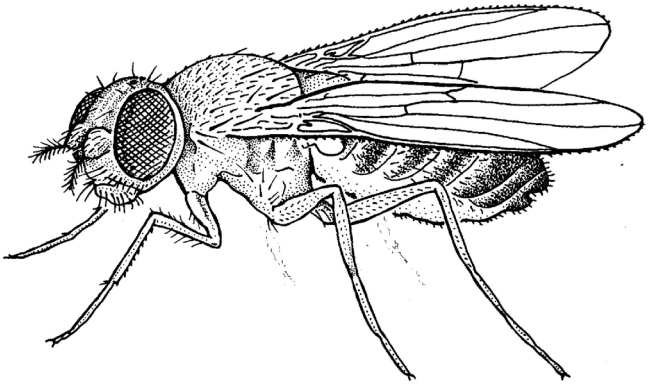
Drosophila Information Service



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Preface

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

The production of this volume of DIS could not have been completed without the generous efforts of many people. In particular, George Davis continued to assist in our transition to a more completely web-based publication format. Except for the special issues that contained mutant and stock information now provided in detail by FlyBase and similar material in the annual volumes, all issues are now freely-accessible from our web site: www.ou.edu/journals/dis. For early issues that only exist as aging typed or mimeographed copies, some notes and announcements have not yet been fully brought on line. But we intend to fill in those gaps.

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

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Can the *P* elements *TP5* and *TP6* affect repressor-sensitive alleles of the *singed* gene in *Drosophila melanogaster*?

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The incomplete *P* elements *TP5* and *TP6* were discovered as insertions in the telomere associated sequences (TAS) of chromosome XL in *D. melanogaster* (Stuart *et al.*, 2002). In their telomeric location, each of these elements brings about the P cytotype, a maternally inherited state that represses the movement of *P* elements in the germline. Cytotype regulation is mediated by small RNAs that interact with the Piwi family of proteins (Brennecke *et al.*, 2008, Jensen *et al.*, 2008). These Piwi-interacting (pi) RNAs are generated from many loci, including the one in the TAS of chromosome XL (Brennecke *et al.*, 2007). Before the discovery of telomeric *P* elements and piRNAs, cytotype regulation was thought to be mediated by repressor proteins encoded by the *P* elements themselves (Rio 1990). For example, complete *P* (*CP*) elements produce a 66 kd repressor polypeptide through alternate splicing of transcripts that encode an 87 kd transposase, and incomplete *P* elements first discovered in flies from Krasnodar, Russia (Black *et al.*, 1987)—hence, called *KP* elements—produce a 22 kd repressor polypeptide. These polypeptides act zygotically rather than maternally and, therefore, cannot explain cytotype regulation of the *P*-element family (Simmons *et al.*, 2002a, b). However, they may provide secondary controls on *P*-element activity in nature.

P-encoded repressor polypeptides affect the expression of some *P*-insertion mutations of the *singed* gene such as sn^{50e} and sn^{w} (Robertson and Engels 1989; Simmons *et al.*, 2004, Paterson *et al.*, 2007). The sn^{50e} allele exhibits repressor sensitivity in the soma, where the singed polypeptide is needed for normal bristle formation. In the absence of other *P* elements, sn^{50e} causes an extreme singed phenotype (very short, twisted bristles), but in the presence of repressor-encoding *P* elements, sn^{50e} flies have a moderate singed phenotype (longer, wavy bristles). The sn^{w} allele exhibits repressor sensitivity in the female germ line, where the singed polypeptide is needed for egg formation. In the absence of other *P* elements, homozygous sn^{w} females produce normal eggs, but in the presence of repressor-encoding *P* elements, sn^{w} homozygotes produce ill-formed eggs, which causes them to be less fertile or even sterile. This effect of repressor-encoding *P* elements, called "singed sterility," is also seen when sn^{w} is heterozygous with the radiation-induced null allele sn^{x2}.

In their telomeric locations, *TP5* and *TP6* have no discernable effect on these repressor-sensitive alleles (Simmons *et al.*, 2004). The lack of an effect could be due to the inability of these elements to encode repressor polypeptides, or to their inability to produce repressors because *P* insertions in the TAS generate piRNAs rather than mRNAs. To distinguish between these possibilities, we tested non-telomeric transgenes that contain either *TP5* or *TP6* for the ability to alter the phenotypes of sn^{50e} and sn^{w}. These transgenes, denoted in general as *H(hsp/P)* because they were constructed in a *hobo* (*H*)element vector and contain a fusion of the *P* element (with its promoter) to an ancillary promoter from the *D. melanogaster hsp70* gene, have been described (Simmons *et al.*, 2002a, b; Jensen *et al.*, 2008).

To see if the *H(hsp/P)* transgenes could suppress the sn^{50e} bristle phenotype, we examined hemizyous sn^{50e}; *H(hsp/P)*/+ males. In addition to transgenes containing *TP5* or *TP6*, we tested transgenes containing other types of *P* elements: *SP* (a 0.5 kb-long element, which is too short to encode a repressor polypeptide and could, therefore, serve as a negative control [Rasmussen *et al.*, 1993]), *KP* (which had never been tested in this assay), and *CP* (which is known to suppress sn^{50e} and could, therefore, serve as a positive control [Simmons *et al.*, 2004]). The bristle phenotype was observed in sons of the cross *w* sn^{50e} ♀♀ × w; *H(hsp/P)* ♂♂ which were reared at 25°C. All the crosses were also brooded to produce replicate cultures, which were subjected to a 1 hr treatment at 37°C on days 9, 10, and 11 after the cultures were established. Thus, it was possible to

score the phenotype in flies that had been subjected to a heat shock during the pupal stage, when the bristles develop, as well as in flies that had not (Table 1).

Table 1. Effect of *H(hsp/P)* transgenes on the singed bristle phenotype of males hemizygous for the repressor-sensitive allele sn^{50e}.

Transgene	Chromosome	Singed phenotype
H(*hsp*/*SP*)*A*	3	Extreme
H(*hsp*/*SP*)*B*	2	Extreme
H(*hsp*/*KP*)*3*	2	Extreme
H(*hsp*/*KP*)*7*[a]	2	Moderate
H(*hsp*/*KP*)*11*	2	Extreme/Moderate [b]
H(*hsp*/*KP*)*14*	2	Quasi-moderate
H(*hsp*/*TP5*)*A*	3	Extreme
H(*hsp*/*TP5*)*B*	2	Extreme
H(*hsp*/*TP5*)*C*	3	Extreme
H(*hsp*/*TP5*)*D*	2	Extreme
H(*hsp*/*TP6*)*A*	2	Extreme
H(*hsp*/*TP6*)*B*	3	Extreme
H(*hsp*/*TP6*)*C*	3	Extreme
H(*hsp*/*CP*)*2*	2	Moderate
H(*hsp*/*CP*)*3*	3	Moderate

[a] Two insertions of the transgene, separable by recombination, are located on chromosome 2.
[b] The moderate phenotype was seen only after heat shock treatments.

None of the *H(hsp/TP5)* or *H(hsp/TP6)* transgene insertions—7 insertions altogether—suppressed the sn^{50e} phenotype, even after heat shocks. The bristles of the flies that carried these transgenes were indistinguishable from those of the two *H(hsp/SP)* negative controls. By contrast, three of the four stocks with *H(hsp/KP)* insertions partially suppressed the mutant bristle phenotype, although one did so only after heat shock. In these cases, the bristles were identical to those of the two *H(hsp/CP)* positive controls. Thus, *KP*, but not *TP5* or *TP6*, produces a polypeptide that acts on the sn^{50e} *P* element to enhance expression of the *singed* gene in somatic cells.

To assess the effects of *P*-encoded polypeptides in germ-line cells, we examined the fertility of females heterozygous for sn^{w} and sn^{x2}. These sn^{w}/sn^{x2} females were obtained by crossing *FM7*, w^{81g} sn^{x2} *B*; *H(hsp/P)*/+ males to *w* sn^{w} females; w^{81g} is a null mutation of the *white* gene that arose spontaneously in the *FM7* balancer chromosome. Both types of daughters—those that inherited the *H(hsp/P)* transgene (recognized by their colored eyes) and those that did not (white eyes)—were allowed to mate with their brothers for 3-4 days, and then placed individually in 13 × 100 mm culture tubes supplied with a sugar-dried yeast medium (Simmons *et al.*, 1980); these tubes were then incubated at 25°C. Each female's fertility was quantified by counting the number of pupae present in the tube 9 days later.

The sn^{w}/sn^{x2} females that did not carry an *H(hsp/P)* transgene (right side of Table 2) were almost all fertile. The median number of offspring produced by these females ranged from 21 to 34, and the mean ranged from 21.4 to 34.8. The females that carried the *SP*, *TP5*, or *TP6* transgenes also largely conformed to this pattern. The highest frequency of sterility among these females was 0.13 for *H*(*hsp*/*TP6)B*; however, given the uncertainty in the data (binomial standard deviation 0.04), this frequency is not out of line with some of the frequencies observed among females that did not carry a transgene. Furthermore, the median and mean numbers of progeny produced by fertile females that carried the *SP*, *TP5*, or *TP6* transgenes were comparable to those of their transgene-free sisters. Thus, none of these transgenes appeared to cause singed sterility. In contrast, a considerable fraction of the females that carried the *KP* or *CP* transgenes were sterile, and among those that were fertile, the number of progeny was meager (median and mean numbers less than 10). The *H*(*hsp*/*KP*) insertions in two of the stocks (*3* and *7*) caused many females to be completely sterile (37 and 63 percent, respectively), and the insertions in all four of the *H*(*hsp*/*KP*) stocks caused significant reductions in fertility when compared to transgene-free flies. Both insertions of the *CP* transgene also caused some complete sterility, and they reduced the fertility of the non-sterile females significantly.

As a check on the specificity of this test for effects on the germ-line function of the sn^{w} allele, we also assessed the fertility of sn^{50e}/sn^{x2} females in the presence and absence of the *H(hsp/CP)* transgenes. Previous studies had indicated that the sn^{50e} allele is not associated with singed sterility—that is, sn^{50e}/sn^{x2} flies are fertile in the presence of P repressor polypeptides (Robertson and Engels 1989). Accordingly, we found that neither insertion of the *H(hsp/CP)* transgene caused sterility or reduced fertility in sn^{50e}/sn^{x2} females (last two rows in Table 2). In fact, the fertility of these females was greater than that of transgene-free sn^{w}/sn^{x2} females, suggesting that the sn^{w} allele has a fertility-reducing effect even in the absence of a *KP* or a *CP* transgene.

Table 2. Fertility of sn^{w}/sn^{x2} or sn^{50e}/sn^{x2} females in the presence and absence of *H(hsp/P)* transgenes.

	Transgene present					Transgene absent				
	No. of Females	Prop. sterile	No. of progeny per fertile female			No. of Females	Prop. Sterile	No. of progeny per fertile female		
			Median	Mean	SD[a]			Median	Mean	SD[a]
H(*hsp*/*SP*)*A*	53	0.02	22.5	22.8	9.1	44	0.00	27	26.3	9.1
H(*hsp*/*SP*)*B*	62	0.00	22.5	21.8	8.8	59	0.02	22.5	23.5	9.5
H(*hsp*/*KP*)*3*	62	0.37	2	3.2	3.5	57	0.02	27	26.3	9.4
H(*hsp*/*KP*)*7*	60	0.63	2.5	3.4	3.2	55	0.00	21	21.4	8.2
H(*hsp*/*KP*)*11*	51	0.04	8	9.6	6.9	58	0.09	26	25.1	8.7
H(*hsp*/*KP*)*14*	49	0.06	8	9.1	6.3	54	0.00	25	24.7	8.0
H(*hsp*/*TP5*)*A*	48	0.04	23	22.8	11.1	65	0.03	30	32.9	15.7
H(*hsp*/*TP5*)*B*	28	0.00	28.5	31.2	13.7	38	0.00	27.5	29.7	16.8
H(*hsp*/*TP5*)*C*	61	0.00	25	25.5	7.2	64	0.02	24	24.6	7.2
H(*hsp*/*TP5*)*D*	58	0.00	25.5	25.8	7.9	45	0.00	28	30.2	12.3
H(*hsp*/*TP6*)*A*	59	0.02	26	27.1	10.6	57	0.00	29	31.2	11.5
H(*hsp*/*TP6*)*B*	70	0.13	24	23.3	8.7	66	0.06	27	26.1	8.3
H(*hsp*/*TP6*)*C*	34	0.03	31	29.9	10.7	32	0.00	27.5	26.8	8.0
H(*hsp*/*CP*)*2*	84	0.11	5	6.8	5.8	57	0.02	34	34.8	13.1
H(*hsp*/*CP*)*3*	31	0.19	7	7.4	5.1	24	0.08	24	26.3	11.3
H(*hsp*/*CP*)*2*	36[b]	0.00	38.5	40.1	7.8	24[b]	0.00	44	45.1	6.3
H(*hsp*/*CP*)*3*	38[b]	0.00	39.5	37.0	10.7	30[b]	0.00	40	41.4	8.6

[a] Standard deviation
[b] These females were sn^{50e}/sn^{x2}; all others in the Table were sn^{w}/sn^{x2}.

Although the *KP* and *CP* transgenes significantly impaired the germ-line function of the sn^{w} allele, neither they nor any of the other transgenes affected its somatic function. In the presence of any of the *H(hsp/P)* transgenes, sn^{w} males have moderately twisted bristles that are somewhat shorter than wild type. Females heterozygous for sn^{w} and sn^{x2} also have moderately twisted bristles, regardless of the presence of an *H(hsp/P)* transgene. Thus, at the phenotypic level, the repressor sensitivity of the sn^{w} allele is limited to the female germ line. Furthermore, only *P* elements that encode known repressor polypeptides—*KP* and *CP*—bring out this sensitivity; the *TP5* and *TP6* elements, either in their native telomeric positions (Simmons *et al.*, 2004) or in transgenes designed to express them, do not.

Both *KP* elements and *CP* elements encode polypeptides that repress some aspects of hybrid dysgenesis, a syndrome of germ-line abnormalities caused by *P* activity, and both of these elements induce changes in the phenotypes of sn^{50e} and sn^{w}. This phenotype-changing ability is thought to involve the KP and 66 kD repressors binding to the *P* elements inserted in these mutant alleles. In one case (sn^{50e}), the phenotype is ameliorated, whereas in the other (sn^{w}), it is worsened. Although the bound repressors presumably influence transcription of the mutant *singed* gene—for better or for worse—the specific reasons for these opposite effects are unknown.

