i.e*., it utilizes diverse food resources), and this assists it to invade new locations. This species is typically tropical and its geographical distribution is limited by cold temperatures in wintertime. Some data suggest that its fertility is reduced at temperatures below 20°C and that development ceases entirely at 15°C (Medeiros *et al*., 2004).

Drosophila malerkotliana was registered in the city of Porto Alegre in the month of April when the average urban temperatures there, (according to the 8th Meteorological District of the Ministry of Agriculture), range from 11 to 20°C. During the coldest months of the year (June, July, and August) the city temperatures have traditionally oscillated between –3°C and 18°C (Hasenack and Ferraro, 1989). However, in recent years Menegat *et al.* (1999) found that the temperature in Porto Alegre is changing, because in the period between 1968 to 1997 the mean minimal annual temperature increased half of one percent - from 15 to 15.5ºC. This could facilitate the expansion of this invader species to reach points still farther south in South Brazil.

This situation could be related to the existence of heat islands in Porto Alegre because of growing urbanization (Danni, 1980). These thermal islands probably provide warmer refuges for urban populations of insects during periods of unfavorable weather. This could explain the already existing *Z. indianus* invasive species colonization and expansion (Machado *et al.*, 2005) and our recent detection of *D. malerkotliana*.

We do not know exactly why *D. malerkotliana* has this capability to expand its distribution in both the North and South regions. It may be that its generalist food habits combined with an ability to withstand different temperatures and the absence of natural enemies sufficiently explains this phenomenon. However, continued study of the natural populations of this species is required for the complete understanding of its colonization dynamic and expansion mechanisms throughout the southern region.

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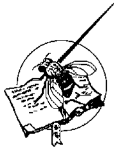

Chromosomal polymorphism in natural populations of *Drosophila willistoni* from Eastern Mexico. Preliminary report.

Salceda, Víctor M. Departamento de Biología, Instituto Nacional de Investigaciones Nucleares. Salazar, Edo. de México. México. Apartado Postal 111, Lerma, Edo de México. C.P. 52000. MEXICO. vmss@nuclear.inin.mx.

One of the most widely distributed and abundant species of *Drosophila* in America is *D. willistoni.* Geographically, it has been found from Southern Florida and Central Mexico south to Buenos Aires, Argentina (Townsend, 1952). Similar to other natural populations of *Drosophila,* it presents a great degree of polymorphism in the gene arrangements of its chromosomes. According to Valente and Morales (1985), *D. willistoni* could be considered as the most polymorphic of all, with up to 70 different known inversions evenly distributed in the complete genome as shown in its polytene chromosomes. In Mexico this species has been poorly studied.

In a search for appropriate areas in which to study the inversion polymorphism of the species, we performed five one-day collections along the Eastern Coast of Mexico, namely north to south in the transect: Poza Rica, Arroyo Agrio, Comalcalco, Teapa, and Kolemjaa plus a single one on the Pacific Coast, Tepic. In doing the collections we used fermenting bananas placed into 5-10 plastic buckets as baits, and after waiting several minutes to allow the flies to come, rounds of collections were started using for this purpose an entomological net. From the trapped flies we separate those females belonging to the *D. willistoni* species, carried them in vials with fresh food to the laboratory in Mexico City, and once there placed each captured female individually in a half pint bottle with fresh medium. We let them oviposit and incubate at 25ºC for a week. When larvae started to crawl out of the food and using the regular aceto - orcein staining technique, a single larva from each isofemale line was dissected, a salivary gland smear was prepared, and its chromosomes analyzed through the light microscope. The different inversions present in each collection were recorded using as a guide the chromosome maps of this species done by Dobzhansky (1950) and da Cunha *et al.* (1950). The results of our observations are shown in Table 1.

The number of flies captured in most of the samples was too small for the determination of the relative frequencies of the several inversions found. Nevertheless, the gathered data proved to be useful in providing information on the genetic variability present in the different locations. Altogether, we were able to karyotype from 102 females captured in nature 1026 chromosomes corresponding to the five chromosome arms, as shown in the table. Small discrepancies in the number of chromosomes are due to the occasional occurrence of more than two inversions in a particular

Table 1. Relative frequencies of the different heterozygous inversions in the five chromosome arms in six natural populations of *Drosophila willistoni* from Mexico.