Both the KP and 66 kD repressors possess a domain that recognizes and binds to *P* elements at three different sites: the transposase-binding sites near the 31-bp terminal inverted repeats, the 11-bp internal inverted repeats, and—at high protein concentrations—the terminal inverted repeats (Lee *et al.*, 1996, 1998). It is plausible that when either type of repressor interacts with any of these sites, it affects transcription from the *P* promoter. Indeed, both types of repressors have been shown to silence the expression of *P-lacZ* transgenes *in vivo* (Lemaitre *et al.*, 1993; Lemaitre and Coen 1991). Repressor binding to complete *P* elements has been hypothesized to limit the production of the P transposase, and ultimately to reduce the amount of *P*-element movement. However, *P*-element movement might also be reduced if repressor binding

simply prevents the transposase from attacking potentially mobile *P* elements. The domain for *P*-element binding is located within the first 88 amino acids of the repressor polypeptide sequence (Lee *et al.*, 1998). Artificially engineered polypeptides with these amino acids are able to repress *P*-element transposition *in vitro*, although not as well as longer polypeptides. Thus, other regions in the KP and 66 kD polypeptides contribute significantly to repressor function.

Both the *TP5* and *TP6* elements could encode polypeptides longer than 88 amino acids, and transgenes designed to express the TP5 polypeptide, but not those designed to express the TP6 polypeptide, have a modest ability to repress transposase-induced sn^{w} mutability (Jensen *et al.*, 2008). However, this repression is not nearly so strong as that seen with the *H*(*hsp*/*KP*) transgenes, and it is very much weaker than that of the native *TP5* element situated in the TAS of the XL telomere. The TP5 polypeptide, which is 113 amino acids long, may, therefore, be a bona fide repressor, but it is not as effective as the KP repressor. Furthermore, unlike the KP and 66 kD repressors, the TP5 polypeptide has no ability to alter the phenotypes of sn^{50e} and sn^{w}. Repressors that alter mutant singed phenotypes may do so because they possess downstream amino acid motifs that augment their ability to bind to *P* elements. For instance, a leucine zipper in the KP polypeptide has been implicated in its ability to form dimers, and dimerization may enhance the ability of KP repressors to bind to *P*-element DNA. Andrews and Gloor (1995) demonstrated that this leucine zipper is important for repression of hybrid dysgenesis. One other fact argues that *TP5* and *TP6* do not produce polypeptides with significant repressor function: Unlike *KP*, neither of these elements is widespread in natural populations (Stuart *et al.*, 2002). If *TP5* and *TP6* encoded effective repressor polypeptides, we would expect natural selection to have brought them to noticeable frequencies in many places in the world.

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Evidence for action of the KP repressor polypeptide in the germ line of female *Drosophila melanogaster* carrying piRNA-generating telomeric *P* elements.

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The transposable *P* elements of *D. melanogaster* are responsible for a syndrome of germ-line abnormalities that includes sterility and high frequencies of mutation and chromosome breakage (Engels, 1989). This syndrome, called hybrid dysgenesis, occurs in the offspring of crosses between paternally contributing (P) and maternally contributing (M) strains. Thus, P male × M female produces dysgenic offspring, but P female × M male usually does not. Hybrid dysgenesis is repressed by small *P*-derived RNAs,

called piRNAs, because they interact with the Piwi family of proteins (Brennecke *et al.*, 2008; Jensen *et al.*, 2008). These RNAs are generated by *P* elements inserted in the telomere associated sequences (TAS) at the left end of the X chromosome. *TP5* (1.8 kb long) and *TP6* (1.9 kb long) are two such elements (Stuart *et al.*, 2002). Analyses of *TP5* and *TP6*—and of other *P* insertions in the TAS as well (Marin *et al.*, 2000)—have shown that piRNAs are the basis of the P cytotype, a maternally transmitted state that regulates *P*-element activity in the germ line.

Although piRNAs are the primary means of repressing hybrid dysgenesis, some *P*-encoded polypeptides may also play a role. For example, complete *P* elements (*CP*, 2.9 kb long) encode a 66 kd repressor polpeptide as well as an 87 kd transposase (Rio, 1990). These two polypeptides are produced through alternate splicing of *CP* mRNA; the transposase is synthesized from mRNA that has lost all three of *CP*'s introns, whereas the repressor is synthesized from mRNA that retails the last of these introns (denoted as the *2-3* intron because it lies between exons *2* and *3*). In the germ line, both polypeptides appear to be made, but in the soma, the 66 kd polypeptide is the only *CP* product. This tissue-specific expression of the *CP* polypeptides explains why *P*-element movement, which is catalyzed by the transposase, is restricted to the germ line (Laski *et al.*, 1986).

An element called *KP* (1.15 kb long) also encodes a repressor polypeptide (Rio, 1990; Andrews and Gloor, 1995; Lee *et al.*,1996, 1998). This repressor is 207 amino acids long and shares 199 amino acids with the transposase. It is able to bind to *P* elements and may also interact with other *P*-encoded polypeptides, including itself and the transposase. Because *KP* elements are widespread in natural populations, they appear to confer a selective advantage (Boussy *et al.*, 1988; Itoh and Boussy, 2002; Itoh *et al.*, 2007). A recent report (Simmons *et al.*, 2015) indicates that repression of hybrid dysgenesis by *KP* elements is enhanced by cytotype-anchoring telomeric *P* elements such as *TP5* and *TP6*. This *KP* effect is zygotic rather than maternal and presumably results from the action of the KP polypeptide. Models of transposon regulation have assumed that piRNAs act by destroying transposon mRNAs or by blocking their translation. However, the *KP* effect implies that some *P* mRNAs survive and are translated, which in turn implies that piRNA-mediated regulation is more complicated than simply destroying transposon mRNAs or blocking their translation.

This report provides additional evidence for the action of the KP repressor polypeptide in the face of piRNA-mediated cytotype regulation. First, it confirms that the telomeric elements *TP5* and *TP6* confer the P cytotype in males and females. Second, it demonstrates that the KP repressor polypeptide is active in flies that have inherited these elements from their mothers.

Two assays were used to demonstrate that *TP5* and *TP6* confer the P cytotype. The strains used in these assays were homozygous for one of the telomeric *P* elements (*TP*), a null allele of the closely linked *white* (*w*) locus, and a double *P*-element insertion allele of the X-linked *singed* (*sn*) locus. This last allele, called *weak singed* (sn^w), causes a mild malformation of the bristles in hemizygous males but has little or no effect on the bristles of homozygous females.

The first assay measured the incidence of sterility in females from testcrosses between *TP w* sn^w or *w* sn^w (control) females and males from a strong P strain (Harwich). This sterility results from the failure of the ovaries to develop properly, a condition called gonadal dysgenesis (GD). It is induced specifically by the action of the Harwich-derived *P* elements in the germ lines of testcross offspring, and is easily recognized in females by their inability to produce eggs. GD was induced by crossing females from the *TP* and control strains *en masse* to Harwich males at 21°C. After three days, each female was individually placed in a fresh culture vial, which was incubated at 29°C, a temperature that maximizes the occurrence of GD. On day 11, the offspring were transferred to a holding vial, where they matured for two days at 21°C. The females were then screened for GD by squashing them between two glass slides. Females that did not extrude eggs were classified as dysgenic. A solution of green food coloring was placed between the slides to make the eggs easier to see. The data on the left side of Table 1 show that both *TP5* and *TP6* strongly repressed Harwich-induced GD.

The second assay measured the frequency of *P*-element excisions from sn^w in the male germ line. Transposase-catalyzed excision of one or the other of the *P* elements in the sn^w allele creates alleles with a phenotype more or less severe than that of the parent allele (Roiha *et al.*, 1988). Germ-line excisions of these elements can be detected by scoring for the extreme singed (sn^e) or pseudo-wild phenotypes ($sn^{(+)}$) in the next generation. To induce the excisions, homozygous sn^w females were crossed to males that carried *P*(ry^+, Δ*2*-

3)*99B*, a stable *P* transgene that makes the P transposase in the soma as well as in the germ line, because the *2-3* intron has been deleted by construction (Robertson *et al.*, 1988). These crosses were incubated at 18°C, and the sn^w; Δ*2-3*/+ sons from them were crossed at 25°C to *C*(*1*)*DX, y w* females with attached-X chromosomes to enforce patroclinous transmission of the sn^w allele or its sn^e or $sn^{(+)}$ derivatives. These attached-X females came from a P strain to prevent transposase activity in their offspring's soma (Robertson and Engels, 1989). The sons of these crosses were scored for the three singed phenotypes and the proportion that were sn^e or $sn^{(+)}$ was calculated to measure the mutability of the sn^w allele. The data on the right side of Table 1 show that both *TP5* and *TP6* strongly repressed *P* excisions from sn^w in the male germ line. \

Table 1. Repression of gonadal dysgenesis (GD) and sn^w-mutability by strains with telomeric *P* elements.

	Gonadal dysgenesis in females			Mutability of sn^w in males		
Strain	No. vials	No. daughters	%GD ± SE[a]	No. vials	No. sons	Mutability ± SE[b]
w sn^w	25	344	98.4 ± 0.7	30	819	0.340 ± 0.029
TP5 w sn^w	29	455	2.2 ± 1.4	28	577	0.041 ± 0.012
TP6 w sn^w	28	245	37.3 ± 6.2	29	1186	0.059 ± 0.014

[a] Unweighted average percentage of GD among daughters ± standard error.
[b] Unweighted average frequency of sn^e and $sn^{(+)}$ among sons ± standard error.

Together these two assays show that both *TP5* and *TP6* repress *P*-element activity in the germ line—that is, they confer the P cytotype. This activity is catalyzed by the P transposase. The telomeric *P* elements are thought to repress *P* activity by targeting *P*-specific piRNAs to the transposase mRNA, which is then either degraded or blocked from serving as a template for polypeptide synthesis. Either way, transposase activity would be reduced, although, as the data in Table 1 show, it is not completely abolished. It is worth noting that RT-PCR data have shown that the transposase mRNA, though diminished, is not entirely eliminated in females that inherit a *TP* from their mothers (Jensen *et al.*, 2008).

A different assay was used to see if *TP5* and *TP6* could undercut germ-line activity of the KP repressor polypeptide. This assay makes use of mutant alleles of the *singed* gene that impair female fertility. Females carrying these alleles produce ill-formed eggs that are unlikely to develop normally after fertilization; thus, they produce few viable offspring (Robertson and Engels 1989). This "singed sterility" results from defects in the ring canals, which contain the singed protein and which normally allow materials to pass from the nurse cells into the oocyte during oogenesis (Cant *et al.*, 1994). *P*-insertion alleles such as sn^w cause singed sterility, but only when P repressor polypeptides are present, presumably because the repressors interfere with the expression of the mutant *singed* gene by binding to its inserted *P* elements. These "repressor-sensitive" alleles are, therefore, useful detectors of P repressor polypeptides.

The issue is whether or not a *KP* element can produce the KP repressor in flies that have inherited a *TP* element maternally. To resolve this issue, females that were heterozygous for sn^{x2} (a null mutation) and sn^w were tested for fertility in the presence and absence of *H(hsp/KP)3*, an *hobo* transgene that contains a *KP* element fused to an ancillary promoter from the *hsp70* gene (Simmons *et al.*, 2002). These females were obtained by crossing *FM7*, w^{81g} sn^{x2} *B*; *H(hsp/KP)3*/+ males to *TP w* sn^w females; w^{81g} is a null mutation of the *white* gene that arose spontaneously in the *FM7* balancer chromosome. Both types of daughters—those that inherited the *H(hsp/KP)3* transgene (recognized by their colored eyes) and those that did not (white eyes)—were allowed to mate with their brothers for 3-4 days, and then placed individually in 13 × 100 mm culture tubes supplied with a sugar-dried yeast medium (Simmons *et al.*, 1980); these tubes were then incubated at 25°C. Each female's fertility was quantified by counting the number of pupae present in the tube 9 days later. Previous work had established that the *H(hsp*/*KP)3* transgene induces singed sterility by acting zygotically, presumably because it produces the KP repressor polypeptide (Paterson *et al.*, 2007). If the telomeric *P* elements undercut *KP* expression, they would be expected to suppress singed sterility in sn^w/sn^{x2} females that carry *H*(*hsp*/*KP*)*3*.

Table 2. Effect of maternally inherited telomeric *P* elements on *H*(*hsp*/*KP*)*3*-induced singed sterility in sn^{w}/sn^{x2} females.

	H(*hsp*/*KP*)*3* present					*H*(*hsp*/*KP*)*3* absent				
			No. of Progeny per fertile female					No. of Progeny per fertile female		
Strain	No. of females	Prop. sterile	Median	Mean	SD[a]	No. of females	Prop. sterile	Median	Mean	SD[a]
w sn^{w}	91	0.86	2	2.5	1.5	83	0.01	23	23.2	9.2
TP5 w sn^{w}	64	0.55	2	5.0	5.7	75	0	39	37.5	13.8
TP6 w sn^{w}	83	0.48	4	5.5	5.2	72	0	35	35.4	13.2

[a] Standard deviation

The data (Table 2) show that many of the sn^{w}/sn^{x2} females that carried this transgene were completely sterile, and of those that were not, the number of progeny was meager. The telomeric elements did seem to alleviate complete sterility in the *KP*-bearing females somewhat (55% for *TP5* and 48% for *TP6*, compared to 86% for the control), but they did not improve progeny numbers significantly. By contrast, almost all the sn^{w}/sn^{x2} females that did not carry the *KP* transgene were fertile. These females produced many offspring, especially when a *TP* was present. The increased number of progeny when a *TP* was present suggests that the sn^{w} allele has an intrinsic fertility-reducing effect, and that this effect is mitigated by piRNAs generated from *TP5* and *TP6*. Perhaps these piRNAs interact with the *P* elements inserted in sn^{w} and boost the expression of the *singed* gene, thereby bringing about more nearly normal levels of singed protein. However, no such boosting occurs in sn^{w}/sn^{x2} females that carry the *KP* transgene. In these females, the KP repressor polypeptide dramatically impairs singed expression, even when the telomeric *P* element is present.

The paramount effect of the KP repressor polypeptide in the flies that carry the *H(hsp/KP)3* transgene is *prima facie* evidence that cytotype-anchoring telomeric *P* elements do not abolish the production of P polypeptides; the mRNAs that encode these polypeptides must survive and be translated despite attacks by *TP*-generated piRNAs. The piRNA-mediated repression of hybrid dysgenesis must, therefore, involve something more than the destruction or translational arrest of *P*-element mRNAs. One possibility is that piRNAs alter the chromatin in and around *P* insertions in such a way that these insertions can no longer be mobilized by the P transposase. It is known that chromatin can be reorganized by piRNA action (Grewal 2010), and there is some evidence that *TP*-generated piRNAs affect the status of the chromatin that contains *P* elements (Josse *et al.*, 2007).

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Oscillations in the ERG of the *Drosophila trpl*^{302} mutant are caused by an additional mutation in the *inebriated* gene.

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Introduction

Light-absorption in the *Drosophila* compound eye triggers a G protein- and PLCβ-mediated signalling cascade that finally leads to the opening of the cation channels TRP and TRPL. The influx of cations causes a depolarisation of the photoreceptor cells and, via histamine-dependant synaptic transmission, an activation of downstream neurons. Electroretinogram recordings (ERGs) reveal the depolarization of photoreceptor cells as a prominent voltage change while electrical responses of laminar neurons are recorded as on- and off-transients. Mutations in the *inebriated* (*ine*) gene, that encodes a putative neurotransmitter transporter, induce defects at the photoreceptor synapse and result in electrical oscillations superimposed on the depolarizing receptor potential (Gavin *et al.*, 2007). Gavin *et al.* have proposed a mechanism for the oscillations in the *ine* mutant. Histamine stored in the photoreceptor synapse is released into the synaptic cleft. Here, it activates postsynaptic Cl^- channels as well as presynaptic H_3 receptors. The activation of the H_3 receptor inhibits presynaptic Ca^{2+} channels and down-regulates histamine release. In the glia cells, histamine is converted to carcinine, which is transported into the photoreceptor cell by Inebriated. If Inebriated is missing, carcinine accumulates in the synaptic cleft and competes with histamine binding to the H_3 receptor. This leads to fluctuations of the current through the presynaptic Ca^{2+} channel. The resulting Ca^{2+} fluctuations finally cause oscillations in the response of downstream neurons. Similar oscillations were observed in the *trpl*^{302} mutant (Leung *et al.*, 2000), giving rise to speculations whether TRPL, in addition to its function in the phototransduction cascade, might have a function at the photoreceptor synapse. Interestingly, the *arrestin1*^{1} mutant also displays ERG oscillations. Here we show that oscillations observed in the ERG of *trpl*^{302} or *arr1*^{1} mutant flies are due to a secondary mutation in the *ine* gene.

Material and Methods

Drosophila stocks

Oregon R *w*^{1118} (here referred to as wild type), *y*; *ine*^{MI05077} [*y*$^{+}$], and the deletion strain *w*^{1118}; Df(2R)BSC131/CyO (46A1-46B4 covering trpl at 46B2) were obtained from the Bloomington *Drosophila* Stock Center. *trpl*^{302} *cn bw* (Niemeyer *et al.*, 1996), *arr1*^{1} *cn bw* (Dolph *et al.*, 1993) were obtained from C.S. Zuker. For the generation of a pure *trpl* mutant, *trpl*^{302} *cn bw ine* flies were crossed with *yw;* + mutant flies and female *trpl*^{302} *cn bw ine* / + flies were crossed with a *CyO*-balancer stock. Single male offspring flies carrying a recombined second chromosome and the *CyO* balancer were crossed with a *Sco*/*CyO* balancer stock. In the next generation, flies carrying the recombined second chromosome and *CyO* were crossed *inter se* and finally stocks carrying the recombined second chromosome homozygously were established. The mutant carrying the *trpl*^{302} mutation but lacking the *ine* second site mutation is now called *trpl*^{302NO} (No Oscillations) and still carries the *cn* and *bw* mutations (*trpl*^{302NO} *cn bw*). Flies were raised on standard cornmeal food at 25°C and crossings were carried out using standard *Drosophila* genetics.

Electroretinogram recordings (ERG)

For electroretinogram recordings, flies were immobilized in a pipette tip and mounted with a mixture of colophonium and bee's wax (1:3). Electroretinogram recordings were performed at room temperature after 3 minutes of dark adaptation prior to the first orange light-stimulus. Light-stimuli of 5 s duration were delivered by an orange light-emitting diode (580 nm, Roithner, Austria) and a blue light-emitting diode (470 nm, Roithner, Austria), collimated and combined by a 50% beam splitter (Linos, Germany) within the light path. The light intensity at the position of the fly eye was 2.15 mW/cm^2 for orange light and 1.3 mW/cm^2 for blue light. A DPA-2FS amplifier (NPI electronic, Germany) with a low pass filter (700 Hz) was used for signal amplification. Analog-to-digital conversion was accomplished with a PCI-6221 PC card (National Instruments, Germany). Data recording was achieved by the Whole Cell Analysis Program software WinWCP 4.7.6. (University of Strathclyde). The recording electrode glass capillary was filled with Davenport solution (100 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1.8 mM $NaHCO_3$, pH 7.2). In all experiments the following stimulus protocol (OBBOO) was used: 5 s orange light, 10 s dark, 5 s blue light, 10 s dark, 5 s blue light, 10 s dark, 5 s orange light, 10 s dark, 5 s orange light.

Western Blot analysis

Western blot analysis with anti-TRP and anti-TRPL antibodies was carried out as previously described (Cerny *et al.*, 2015).

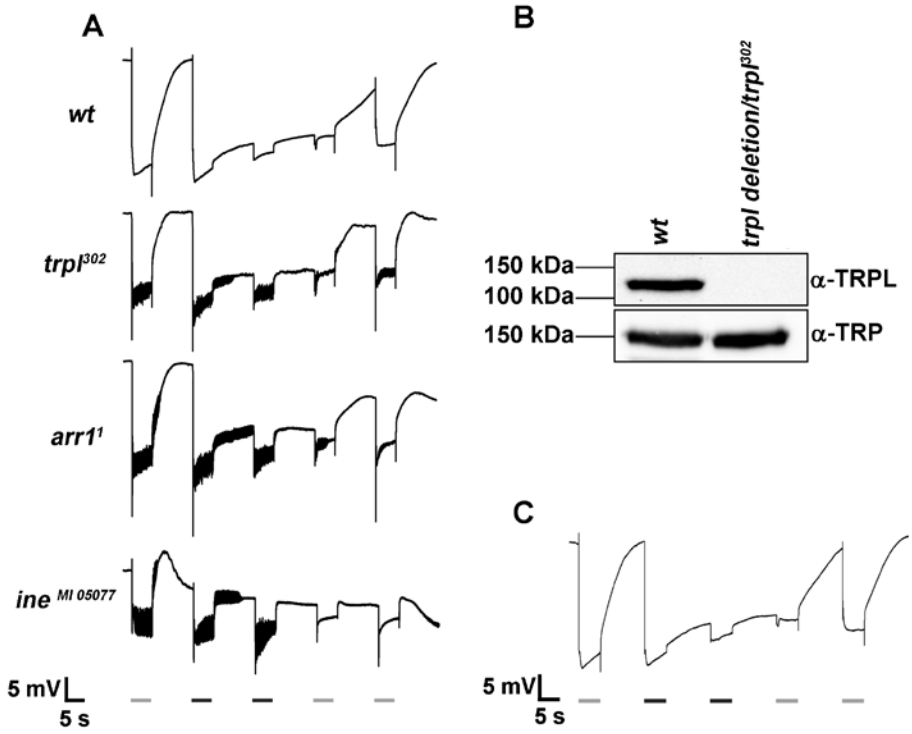

Figure 1. (A) ERGs of 3 day old flies with the indicated genotypes that were subjected to an OBBOO-protocol (see Material and Methods). The *ine*^{MI05077} mutant did not show a prolonged depolarization afterpotential (PDA) after blue light illumination due to its red eye color (*white*$^+$). Oscillations are superimposed on the depolarization and sometimes remain after termination of the light stimulus. (B) Western blot analysis of wild type (wt) and the *trpl deletion/trpl*^{302} mutant (Df(2R)BSC131/*trpl*^{302}). No TRPL protein was detected in the mutant while TRP that served as a loading control was present at wild type levels. (C) ERG of the *trpl deletion/trpl*^{302} mutant exposed to OBBOO protocol. No oscillations were observed.

Results

Figure 1A shows the oscillations in the ERG recordings of *trpl*^{302}, *arr1*^{1}, and *ine*^{MI05077} mutants. The oscillations superimposed on the depolarizing receptor potential have a frequency of 50-60 Hz and sometimes remain even after termination of the light pulse. In our stocks 92% of *trpl*^{302} flies, 57% of *arr1*^{1} flies, and 78% of *ine*^{MI05077} flies (*N* = 7-18) but none of the wild type flies showed oscillations.

To analyse whether the oscillations in the *trpl*^{302} mutant are really caused by a mutation in the *trpl* gene, we crossed the *trpl*^{302} mutant with a deletion mutant covering the *trpl* locus. These mutants were tested for presence of TRPL by Western blot analysis (Figure 1B) and analysed by ERG recordings (Figure 1C). Although the mutants lacked TRPL, none of seven flies tested showed oscillations. This finding revealed that the oscillations are not caused by the defective *trpl* gene.

Since *trpl*, *arr1*, and *ine* are all located on the second chromosome, we hypothesized that the oscillations in the *trpl*^{302} mutant as well as in the *arr1*^{1} mutant are caused by an additional mutation in the *ine* gene. To confirm this hypothesis we carried out complementation tests. For complementation analysis we first tested whether *ine*^{MI05077} is a recessive mutation with respect to ERG oscillations. This is the case, because heterozygous *ine* mutants showed no oscillations (Figure 2). However, ERG oscillations were readily observed in *ine*^{MI05077}/*trpl*^{302}, *ine*^{MI05077}/*arr1*^{1}, and *arr1*^{1}/*trpl*^{302} trans-heterozygous mutants (Figure 2).

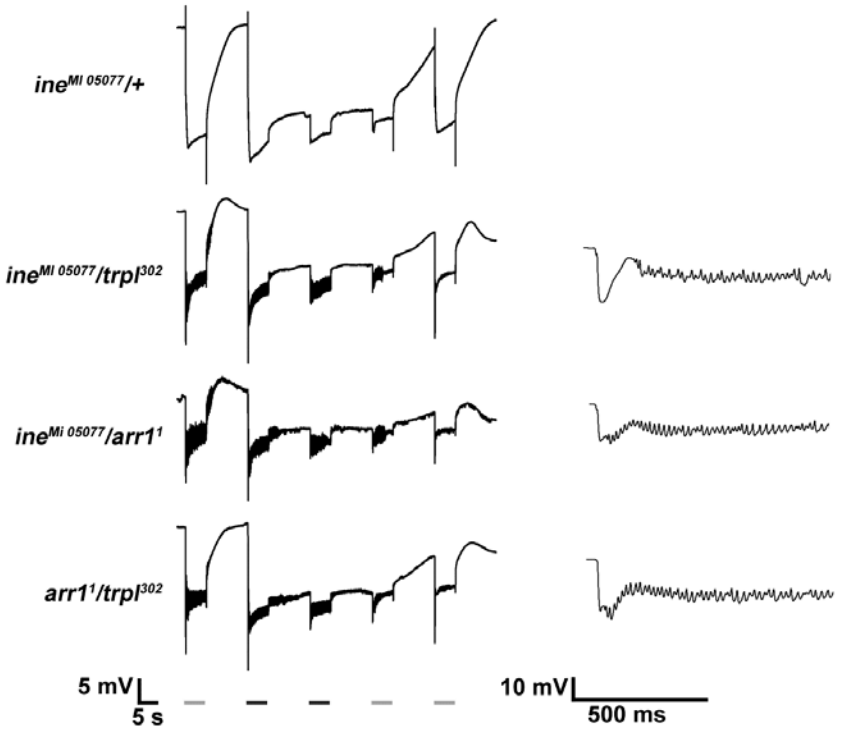

Figure 2. ERG recordings of 3 days old heterozygous *ine*^{MI05077} mutant and trans-heterozygous mutants, measured with an OBBOO protocol. None of the heterozygous *ine*^{MI05077} mutants (6 flies tested) but all of the tested trans-heterozygous mutants (9 flies *ine*^{MI05077}/*trpl*^{302}, 4 flies *ine*^{MI05077}/*arr1*^{1}, and 2 flies *arr1*^{1}/*trpl*^{302}) showed oscillations similar to the oscillations observed in the homozygous mutants. Recordings at the right show magnified views of the ERGs during the first orange light pulse.

As a conclusion, our results indicate that electric oscillations in the ERG recordings of *trpl*^{302} or *arr1*^{1} mutant flies are not caused by the mutations in the respective gene, but instead by an additional mutation in the *inebriated* gene. Therefore, ERG oscillations observed in *trpl*^{302} or *arr1*^{1} mutants cannot be taken as an

indication for a possible role of TRPL and arrestin in the phototransduction or synaptic transmission of photoreceptor cells. In order to generate a pure *trpl* mutant without an *ine* second site mutation, we performed a meiotic recombination between the *trpl* and *ine* loci which are 48 cM apart from each other. Among the 13 stocks carrying a recombined second chromosome, five stocks inherited the $trpl^{302}$ mutation (Figure 3A). Among these stocks, one stock was devoid of oscillations (Figure 3B,C) indicating a loss of the *ine* second site mutation. This mutant is now called $trpl^{302NO}$ (No Oscillations) and can be obtained from the authors.