	Locality					
Inversion	Poza Rica	Arroyo Agrio	Comalcalco	Teapa	Kolemjaa	Tepic
XL						
None	0.36	0.56	0.68	0.72	0.375	0.9
A	0.27	0.09	0.16	0.125	0.275	0.1
C	x	0.02	x	x	x	x
D	x	0.09	0.05	0.09	x	x
E	x	0.02	x	x	x	x
F	0.18	0.14	0.05	x	0.12	x
G	0.18	0.09	0.05	0.03	0.125	x
H	x	x	x	0.03	x	x
# chrom.	11*	117*	38	32	8	2
# females	5	57	19	16	4	1
XR						
None	0.9	0.75	0.74	0.75	1	1
A	x	x	0.05	x	x	x
B	x	x	0.03	x	x	x
C	x	0.04	0.05	x	x	x
D	0.1	0.07	0.08	0.22	x	x
E	x	0.14	0.05	0.03	x	x
F	x	0.009	x	x	x	x
# chrom.	10	114	38	32	8	2
# females	5	57	19	16	4	1
II L						
None	1	0.77	0.68	0.91	1	1
A	x	0.02	0.08	0.03	x	x
B	x	0.009	0.05	x	x	x
C	x	0.05	0.05	x	x	x
D	x	0.009	x	x	x	x
E	x	0.03	x	x	x	x
F	x	0.04	0.08	0.06	x	x
G	x	0.08	0.05	x	x	x
# chrom.	10	114	38	32	8	2
# females	5	57	19	16	4	1
II R						
None	0.4	0.61	0.53	0.53	0.5	1
B	x	0.04	0.05	0.03	0.125	x
C	0.1	0.05	0.08	0.09	0.125	x
D	x	0.09	0.03	0.06	x	x
E	0.5	0.2	0.29	0.28	0.25	x
G	x	0.009	x	x	x	x
H	x	x	0.03	x	x	x
# chrom.	10	114	38	32	8	2
# females	5	57	19	16	4	1

Table 1. Continued

Inversion	Poza Rica	Arroyo Agrio	Comalcalco	Teapa	Kolemjaa	Tepic
III						
None	0.6	0.45	0.55	0.72	0.5	0.9
A	x	0.06	0.05	0.03	x	x
B	x	0.21	0.21	0.03	x	x
C	x	0.03	x	0.03	x	x
D	x	0.009	0.03	0.03	x	x
E	x	x	0.03	x	x	x
F	x	0.04	0.05	0.06	x	0.1
H	x	0.02	x	x	x	x
I	x	0.03	0.03	x	x	x
J	0.1	0.06	0.03	0.03	0.25	x
K	0.1	0.03	x	x	0.125	x
L	0.1	0.05	x	x	0.125	x
M	0.1	0.009	0.05	0.09	x	x
# chrom.	10	116*	38	32	8	2
# females	5	57	19	16	4	1

chromosome pair of some larvae. In our analysis we found 38 different inversions, which corresponds to 54.28% of the total known gene sequences for this species.

Since the sample size of each locality was small, we organized the analysis in a global form when we refer to the relative frequencies of the different inversions found. We present the number of different inversions found per chromosome arm. They are in decreasing order 34 in the III chromosome, 21 in the XL, 19 in the IIR, 14 in the IIL, and 12 in the XR. This information shows the high degree of polymorphism present in each chromosome arm.

As for the relative frequency of each inversion, in its respective chromosome arm and also in a global form, we got the following percentages. In the XL arm, inversion A showed the highest frequency with 32.94% followed by F with 24.71; G with 18.8205; D with 17.65; C and E with 2.35 each; and H with 1.18.

For chromosome arm XR the values are: D and E each with 39.58; C = 12.50; A = 4.17; and B and F with 2.08 each.

In the IIL the values are : G = 28.21; F = 23.08; C = 20.51; A = 10.26; B and E with 7.69 each; and D = 2.56.

The IIR arm showed for inversion E 57.47; for C =16.09; D = 14.94; B = 9.20; and G and H 1.15 each.