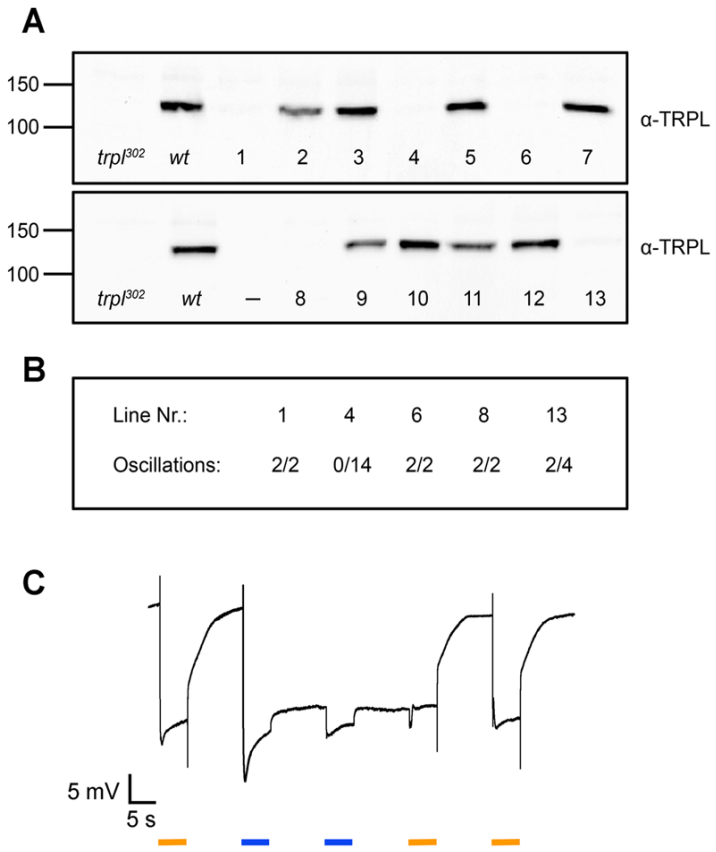

Figure 3. Generation of a pure $trpl^{302}$ mutant. (A) Stocks derived from recombination of the $trpl^{302}$ *cn bw ine* chromosome with a wild type chromosome (see Material and Methods) were analyzed for the $trpl^{302}$ mutation as indicated by the absence of a TRPL signal in Western-Blot analysis. (B) Stocks carrying the $trpl^{302}$ mutation were analyzed for the presence of oscillations in ERG recordings; fraction indicates the number of flies with oscillations/all flies tested. (C) ERG recording of a fly from recombination stock 4 using the OBBOO protocol. No oscillations could be detected.

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Cyp28AI gene variability in *Drosophila eremophila.*

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Abstract

We sequenced and analyzed gene *Cyp28A1* segment in cactophilic fly *Drosophila eremophila* samples obtained from the Mexican states of Guanajuato, Hidalgo, and San Luis Potosi. The results showed high

variability in this gene in *D. eremophila*. This suggests that *D. eremophila Cyp28AI* presents an important role in adaptation to the cactus host.

Introduction

Insects have developed resistance to xenobiotic compounds, including pesticides and allelochemicals ingested from the plants they feed on. The key to this adaptation lies within three detoxification gene super-families: esterases, cytochrome P450 monooxygenases, and glutathione *S*-transferases (Bono *et al.*, 2008).

Cytochrome P450 (CYP) is a family of genes present in all organisms, ranging from *Drosophila* to humans, each coding for an enzyme involved in the detoxification of xenobiotic compounds, as well as in the regulation of various metabolic processes involving hormones and fatty acids (Scott, 2008). Over 20,000 different CYP sequences have been found (Nelson, 2009).

Drosophila melanogaster has 84 different CYP enzymes, and here we evaluated sequence variation in one of these, *CYP28A1,* in the cactophilic species *D. eremophila. D. eremophila* is endemic to Mexico where it breeds in the soil soaked by necrotic cactus (Heed, 1989).

Materials and Methods

Samples and their localities are summarized in Table 1. Total genomic DNA was extracted from individual flies using DNeasy™ (QIAGEN Inc., Valencia, CA) kits. An 1100 bp segment of the *Cyp28AI* gene was amplified by PCR using the following forward and reverse primers: *5'* CTACTACTGCTGGGCCTCTTCTAT *3'* and *5'* CTACTACTGCTGGGCCTCTTCTAT *3'*. The amplifications were performed at 59°C temperature, using Bio Basic Inc. (Canada) *Taq* DNA polymerase. DNA sequencing was performed by Genomic Services, LANGEBIO, Irapuato, Gto., México. Sequences were proofread and aligned in Geneious Pro ver. 4.8.5 software (Biomatters Ltd) followed by manual editing. Analyses were performed on a 352 bp fragment. Phylogenetic analyses were performed using MEGA v. 6.06 software (Tamura *et al.*, 2013), and DNASP ver. 5.10.01 (Librado and Rozas, 2009) software was utilized to measure polymorphism.

Table 1. Summary of number of *Drosophila eremophila* from each locality.

Locality	Number of samples
Barranca de Metztitlán, Hidalgo, Mexico	1
Charcas, San Luis Potosí, Mexico	7
Irapuato, Guanajuato, Mexico	3
San Miguel de Allende, Guanajuato, Mexico	11

Results and Discussion

Cyp28AI is autosomally inherited, found on the third chromosome in *Drosophila mojavensis* (Muller element B). *Cyp28AI* of *Drosophila mettleri*, a close relative of *D. eremophila*, has five introns and with multiple heterozygous sites (Bono *et al.*, 2008). Our 352 bp fragment of *Cyp28AI*, also showed a number of heterozygotes (Bono *et al.*, 2008).

We detected 11 haplotypes and 7 variable sites (Table 2). The only amino acid change associated with these polymorphisms is present in position 279. The 279 residue of CYP28AI of *D. eremophila* can be Asn (10 samples) or Ser (12 samples) (Data not shown). Values for Tajima´s D, Fu and Li´s D, and Fu´s Fs were not significant. Neutrality tests detected no enrichment of rare alleles among the *D. eremophila Cyp28AI* segment ($P > 0.10$).

Table 2. Polymorphism statistics for 352 bp of *Cyp28A1* in *Drosophila eremophila.*

Species	N	S	K	h ± SD	π ± SD	Tajima's D	Fu and Li´s D	Fu'sFs
D. eremophila	22	7	11	0.922± 0.034	0.00685 ± 0.00066	0.81826	0.65029	-4.537

N, number of sequences; S, number of variable sites; K, number of haplotypes; h, haplotype diversity; π, nucleotide diversity.

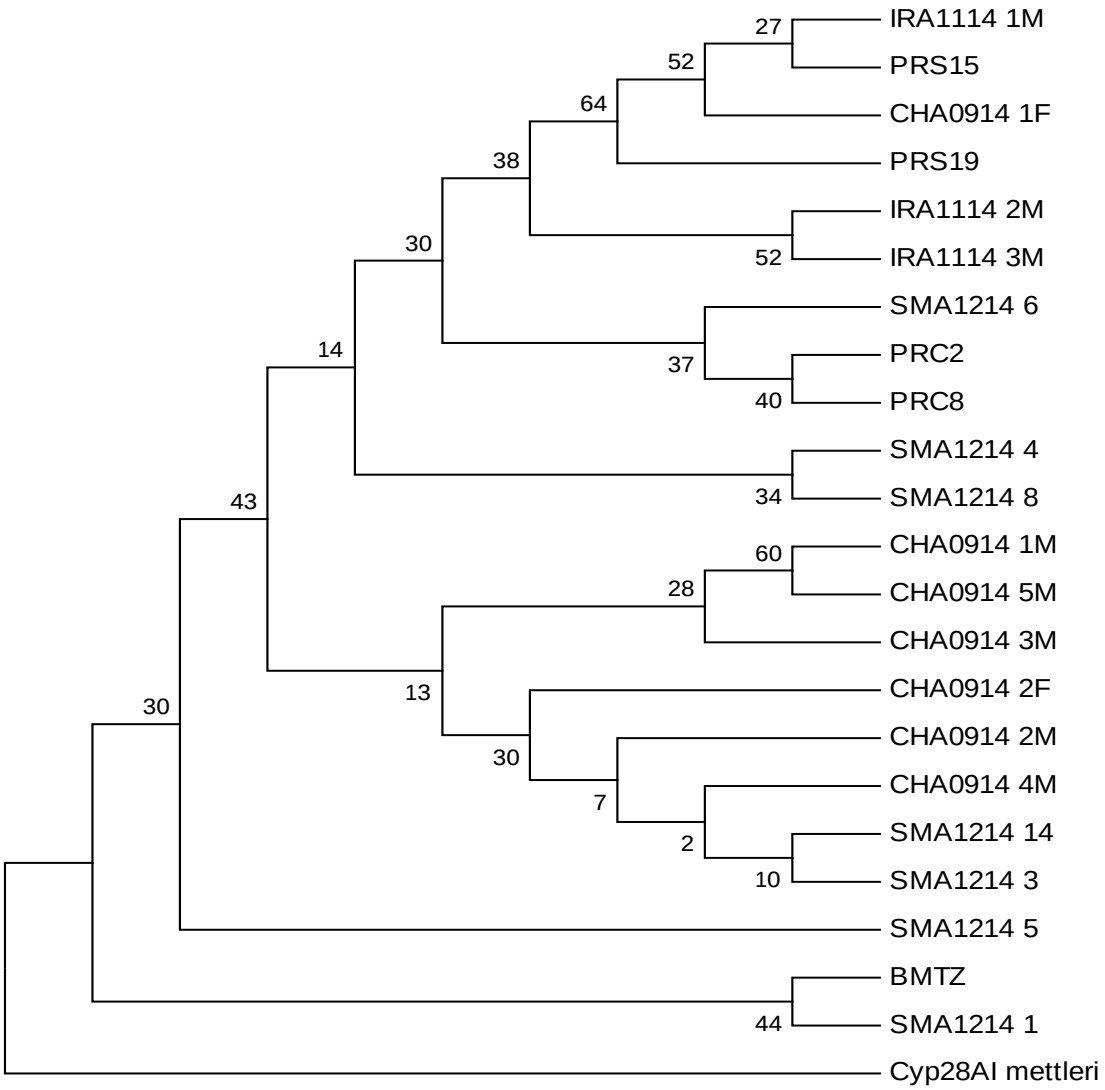

Figure 1. Phylogenetic tree of *Cyp28AI* in *D. eremophila*. (Maximum Likelihood). BMTZ (Barranca de Metztitlán, Hidalgo); CHA (Charcas, San Luís Potosí); IRA (Irapuato, Guanajuato); PRC, PRS, and SMA (San Miguel de Allende, Guanajuato).

We used the Maximum Likelihood method for phylogenetic analysis (Figure 1). The evolutionary model chosen was K80+I (Kimura, 1980). The tree was not well-supported (bootstrap = 2 to 60). We used the *Cyp28AI* of *D. mettleri* (GenBank: EU659006) as the outgroup to root the tree. Our data showed no population structure in the mainland for the 352 bp segment.

While our sample size is small, two things seem clear. One is that the sampled populations of *D. eremophila* comprise a large panmictic population. The other interesting outcome is that one of the substitutions causes an amino acid replacement, which may, upon further ecological work and additional sampling, suggest a degree of adaptation in this gene in *D. eremophila*.

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Co-expression of *Buffy* with *Buffy-RNAi* produces an intermediate phenotype in the *Drosophila* eye.

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Introduction

The use of ribonucleic acid interference (RNAi), a post-transcriptional gene silencing mechanism, to inhibit gene function is widely applied to analyse phenotypic consequences of gene suppression (Izant and Weintraub, 1984; Fire *et al.*, 1998; Ambesajir *et al.*, 2012). RNAi is an evolutionarily conserved cellular mechanism which is present in protozoa, fungi, nematodes, plants, flies and mammals (Agrawal *et al.*, 2003). This method is used in genome-wide screens (Dietzl *et al.*, 2007), functional genomics, genetic therapeutics, crop and animal improvements among many upcoming applications (Ambesajir *et al.*, 2012). In most of the organisms currently being used for studies, RNAi is systemic and cannot, therefore, be restricted to a specific cell type (Dietzl *et al.*, 2007). Using the bipartite UAS/Gal4 expression system (Brand and Perrimon, 1993), RNAi can be triggered in a spacio-temporal manner in *Drosophila melanogaster* (Dietzl *et al.*, 2007). Gene function can be analysed using an appropriate assay by examining the phenotypic effect of the directed inhibition (RNAi) or the overexpression of the gene. To investigate the phenotypic effects of directed overexpression upon the directed RNA interference of an important cell survival gene, we examined the consequences of the overexpression of *Buffy*, the sole pro-cell survival *Bcl-2* homologue (Quinn *et al.*, 2003), and a corresponding RNAi in the developing *Drosophila* eye. We investigated the possibility that an intermediate developmental phenotype can be generated from this interaction that may be subject to modification by other genes.

Materials and Methods

Drosophila stock and culture: *UAS-Buffy* (Quinn *et al.*, 2003) was kindly provided by Dr. Leonie Quinn (University of Melbourne). *UAS-Buffy-RNA*$_i$ (w[*]; P{w[+mC]=UAS-Buffy.RNAi}c3), *GMR-Gal4* (Freeman, 1996) and *UAS-lacZ* flies were obtained from the Bloomington Drosophila Stock Center at Indiana University. The *UAS-Buffy/CyO; GMR-GAL4* line was generated using standard recombination methods and was used to overexpress *Buffy* in the developing eye under the direction of the *GMR-Gal4* transgene. Stocks and crosses were maintained on standard medium containing cornmeal, molasses, yeast, and agar. Stocks were kept at room temperature (23^{o}C ± 2^{o}C) while crosses and experiments were carried out at 29^{o}C.

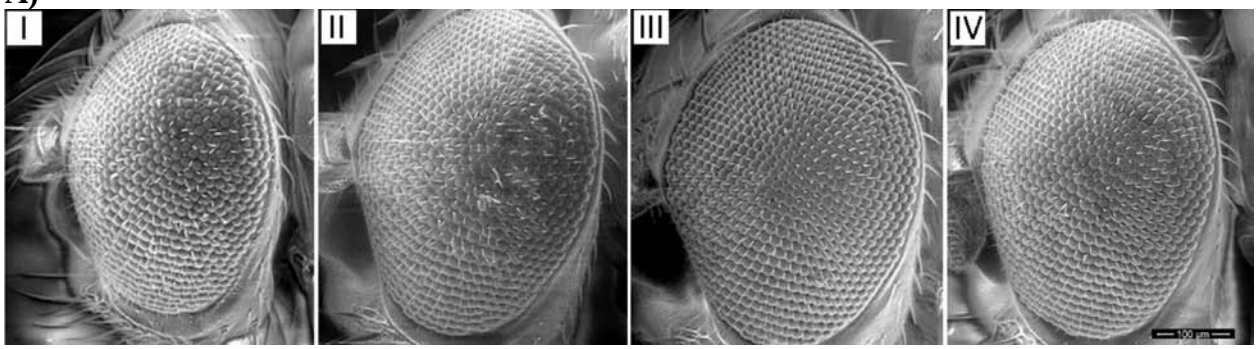
Biometric analysis of the Drosophila eye: A number of single vial crosses of each genotype were made at 29^{o}C, a cohort of the critical class male flies was collected upon eclosion and aged for three days before being frozen at -80^{o}C. Whole flies were mounted on scanning electron microscope stubs, desiccated overnight and photographed with a FEI Mineral Liberation Analyzer 650F scanning electron microscope. For each cross ten eye images were analysed using the National Institutes of Health (NIH) ImageJ software (Schneider *et al.*, 2012) and biometric analysis performed using GraphPad Prism version 5.04. The percentage

area of eye disruption was calculated as previously described (M'Angale and Staveley, 2012).

Results and Discussion

The directed expression of *Gal4* in the *Drosophila* eye at 29°C results in a roughened eye phenotype characterised by uneven, enlarged and fused ommatidia (Kramer and Staveley, 2003; Todd and Staveley, 2015). Analysis of scanning electron micrographs shows ommatidial disarray as a result of the expression of *Gal4* and the inhibition of *Buffy* in the developing eye (Figure 1A, I-II). *Gal4*-expressing flies show a disrupted ommatidia morphology, with 20% disruption of the eye (Figure 1B, I), whereas *Buffy-RNAi* flies display a much more severe phenotype of 45% disruption (Figure 1B, II). The co-expression of *Buffy* along with *Gal4* results in suppression of the roughened eye phenotype with a disruption of 3% (Figure 1B, III). The overexpression of *Buffy* along with its inhibition results in a disruption of the eye with a mean of 22% (Figure 1B, IV), intermediate between *Buffy* overexpression (3%) and its inhibition (45%). While similar to the control, this intermediate phenotype results from a balance of a rescue of the RNAi inhibition by the directed expression of *Buffy* and interference of the overexpression of *Buffy* by the RNAi transgene.

A)



B)

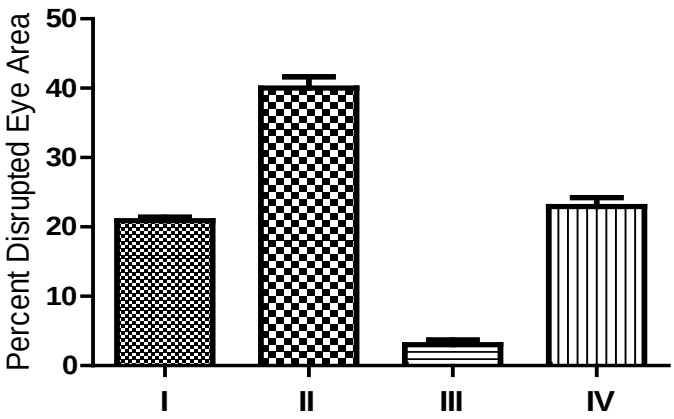


Figure 1. The phenotypic consequences of the directed expression and inhibition of *Buffy* in the Drosophila eye. A) Scanning electron micrographs of the eye of the following genotypes (I) *GMR-Gal4/ UAS-lacZ;* (II) *GMR-Gal4/ UAS-Buffy-RNAi;* (III) *GMR-Gal4 UAS-Buffy/ UAS-lacZ;* and (IV) *GMR-Gal4 UAS-Buffy/ UAS-Buffy-RNAi.* B) The biometric analysis of the eye showing the percent area of disruption (I-IV). There is suppression of the *Gal4*-expression phenotype by *Buffy* (III) and an intermediate phenotype when *Buffy* is overexpressed along with *Buffy-RNAi* (IV) as determined by one-way ANOVA and Dunnett's multiple comparison test ($P<0.05$ and 95% CI), error bars indicate the SEM and $n=10$. *(Data for GMR-Gal4/UAS-Buffy-RNAi is adapted from M'Angale and Staveley, under review.)*

Previously, our research group demonstrated the expression of the yeast transcription factor *Gal4* in the *Drosophila* eye results in apoptosis-dependent developmental defects of the ommatidial array (Kramer and Staveley, 2003). The overexpression of *Buffy*, a *Bcl-2* pro-cell survival homologue, results in the suppression of this phenotype, similar to the suppression of these developmental defects by *Pink1* (Todd and Staveley, 2015). These results suggest that the alteration of *Buffy* expression in the developing eye may subtly influence

neurogenesis. The overexpression of *Buffy* along with its inhibition results in disrupted area of the eye that is intermediate to the two extremes. Intermediate phenotypes are important in determining gene function, neuropathology of neurological diseases, and therapeutics (Civelek and Lusis, 2014; Leuchter *et al*., 2014). The inhibition of gene function by RNA interference relies on the degradation of the mRNA by the introduction of a dsRNA molecule (Boettcher and McManus, 2015). One consequence of using RNAi, for better or worse, is the generation of phenotypes that may or may not be the equivalent of null mutants

A priori, if the inhibition of *Buffy* is extremely efficient, coupled with directed overexpression of *Buffy* might be expected to generate a phenotype similar to *Buffy-RNAi* expression. Interestingly, the resulting intermediate phenotype reveals 1) that *Buffy* partially rescues the effects of *Buffy-RNAi*; 2) that *Buffy-RNAi* reduces the consequences of the directed expression of *Buffy*, and 3) that both transgenes are biologically functional. Alternatively, the overexpression of the pro-cell survival *Buffy* might be acting in a general manner to counteract the downstream effects of an overloaded "RNAi system", with the elevated levels of *Buffy* gene product being sufficient to abrogate the *Buffy-RNAi*-induced developmental eye defects. In conclusion, *GMR-Gal4* produces a cell death-dependent rough eye phenotype that can be suppressed by the pro-survival *Buffy*, enhanced by its loss of function, and the co-expression of *Buffy* along with its inhibition by RNAi results in an intermediate phenotype.

Acknowledgments: This research was funded by Department of Biology of Memorial University of Newfoundland Teaching Assistantships and School of Graduate Studies Fellowships to PGM and by a Natural Sciences and Engineering Council of Canada (NSERC) Discovery Grant to BES.

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Transferability of SRR primers developed for *D. mediopunctata* to the species *D. sturtevanti.*

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Drosophila sturtevanti (Duda, 1927) belongs to the *sturtevanti* subgroup of the *saltans* group (Magalhães, 1962). It presents a wide geographic distribution, occurring from Mexico to southern Brazil, including the Caribbean islands (Magalhães, 1962). Due to the high cost of developing microsatellite markers (SSR) for each species, the transferability of SSR primers between related species has been tested and used in population and evolutionary studies (White and Powell, 1997; Roa *et al*., 2000; Zucchi *et al*., 2002). The aim of this work was to analyze the transferability of SSR primers originally described for *D. mediopunctata* to *D. sturtevanti* (Laborda *et al*., 2009a). Twenty primers that were successfully amplified using a sample composed of a pool of individuals from an isofemale line of *D. sturtevanti*, were selected (Table 1).

Table 1. Microsatellite loci described by Laborda et al. (2009) that have been tested in populations of *D. sturtevanti*. Highlighted in bold are the ones that were amplified in *D. sturtevanti.*

Locus	Genbank acession	Size (bp)	Locus	Genbank acession	Size (bp)
Dmed^{UNICAMP}_033	GQ344866	245	Dmed^{UNICAMP}_096	GQ344919	152
Dmed^{UNICAMP}_034	GQ344867	115	Dmed^{UNICAMP}_099	GQ344922	299
Dmed^{UNICAMP}_039	GQ344872	182	Dmed^{UNICAMP}_102	GQ344925	197
Dmed^{UNICAMP}_041	GQ344873	286	Dmed^{UNICAMP}_107	GQ344930	179
Dmed^{UNICAMP}_053	GQ344883	122	**Dmed^{UNICAMP}_115**	**GQ344938**	**207**
Dmed^{UNICAMP}_054	GQ344884	214	**Dmed^{UNICAMP}_118**	**GQ344941**	**136**
Dmed^{UNICAMP}_056	GQ344886	142	Dmed^{UNICAMP}_121	GQ344943	144
Dmed^{UNICAMP}_057	GQ344887	137	Dmed^{UNICAMP}_124	GQ344946	156
Dmed^{UNICAMP}_065	GQ344892	198	Dmed^{UNICAMP}_126	GQ344947	291
Dmed^{UNICAMP}_095	GQ344918	147	Dmed^{UNICAMP}_133	GQ344951	245

The amplifications were performed under the same conditions and reagent concentrations reported in the literature (Laborda, *et al*., 2009b), using individuals of *D. sturtevanti* from Matão/São Paulo/Brazil (21°37’14”S; 21°37’14”W), Nova Granada/ São Paulo/Brazil (20°32’37”S; 49°14’47”W) and João Pessoa/Pernambuco/Brazil (7°3'49”S; 34°51'25”W). Only two loci amplified in 15 individuals out of 45 tested (Figure 1), even after applying protocol modifications to increase the transferability success.

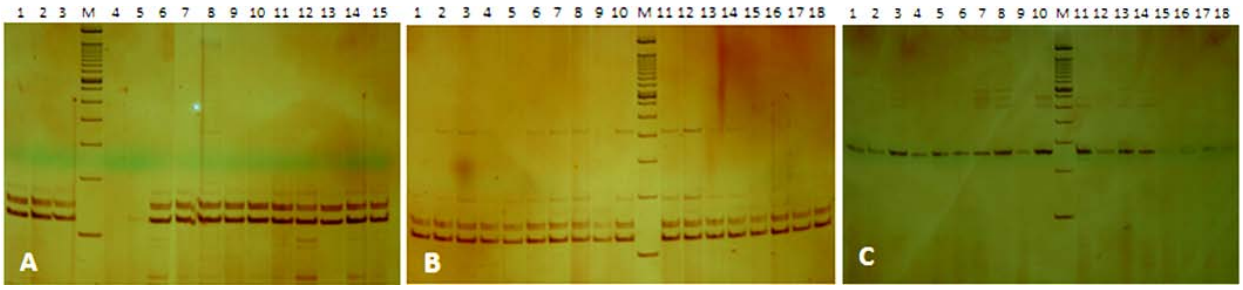

Figure 1. PAGE 6% of microsatellite DNA loci amplified on *Drosophila sturtevanti*. M: Molecular weight marker of 100/100 bp; A. Locus SSR118 – Matão/SP; B. Locus SSR118 – Pernambuco/PB; C. Locus SSR115 – Pernambuco/PB.

The optimal annealing temperatures for these two loci were tested. However, no pattern was obtained. Despite the original amplification of the 20 loci using a pool sample of one isofemale line of *D. sturtevanti*, our work showed that their transferability in individual samples of natural populations is rather difficult. *Drosophila mediopunctata* and *D. sturtevanti* belong to different subgenera, being, therefore, phylogenetically distant. Thus, throughout both species evolutionary processes, mutations could have become fixed in the flank region of the SSR loci, resulting in null alleles, which could be responsible for the low transferability (only 10%). Previous work using some of these loci described for *D. mediopunctata* in other two species of *Drosophila*, *D. maculifrons* and *D. ornatifrons*, which are more closely related to *D. mediopunctata* than *D. sturtevanti*, also showed that as more distant the species is, the less successful is the transferability. *Drosophila maculifrons* is more closely related to *D. mediopunctata* than *D. ornatifrons*, and the rates of

transferability were 50% and 28%, respectively (Tractz *et al*., 2012). Therefore, the tested markers were not suitable for genetic variability analyses of *D. sturtevanti* natural populations, requiring more tests with other primers or the use of strategies for obtaining and synthesizing specific primers for this species.

Keywords: saltans group, molecular markers, genetic variability, microsatellite DNA, transferability.

Financial support: FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) 2014/14059-0; Bolsista CAPES.

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Drosophilids from the Font Groga site (Barcelona, Spain): a new collection.

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On 6th October 2015, we obtained a new collection of drosophilids from the Font Groga site (Barcelona, Spain). This location presents excellent environmental conditions for the study of drosophilid diversity, and a complete description can be found in Araúz *et al.* (2009). This research completes the studies of Canals *et al.* (2013), Pineda *et al.* (2014), and Esteve and Mestres (2015). The results of our collection are presented in Table 1.

Table 1. Number and percentage for each species and sex collected from Font Groga site (Barcelona, Spain) on 6th October 2015.

Species	Number	Percentage
D. subobscura (♂)	107	3.14
D. subobscura (♀)	155	4.55
D. simulans (♂)	836	24.52
D. melanogaster (♂)	16	0.47
D. menalo/simulans (♀)	1953	57.29
D. suzukii (♂)	86	2.52
D. suzukii (♀)	220	6.45
D. cameraria (♂)	13	0.38
D. cameraria (♀)	20	0.59
D. phalerata (♂)	1	0.03
D. buzzatii (♀)	1	0.03
Scaptomyza sp.	1	0.03
Total	3409	100

Comparing these data with those previously obtained in 2012, 2013, and 2014 (Canals *et al.*, 2013; Pineda *et al.*, 2014; and Esteve and Mestres, 2015), it is worth noting the large fluctuation of the frequency of species belonging to *melanogaster* group (*D. melanogaster* and *D. simulans*). This frequency was high in 2012 (81.01%) and again in the present sample (82.28%), whereas it was 32.14% and 38.94% in 2013 and 2014, respectively. Usually, a hot and dry summer produces an increase of this group frequency during autumn. However, *D. subobscura* shows the opposite behavior, being abundant in autumn if the summer has been relatively cold and humid. It was scarce in the present sample (7.69%) and in 2012 (6.85%), but reached 62.60% and 30.53% in 2013 and 2014, respectively. It is interesting to observe the relative abundance of the invasive species *D. suzukii*, presenting similar values in the present research (8.97%) and also in 2012 (9.20%) and 2013 (7.98%). However, its frequency had a peak in 2014 (20.35%). Finally, commenting that for the first time we didn't find *D. immigrans* in the Font Groga site, but a *D. buzzatii* female was sampled for the first time.

With data of Table 1, we computed the *H'* (Shannon diversity index) and *J* (Shannon uniformity index), their values being 0.62 and 0.35, respectively. These values are the lowest recorded in our time series

at the Font Groga site. Likely, this is due to the relative high abundance of individuals belonging to the *melanogaster* group.

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Metabolic activity of diuron by *Zea mays* detected through the wing spot assay in *Drosophila melanogaster*.