Finally for the third chromosome the frequencies are: B = 34.02; J = 12.37; A and F with 10.31 each; L = 8.24; K and M with 6.18 each; C = 5.15; I = 4.12; D = 3.09; H = 2.06 and E = 1.03.

We found in a total of 361 inversions among 102 females analyzed indicating that each female carries an average of 3.54, which is in the range of values found in other populations of the species, as shown by da Cunha and Dobzhansky (1954) and Ayala *et al.* (1971).

In order to confirm the above unexpected results, it is recommended that the above collections be repeated in order to get more accurate information as to the relative frequencies of the different inversions. Also it is important to perform similar studies in other Mexican populations in order to have a better understanding of the populations of this species that inhabits the country.

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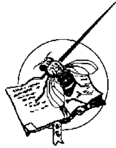

First record of *Drosophila parthenogenetica* and *D. neomorpha, cardini* group, Heed, 1962 (Drosophila, Drosophilidae), in Brazil.

De Toni, Daniela Cristina,[1,3] Jennifer A. Brisson,[2] Paulo R.P. Hofmann,[3] Marlucia Martins,[4] and Hope Hollocher.[1] [1]University of Notre Dame, Notre Dame, IN, USA; [2]Princeton University, Princeton, NJ, USA; [3] Universidade Federal de Santa Catarina, SC, Brazil; [4]Museo Emilio Goeldi / Universidade Federal do Para, PA, Brazil; *Correspondence to: Hope Hollocher, Department of Biological Sciences, University of Notre Dame, Galvin Life Sciences, Notre Dame, IN, 46556; email: Hope.Hollocher.1@nd.edu.

The *cardini* group of the Quinaria section of the genus *Drosophila* is composed of 16 Neotropical species. The two focal species of this report, *Drosophila parthenogenetica* and *D. neomorpha,* are included in this group, and are placed in the *cardini* subgroup with seven other species: *D. polymorpha, D. cardinoides, D. cardini, D. neocardini, D. acutilabella, D. bedicheki* and *D. procardinoides* (Heed and Krishnamurthy, 1959; Heed, 1962; Heed and Russell, 1971; Wilder *et al.*, 2003). The group is characterized by medium-sized flies with a shiny thorax (Vilela *et al.*, 2002). Different species of the group display different abdominal pigmentation patterns (Hollocher *et al.*, 2000a, b), different degrees of divergence and population structure (Wilder *et al.*, 2003; Wilder *et al.*, 2004) as well as different abilities to colonize multiple niches (Rhode and Valente, 1986; Machado *et al.*, 2001). We have been investigating the southern Brazilian Drosophilid communities since the 1990's as a system for understanding the impact of territorial range expansions. In this article, we report the movement of two species of the *cardini* group, *D. parthenogenetica* and *D. neomorpha*, into Brazil and discuss the potential impacts of these range expansions may have on the existing Drosophilid communities.

Drosophilid samples were obtained from collections of adults over fruit and /or vegetable baits in traps in both Atlantic and Amazonian forests in Brazil. The Atlantic sites were within forest in a secondary stage of regeneration at Caldas da Imperatriz (27º 44.480'S; 48º 48.440'W), Joinville (26º 17.150S; 49º 01.00'W), and Campeche Island (27º 41.310' S; 48º 28.880'W), all in the Brazilian State of Santa Catarina. This region is within the transition between subtropical and temperate climatic zones (Moreno, 1961), and, thus, has well-defined seasons with temperatures near 0°C commonly measured during the winter months (June, July, and August) and temperatures reaching 40ºC during the summer (December, January and February). The Amazonian sites were at Caxiuana station (S 01º 44.258'; W 51º 27.352') within the northern Brazilian State of Pará, Urucu in the state of Amazonas (S 08º 17.150'; W 35º 0.200') and Alta Floresta in the state of Mato Grosso (S 09º 53.020'; W 56º 14.380'). These central and northern Brazilian regions exist within a tropical climate such that there are not well-defined seasons, with temperatures varying between 25ºC and 40ºC all year.

At the Atlantic forest site from Caldas da Imperatriz and Campeche Island, we caught three and ten individuals of *D. parthenogenetica*, respectively. This collection brought together *D.*