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Abstract

Diuron is a phenylurea herbicide compound amply used in agriculture to control a wide variety of annual and perennial broadleaf and grass weeds, algae, and mosses. The aim of the present study was to evaluate the genotoxicity of diuron in the wing spot assay of *Drosophila melanogaster* after metabolic activation of an aqueous extract from the roots of *Zea mays* treated with different concentrations of the herbicide. Bentazone was used as positive control. The wing spot assay that assesses for somatic mutation and recombination events was carried out with standard (ST) and high-bioactivation (HB) crosses given chronic treatment to third instar larvae. Results showed that larvae treated with the aqueous extracts caused a similar positive response in both crosses.

Introduction

Diuron is an herbicide used to control a wide variety of weeds affecting maize, sugarcane, cotton, sorghum, among other crops and for fallow and idle cropland use. Diuron also has a widespread use in non-agricultural applications like industrial and rights of way uses. Due to its chemical structure, diuron has been classified as a substituted phenylurea compound. This compound can be readily taken from soil by the root system of plants and translocated into stems and leaves moving primarily via the xylem. The mechanism of action of diuron is through inhibiting photosynthesis by blocking the electron transfer at photosystem II (Wessels and van der Veen, 1956). This compound is able to bind to D-1 protein located at the reactive center of photosystem II (Arnaud *et al*., 1994; Duke, 1990).

The basic metabolism of phenylureas include N-demethylations followed by oxidation of aromatic groups (Engelhardt *et al*., 1972). In mammal cells, diuron is mainly metabolized through de-alkylation of methyl-urea groups (Abass *et al*., 2007). Diuron is capable of increasing cytochrome P450 enzymes (CYPs) activity as well as other enzymes including glutathione-S-transferase, epoxide hydrolase, and UDP-glucuronyl transferase (Schoket and Vincze, 1985, 1990). In plants, phenylureas are metabolized through N-demethylation of the nitrogen atom and hydroxylation of the aromatic group (Fonné-Pfister and Kreuz, 1990). It has been demonstrated that some enzymes belonging to CYP71 and CYP76 families are involved in diuron metabolism in plants (Fonné-Pfister and Kreuz, 1990; Höfer *et al*., 2014; Robineau *et al*., 1998; Siminszky *et al*., 1999).

Many pesticides are promutagens and become active after metabolic biotransformation by plants (Plewa, 1978). Herbicides and their metabolites represent potential health risks to humans since they are applied to food crops and may exert a genotoxic effect when they are consumed by the population. The US Environmental Protection Agency has listed diuron as a likely human carcinogen (US EPA, 1997, 2004). Also, several studies have demonstrated that diuron is a genotoxic and carcinogenic compound (Akcha *et al*.,

2012; Canna-Michaelidou and Nicolaou, 1996; Cardoso *et al*., 2010; Huovinen *et al*., 2015; Rodríguez-Arnaiz *et al*., 1989; Seiler, 1978); thus, it is important to determine the biological mechanisms involved in such processes.

In this work we wanted to determine the genotoxic properties of diuron metabolites produced by maize roots exposed to this compound by means of the wing spot assay in *Drosophila melanogaster*.

Materials and Methods

Chemical compounds

Diuron, (Koa 80WDG®) (3-(3,4-diclorofenil)-1,1-dimetilurea), Makhteshim Agan, Mexico, and Bentazon (Basagran 480 - 3-isopropil-1H-2,1,3-benzotiadiazin-4 (3H)-ona 2,2-dioxide) BASF Mexicana, were purchased from "El Sembrador" pesticide store, Mexico City.

Plant material

Maize white seeds (*Zea mays)* were obtained from "Molino de chiles y harinas El Pesado" at Santa Úrsula local market, México City.

Toxicity of Diuron

The median lethal concentration (LC_{50}) of diuron was obtained by treating third instar larvae with diuron. The herbicide was prepared at the following concentrations (mM): 31, 62.3, 125, 250, 500, and 1000. Larvae were treated in vials with the herbicide for 48 h. Three replicas were performed. With the obtained data of mortality, a polynomial regression was performed with $R^2 = 0.9739$ and a final value for the LC_{50} of 162.6 mM. However, we observed a significant increase in the duration of larval development and size reduction when larvae were exposed to concentrations above 125 mM. In order to avoid this issue, we tested 30, 60, and 120 mM concentrations.

In vivo metabolic activation of diuron by Zea mays

Five day-old primary *Zea mays* roots 3 - 5 cm long were immersed in different concentrations of diuron for 4 hours at room temperature in dark conditions. The concentrations of diuron used were 30, 60, and 120 mM. The herbicide bentazone [basagran 48%] was used as a positive control. This herbicide has been demonstrated to be genotoxic in *Drosophila* (Kaya *et al*., 2004). After treatment, the primary roots were washed with deionized water. The 3 cm tips of the main roots were homogenized at 4°C in PBS buffer, pH 7.4. The ratio of the volume of buffer solution to fresh weight of roots was 1:1 (Calderón-Segura *et al*., 2007; Takehisa *et al*., 1988). The homogenate was centrifuged at 10,000 × *g* for 15 min at 4°C. The extracted supernatant was measured for protein concentration using a Beckman spectrophotometer prior to being used in *Drosophila melanogaster* third instar larvae (Bradford, 1976).

Fly stocks and crosses

Two different stocks of flies were used, both carrying visible wing genetic markers on the third chromosome, multiple wing hairs (*mwh*, 3-0.3) and flare (*flr*^{3}, 3-38):

(1) *flr*^{3}*/In (3LR) TM3, ri p*^{p} *sep l(3)89Aa bx*^{34e} *e Bd*^{S} (*flr*^{3}/TM3, *Bd*^{S})

(2) *mwh/mwh (mwh)*

Two crosses were used, ST and HB. The ST cross was performed by mating flr^3/TM3, Bb^S females to *mwh/mwh* males. The HB cross was performed with ORR/ORR; flr^3/TM3, Bb^S females mated with *mwh/mwh* males. The ORR strain has chromosomes 1 and 2 from a Dichloro-Diphenyl-Trichloroethane (DDT)-resistant Oregon line (OR-R), which constitutively overexpresses *Cyp* genes (Graf and Van Schaik, 1992; Graf *et al*., 1984, 1989).

Wing spot assay

Eggs from both crosses were collected over 8 h into culture bottles containing a solid agar base (5%, w/v) covered with a layer of live fermenting yeast supplemented with sucrose. After 72 ± 3 h, the third instar larvae were washed from the culture bottles with a solution of 20% (w/v) sucrose and fed for the rest of the

larval development stage (approx. 48 h) with the maize aqueous extracts. For this chronic feeding, the larvae were put in vials containing 0.85 g of Drosophila Instant Medium (formula 4-24; Carolina Biological Supply, Burlington NC, USA) hydrated with the aqueous extracts. After eclosion, the adult flies were collected and stored in 70% ethanol. The wings of the transheterozygous flies from both crosses were mounted on slides and coded before scoring, at a magnification of 400×, for the presence of cell clones showing mutant wing hairs expressing *flr* or *mwh* markers (spots). The different types of spots result from different genotoxic mechanisms: point mutations, deletions, and mitotic recombination. To evaluate the genotoxic effects recorded, the frequencies of spots per wing in a treated series were compared with those of the concurrently processed negative and positive control series. These statistical comparisons were made using a computer program written by Zordan (unpublished), which uses the χ^2 test for proportions, followed by a multiple-decision procedure (Frei and Würgler, 1988, 1995). Statistical analyses were performed for single, large, twin, and total numbers of spots recovered.

Figure 1.

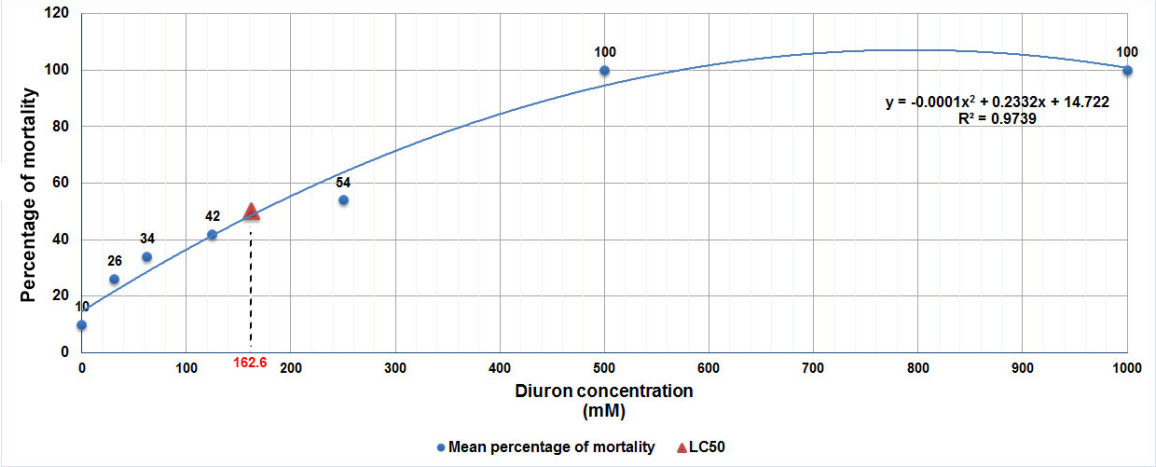
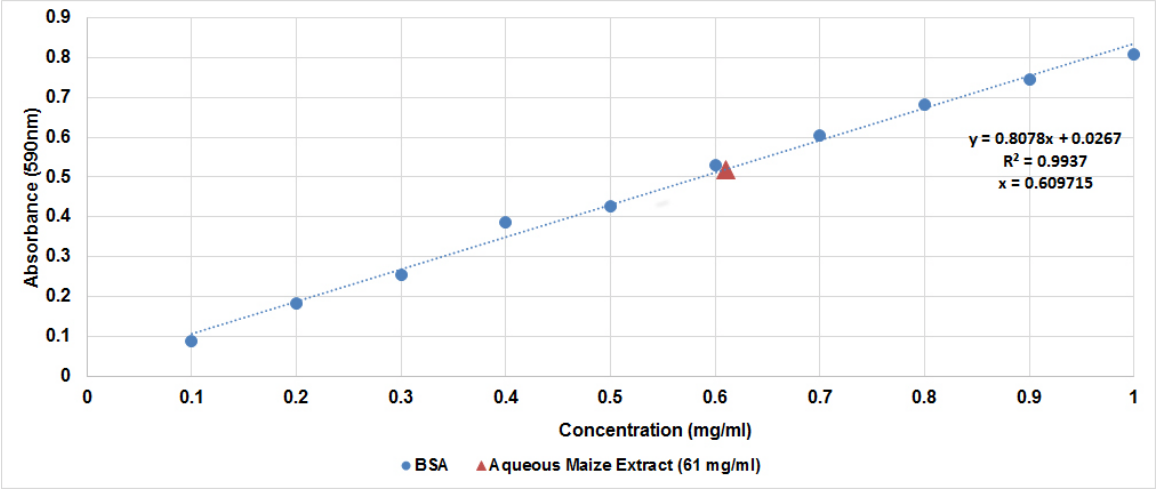


Figure 2.



Results

Figure 1 shows the percentage of toxicity induced by diuron. It can be noted that the LD_{50} was 162 mM. The protein concentration of the maize aqueous extracts in the three replicas assayed was almost constant at 61 mg/ml (Figure 2). A summary of the results obtained in the ST and HB progeny after treatment with the maize aqueous extract and the positive bentazone control is shown in Table 1. Notably, the induction of all types of spots is very similar for both crosses, the majority being small single spots. The positive control

bentazone also gave similar results independent of the type of cross. Comparison of the induced effects between both types of treatment shows that the treatment of larvae with the maize extract reduces the number and frequency of small single and total spots at the highest diuron concentration in the ST cross, but not in the HB cross. Despite this reduction, a positive effect in total spot frequency at all concentrations used was found. Thus our study shows that chronic treatment of third instar larvae produced similar dose-response effects in both crosses.

Table 1. Summary of results obtained in trans-heterozygous progeny of ST and HB crosses after chronic treatment of larvae with the maize extract treated with Diuron.

		Spots per fly (Number of spots) Statistical diagnoses[b]					
Concentration (mM)	Cross [a] and Number of flies	Small single spots (1-2 cells) m = 2	Large single spots (>2 cells) m = 5	Twin spots m = 5	Total Spots m = 2	Spots with mwh clone	Mean number of cell division cycles
	ST						
Negative Control	60	0.35 (21)	0 (0)	0 (0)	0.35 (21)	21	1.48
Bentazone	60	0.43 (26)i	0.12 (7)+	0.05 (3)i	0.60 (36)+	36	1.89
30	60	0.52 (31)i	0.08 (5)+	0.05 (3)i	0.65 (39)+	39	1.74
60	60	0.57 (34)i	0.08 (5)+	0.07 (4)i	0.72 (43)+	43	1.93
120	60	0.55 (33)i	0.05 (3)i	0.03 (2)i	0.63 (38)+	38	1.76
	HB						
Negative Control	60	0.28 (17)	0.05 (3)	0 (0)	0.33 (20)	20	1.65
Bentazone	60	0.62 (37)+	0.05 (3)i	0.03 (2)i	0.70 (42)+	42	1.62
30	60	0.50 (30)+	0.20 (12)+	0.03 (2)i	0.73 (44)+	43	2.23
60	60	0.73 (44)+	0.07 (4)i	0.02 (1)i	0.82 (49)+	49	1.73
120	60	0.72 (43)+	0.10 (6)i	0.02(1)i	0.83 (50)+	50	1.76

[a] ST standard cross; HB high bioactivation cross. [b] Statistical diagnoses according to Frei and Würgler (1988; 1995), m: minimal risk multiplication factor for the assessment of negative results. For the final statistical diagnoses of all outcomes: + = positive; - = negative; = inconclusive and w+= weakly positive with the standard SMART software based in the conditional binomial test according to Kastenbaum-Bowman significance levels (a = b = 0.05) (Frei and Würgler,1988). The non-parametric Mann-Whitney Wilcoxon U-test with significance levels (a = b = 0.05; one-sided) was used in order to exclude false positive or and negative diagnoses (Frei and Würgler, 1995).

Discussion

To evaluate the genotoxicity of diuron comparatively, the larvae of ST and HB crosses were directly treated with the maize aqueous extract of the herbicide. The HB cross is characterized by a higher sensitivity to progenotoxins, because the ORR *flr*^{3}/TM3, *Bd*^{S} strain carries chromosomes 1 and 2 from a DDT-resistant Oregon R-(R) line, which has an increased level of CYPs (Graf and Van Schaik, 1992). The majority of spots recovered after treatment were single spots of one or two cells, which could be due to induced genotoxicity at a late stage of development of the wing imaginal discs due to delayed metabolism. In this study, twin spots were not significantly produced at the concentrations assayed. In mammals diuron is able to induce CYP expression of the isoform CYP3A4 (Abass *et al*., 2012).

Herbicide metabolism in plants is largely due to the biotransformation of substrate by CYP450 enzymes. These compounds are absorbed by the roots and accumulate in the meristem. Soybean CYP71A10

catalyses the metabolism of phenylurea herbicides, thus converting the herbicides of this class to more polar compounds. This enzyme functions as a N-demethylase for diuron (Siminszky *et al*., 1999). In *Arabidopsis thaliana*, the CYP76C subfamily is involved in both natural and xenobiotic metabolism, particularly for phenylurea herbicide biotransformation (Höfer *et al*., 2014). In *Zea mays*, a CYP71C family cluster of four CYP genes was first described by Frey and collaborators (Frey *et al*., 1995). The genes are located on the tip of the short arm of chromosome 4 and are involved in biosynthetic pathways of secondary metabolites (Dutartre *et al*., 2012). Because plants do not have an excretion system, we were interested in studying the effects of maize metabolism on the capability of CYPs from *Zea mays* to modulate genotoxicity to diuron, particularly because plants accumulate secondary metabolites from herbicides. Our results showed a similar dose-response effect in both crosses, hence, aqueous extracts of the roots of *Zea mays* caused similar results in both crosses. Thus, no differences were observed between crosses after extract treatment, a result that probably is due to a lack of post-biotransformation in larvae and suggests that the biotransformation of the secondary metabolites is probably due to the CYP71C family of maize. Although plants and animals have phase I and II enzymes (Parkinson and Ogilvie, 2008), conjugated metabolites are generally excreted in animals, but are stored in plants (Sandermann, 1988, 1992). These important differences could be related to the results obtained in this study. Accumulation of the secondary metabolites in maize roots produced a similar effect in both fly crosses due to both having received the same secondary metabolites produced by the biotransformation of diuron in maize. This effect could be related to the metabolism of other reported pesticides (Abass *et al*., 2007, 2010, 2012; Buratti *et al*., 2003; Croom *et al*., 2010; Foxenberg *et al*., 2007; Leoni *et al*., 2008; Mutch and Williams, 2006; Poet *et al*., 2003; Usmani *et al*., 2004).

The positive control bentazon was genotoxic in both crosses and showed a similar trend. Furthermore, maize metabolism did not modulate the biotransformation of bentazon. Our results are quite different from those reported in 2008 by Heres-Pulido and collaborators using wheat extracts. We found similar induction of total spots, independent of the cross that was used (ST 0.60 *vs.* HB 0.70), whereas they found significant differences (ST 0.82 *vs*. HB 1.62).

Conclusions

Maize aqueous extract was shown to be genotoxic at every concentration used for both crosses in the wing spot assay. No dose-response effect was observed. These results indicate that maize roots are able to biotransform diuron and produce metabolites capable of causing the same level of genotoxic effects in the ST and HB crosses of *Drosophila melanogaster*.

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Drosophila species associated with fresh chili peppers.

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Capsicum baccatum var. *Pendulum* (Willd.) Eshbaugh (The plant list 2016) (Solanaceae) is popularly known as "lady's finger chili" or Cambuci chili, in Brazil, or Ají, in Peru. This species is one of the five species belonging to this genus (Eshbaugh, 1968; 1970) and is native to South America, being found in lowlands of Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, and Peru (Jarrett, 2007).

In June of 2016 some Drosophilidae were observed in a sample of *C. baccatum* from a farm located in Alvorada, Rio Grande do Sul, Brazil (30° 0' 1" S, 51° 4' 42" W). This farm has a chili plantation and swine facilities.

The fruits were collected and placed in 500 mL vials containing vermiculite and plugged with synthetic foam stoppers (2 fruits per vial). The vials were kept in an incubator with constant temperature (21±1ºC), humidity (60% rh), and photoperiod (12h:12h). Emerged adults were transferred to vials with

cornmeal medium (Marques *et al*., 1966). The vials with fruit were maintained in the incubator for six weeks. In addition, some *C. baccatum* fruit bought at a local supermarket received the same treatment. The emerged individuals were sexed and identified to species based on external morphology.

Two species emerged from both fresh and rotten *Capsicum* fruits from the plantation: *D. repleta* Wolaston (*Drosophila* subgenus, *repleta* group) (23 males and 8 females) and *D. busckii* Coquillett (subgenus *Dorsilopha*, *busckii* group) (334 males, 355 females). Nothing emerged from fruits bought at the supermarket. In addition to that, we tried raising both species in medium containing the chilies bought in the supermarket, without success. Neither deposited eggs in those fruits.

Drosophila species mostly breeds in rotten leaves, decaying or fermenting fruit, fungi, flowers, sap, and pollen (review in Throckmorton, 1975). It is not common to Drosophilidae species to breed in fresh fruit. *Drosophila suzukii* Matsumura is an exception and considered a pest since it devastates crops of several types of berries (Walsh *et al*., 2011, Cini *et al*., 2012).

Drosophila busckii belongs to *Dorsilopha* subgenus and is a cosmopolitan species (Parsons and Stanley, 1981) with records in the five continents (Bächli, 2016). Pavan (1959) claimed that this species is associated with human populations in South America. According to Powell (1997), "*D. busckii* probably has the broadest niche of any Drosophilidae". This claim is in agreement with Sturtevant (1921), who described various substrates in which he reared this species: bread and milk, moist bran, rotten pigeon egg, stale formalinized chicken, sour milk, spinach leaves, banana, flour paste, decayed onions, rotten fish, rotten potato, tomato, and fungi.

Drosophila repleta is also a cosmopolitan species (Patterson and Stone, 1952; Parsons and Stanley, 1981). More than that, this species became a domestic species, since it is associated with human habitats (Powell, 1997). Larvae of *D. repleta* feed on yeast from decaying fruits and fermenting organic matter (Wegner, 2007). According to Hottel *et al.* (2015), these flies became pests in several sites where food residue is present. *Drosophila repleta* can also be a pest in swine and poultry facilities, where they feed on spilled animal food (Harrington and Axtell, 1994).

Given the habits of these species, it is possible that they were primarily attracted to the swine facilities and used the chili fruits as an alternative for breeding. Should *Drosophila busckii* and *Drosophila repleta* be considered chili crop pests, since they breed in fresh fruits, even with other available breeding sites? Or just occasional fresh fruit breeders? Still, why did the flies fail to breed in the chili fruits acquired in the supermarket? Could it be due to pesticides applied in large commercial crops?

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Preliminary report on electrophoretic pattern of acid phosphatase of a few species belonging to *ananassae* subgroup of *Drosophila* in Dharwad District.

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Abstract

Acid phosphatase in 3 different species of *Drosophila* belonging to *ananassae* subgroup collected from Dharwad district was studied using native page. The isozyme analysis revealed that there are about 9, 8, and 7 different patterns in *D. ananassae*, *D. bipectinata*, and *D. malerkotliana*, respectively. Each pattern of all the species analyzed differs in their number of bands.

Introduction

Isozymes are the multiple forms of enzymes that are specially considered as examples of the structural variations that occur in proteins. These enzymes catalyze the same chemical reaction but differ in amino acid sequence (Kaplan, 1968; Latner, 1970). Genetic variations at different enzyme loci help in characterizing a strain or a species and are analyzed by gel electrophoresis (Ausubel *et al.*, 1993).

Acid Phosphatase (Acph) is a lysosomal enzyme, which is involved in dissociation of phosphoryl groups from other molecules during digestion. It is a ubiquitous enzyme commonly found in all or most all tissues of the body. In *Drosophila* it is found in second and third instar larvae, pupae, and adults. In general, pupae seem to have the highest amount of enzyme activity among all developmental stages. Acid phosphatase has been used by earlier workers to analyze the genetic variations in *Drosophila* (Beckman and Johnson, 1964; MacIntyre, 1966; Prakash *et al.*, 1969; Norman and Prakash, 1980; Kojima *et al.*, 1970; Ayala *et al.*, 1974; Hyytia *et al.*, 1985). Studies on genetic variations of acid phosphatase in *ananassae* subgroup were done by Hegde (1979) and Nagaraj (1985) in different populations of *D. ananassae* (Chitradurga, Sagar, Mysore, Kemmangundi, Jog falls, Dimbam, Kerala, Poona, Bombay), *D. malerkotliana* and *D. bipectinata* (Sirsi, Mavinagundi, Malemane, Karwar). There is no information on genetic variation with respect to acid phosphatase in natural populations of *Drosophila* in Dharwad District. Most of the areas studied by the earlier workers come under the forests of Western Ghats region, whereas geographically Dharwad is considered as a transition zone where western zone consists of deciduous forests and eastern zone consists of plain (arid) lands. Hence the present investigation on genetic variations among three species of *ananassae* subgroup was undertaken.

Materials and Methods

a. Collection of flies

Drosophila flies were collected from different localities of Dharwad district. The study area includes different habitats such as domestic places, fruit markets, agriculture fields, and forests. The wild males collected from natural populations were directly used for the present work. Single fly homogenate was prepared in 40% sucrose solution and stored at 4°C. The samples were prepared as per the procedure described by Bayrami *et al*. (2010) with slight modifications. A total of 10 samples were prepared per locality.

b. Electrophoretic analysis (NATIVE PAGE)

Native polyacryl amide gel electrophoresis of (7.5%) 1 mm thickness was prepared. 20 µl of the sample was loaded into each slot of the stacking gel. For the first slot, Bromophenol blue is added as the tracking dye. Electrophoresis was performed at 4°C with 50 volts for 1 hour and later increased to 80 V until the dye migrated to 7 cm in the small pore running gel. NaOH – Boric acid (0.3M) was used as tray/ tank

buffer (Bayrami *et al*., 2010). After electrophoresis the gels were stained for acid phosphatase using suitable substrate. The staining was carried out at 37°C.

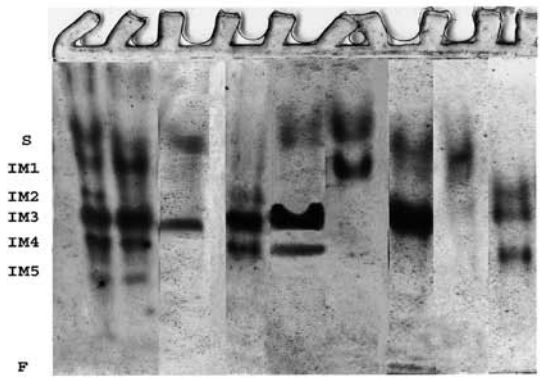

Figure 1a

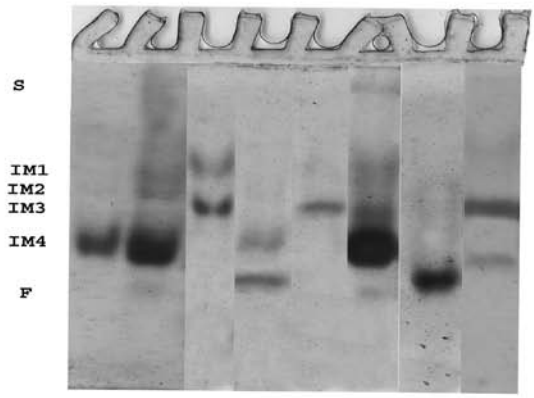

Figure 1b

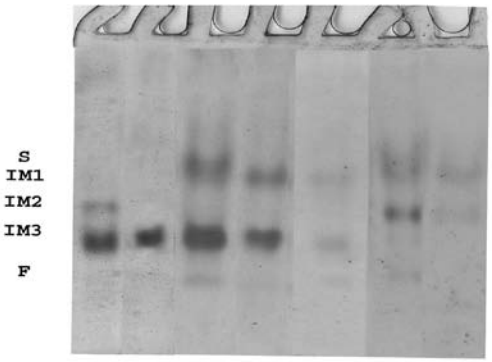

Figure 1c

Figure 1. Native Page Analysis of Acid Phosphatase in different populations of *Drosophila*. a, *D. ananassae*; b, *D. bipectinata*; c, *D. malerkotliana*; S – Slow; IM – Intermediate; F – Fast.

Results

Figure 1a shows the whole body homogenate native page analysis of acid phosphatase in case of *D. ananassae*. It revealed that there are about 9 different patterns. Each pattern differs in number of bands. The total number of bands varies from 1 – 7 based on their mobility and were named as F (Fast), IM (Intermediate)$_{1\text{-}5}$, S (Slow). Pattern 1 has 6 bands, pattern 2 has 4, patterns 3, 5, and 6 have 2, patterns 4, 7 and 9 have 3 bands, and pattern 8 has 1 band. Pattern 1 has the highest, whereas pattern 8 has the lowest, number of bands.

A total of 8 types of patterns were found in *D. bipectinata*. The total number of bands varied from 1 – 6. Patterns 1, 5, and 7 have 1 band; pattern 2 has 4; patterns 3, 4, and 8 have 2; and pattern 6 has 5 bands. Pattern 6 has the highest number of bands whereas patterns 1, 5, and 7 have the lowest number of band (Figure 1b).

D. malerkotliana revealed that there are about 7 different types of patterns; each pattern differed in number of bands (Figure 1c). The total number of bands based on their mobility varied from 1 – 5. Patterns 1, 4, and 7 have 2 bands; pattern 2 has 1; patterns 3 and 6 have 4; pattern 5 has 3 bands. Patterns 3 and 6 had the highest number of bands, whereas pattern 2 had the lowest number of bands.

Discussion

The bands can also be classified based on their movement towards the anode. The S, F, and IM bands represent different banding patterns (Beckman and Johnson, 1964). Studies on molecular variation were based on the genetic basis of allelic frequency of the loci. Ayala *et al*. (1972 a, b) observed allelic variations at 28 and 27 gene loci in natural populations of *D. willistoni* and *D. equinoxialis* from Mexico, Florida, and South

America. They found a great deal of genetic variation, and on an average 58% of loci were found to be polymorphic for *D. willistoni* and 71% polymorphic for *D. equinoxialis*. Acid Phosphatase was also studied in adult *D. melanogaster* and *D. simulans*, which revealed three types of variants in both the species (MacIntyre, 1966). The present study differs from the studies of Hegde (1979) as they have reported a total of 7 bands in *D. ananassae*.

Nagaraj (1985) reported 3 and 2 patterns of acid phosphatase in *D. bipectinata* and *D. malerkotliana*, respectively, from Uttara Kannada district. Dharwad, which is an adjacent district, showed 8 and 7 different patterns. The present study showed more patterns and bands in each pattern compared to earlier studies of Hegde (1979) and Nagaraj (1985). This provides evidence for the polymorphic nature of the acid phosphatase enzyme in *D. ananassae*, *D. bipectinata*, and *D. malerkotliana.*

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Age and sex related change in the heritability of locomotor behavior in *Drosophila melanogaster.*

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Abstract

Locomotor behavior is a crucial and fitness-related trait which has a polygenic basis. Here in this study we estimated basic quantitative genetic parameters of locomotion using isofemale lines of *D. melanogaster*. Negative geotaxis and startle response were used as component traits defining locomotion. We have estimated narrow sense heritabilities and its components for three different age groups for both sexes. Our results show that these indices can change with age and sex, though differently for geotaxis and startle response. Change in heritability of negative geotaxis with age was more or less negligible, whereas the heritability for startle response decreased with age. We infer this difference between the traits in amount of change they had with increasing age could indicate that the putative genes influencing additively each trait phenotype are distinct, and, accordingly, act differently. **Keywords:** Locomotion, aging, genetic variance, heritability, *Drosophila*

Introduction

Locomotor behavior is one the most important evolutionary features of an organism affecting its feeding, mating success, dispersal ability under normal or stresfull conditions, and its ability of predator

avoidance (Alexander, 2006). Locomotion is a complex trait in the sense of quantitative genetics and has been the subject of several genetics and genomic studies focusing on its genetic architecture (Anholt and Mackay, 2010). Decline in locomotor activity of *Drosophila* with age is an indication of functional senescence in which negative geotaxis, a component of locomotion, has been shown to be severely reduced by increasing age (Grotewiel *et al*., 2005). Differences in the level and pattern of decline in locomotor activity with age among the different lines of *Drosophila melanogaster* suggest that the pattern of locomotion with age may be genetic (Fernandez *et al*., 1999). Here in the present study, we measured the change in locomotion of *D. melanogaster* inbred lines with age and calculated the narrow sense heritabilities for both sexes per age category in order to see if the pattern of change with age could have a genetic component.

Material and Methods

Lines

For the negative geotaxis and startle response essays 7 isofemales lines were used. These lines were picked up from a collection of females caught in the eastern part of Black Sea region of Turkey, in Firtina Valley, a couple of kilometers West to the Ardeşen county, in 2012. That collection of isofemale lines has been highly inbred with brother sister matings since then and the 7 lines of our study were picked up after checking the status of inbreeding (not shown). They were all found completely inbred.

Locomotion

Negative geotaxis and startle response were measured to phenotype locomotion. For both negative geotaxis and startle response, we measured 7 highly inbred lines in which 10 individual measurements were made per line per sex. In negative geotaxis, banging essay was performed: individual flies were taken into empty vials and, after banging, each fly was observed for climbing distance in a period of 10 seconds. In startle response essays, after banging the empty vials each containing an individual fly, each fly was observed for moving activity time through a fixed duration of 45 seconds. 10 flies per sex and line were assayed both for negative geotaxis and startle response, and mean line phenotypic scores for each trait for each sex were estimated. All measurements were performed for three ages, namely, at 0 (eclosion), 15, and 30 days after emergence.

Heritability

For each age category, we estimated narrow-sense heritability (h^2) and associated parameters for each sex. Narrow-sense heritability estimates were performed after computing the genotypic variance (V_G) from the single classification ANOVA performed per sex. Additive genetic variance, V_A, was calculated as $V_G = 2 \times F \times V_A$, where F is the inbreeding coefficient (taken as unity as we used an isofemale line design)(Falconer and Mackay, 1996). Heritability was estimated from the general formula of $h^2 = V_A/V_P$, where V_P is the phenotypic variance.

Results

I. Among line variation and sex differences through aging

We found considerabe variation in mean scores of negative geotaxis and startle response both within an age category and across ages (Figures 1 and 2). Sexes also varied: there was a marked (and significant) difference between female and male scores for both traits. Interestingly, magnitude and direction of change between sexes seemed to be enlarged and reversed with increasing age for most of the lines. At zero age (eclosion), the number of the lines that gave female mean negative geotaxis scores larger than those of the males was almost half the total lines, but at age 30 almost all the lines had larger male scores (Figure 1). Moreover, the magnitude of the difference between female and male was conspicuously increased in the male direction compared to age 0. The same was almost true for startle response, though with a less emphasized pattern (Figure 2).

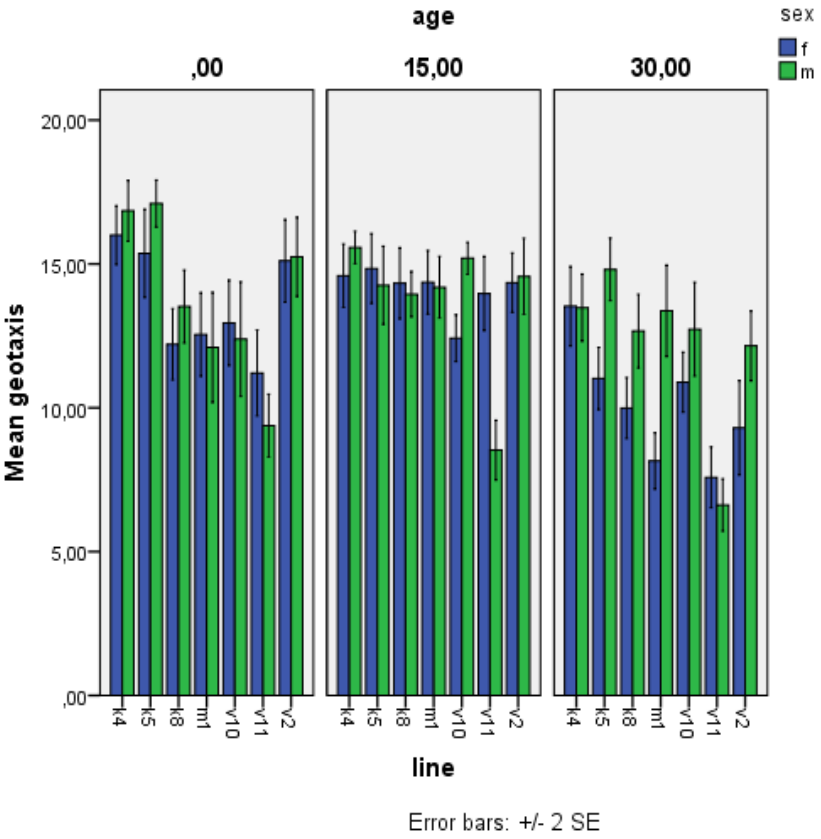


Figure 1. Among line variation in negative geotaxis and its response to aging.

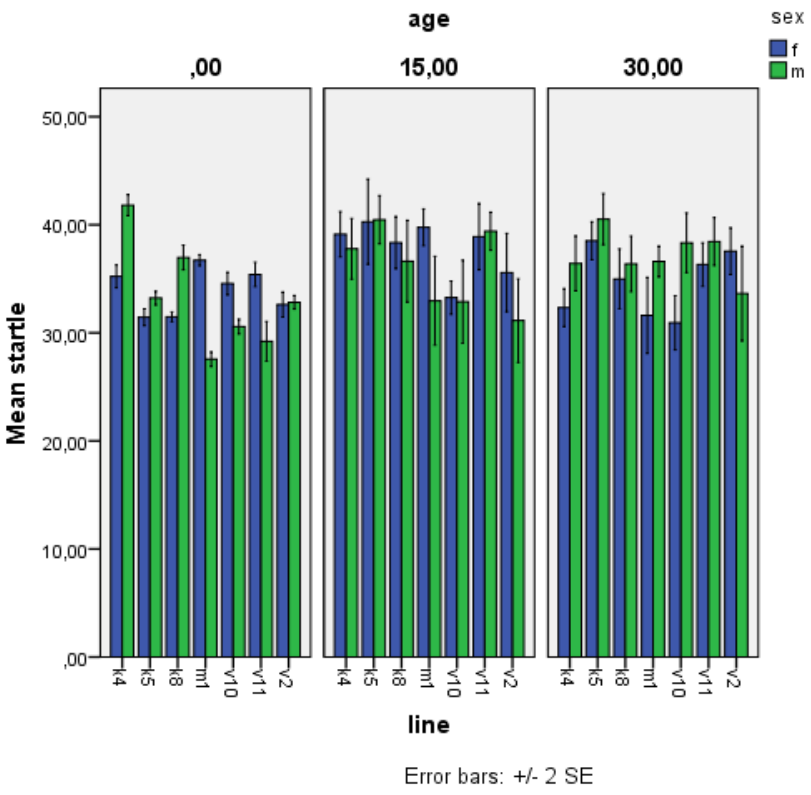


Figure 2. Among line variation in startle and its response to aging.

Table 1. Heritability and associated parameter estimations for negative geotaxis. V_P: phenotypic variance. V_G: genotypic variance. V_A: additive genetic variance. h^2: narrow sense-heritability.

Age (days) ♀♀	V_P	V_G	V_E	V_A	h^2
0 (eclosion)	7.4	2.9	**4.8**	1.5	**0.20**
15	3.4	0.3	**3.1**	0.2	**0.05**
30	6.8	3.6	**3.6**	1.8	**0.27**
Age (days) ♂♂					
0 (eclosion)	11.4	7.3	**5.0**	3.6	**0.32**
15	7.2	5.4	**2.5**	2.7	**0.37**
30	9.8	6.5	**4.2**	3.2	**0.33**

Table 2. Heritability and associated parameter estimations for startle-response. V_P: phenotypic variance. V_G: genotypic variance. V_A: additive genetic variance. h^2: narrow sense-heritability.

Age (days) ♀♀	V_P	V_G	V_E	V_A	h^2
0 (eclosion)	5.7	4.1	**2.1**	2.1	**0.36**
15	23.1	4.5	**19.1**	2.3	**0.10**
30	21.4	7.9	**14.7**	3.9	**0.19**
Age (days) ♂♂					
0 (eclosion)	23.1	23.6	**2.6**	11.8	**0.51**
15	36.3	10.2	**27.4**	5.1	**0.14**
30	21.2	2.8	**18.8**	1.4	**0.07**

II. Heritability and its change with age

The second part of our work entailed the genetic description of the age and sex dependent line variability in locomotion. For this purpose we estimated narrow sense heritability (h^2) and related parameters for each sex in each age category. Tables 1 and 2 show estimations in this respect.

Traits showed distinct patterns of change with age and sex for heritability. For negative geotaxis the amount of genetic variation as *h2* was mostly invariant between sexes across ages (Table 1), while the h^2 of startle response was consistenly decreased with age in both sexes (Table 2). This trait specifity in change of heritability with age may be highly likely to have resulted from the effect of differential amount of increase in environmental variance (V_E) through age. Although the amount of change in V_E was smaller and similar across ages for geotaxis, it seems that increasing age created increasing environmental variance for the startle (Table 1 and 2, V_E). The magnitude of the environment effect through age on heritability can be seen in Figure 3A and B.

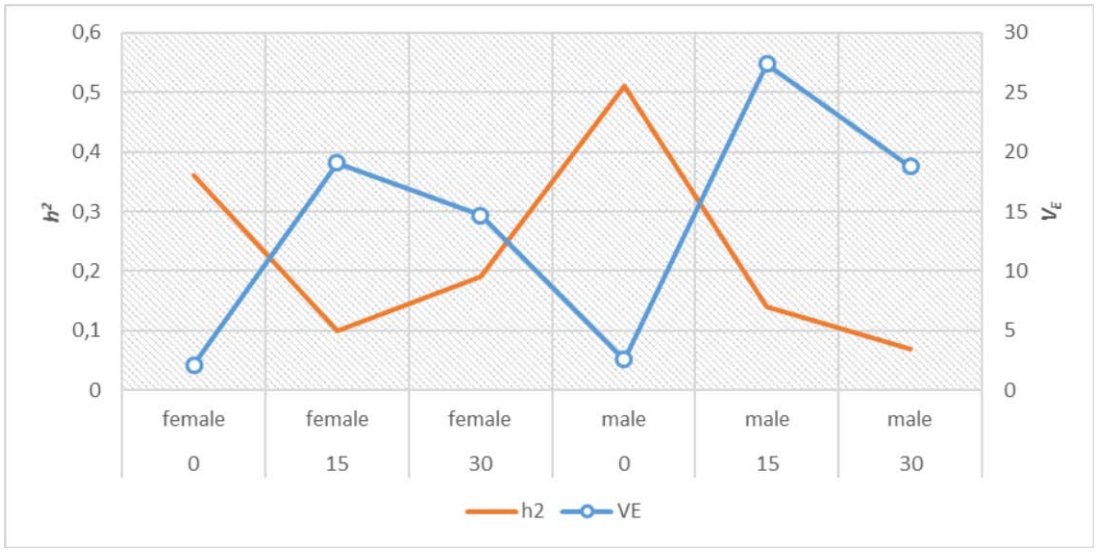
Discussion

In summary, both the high variability among lines and the conspicuous sex dependent change in that variability through aging suggest that the genes underlining locomotion may have been undergoing drastic change in expression through aging (Figures 1 and 2). This change should have contributions from many locations from the genome, as locomotion is a complex, polygenic trait. Consequently, this sex and trait based variability in locomotion with age among lines could be translated into variation in the direction and magnitude of narrow sense heritability with increasing age. However, we have found that in only one of the traits, startle response, the change in heritability seems to track aging (Table 2). Narrow sense heritability (h^2) is an indication of additive genetic variance that contributes to phenotypic variation in a trait (Falconer and Mackay, 1996). In this respect, our finding that this genetic variance for startle response can be different for different age categories suggests that the expression of the putative genes contributing to locomotion might be changing through age. Indeed it has been shown that the pattern of change in locomotion with age can be line specific, hence genetic (Fernandez *et al*., 1999). Our results for startle response could enforce this previous inference. On the other hand, we have found that the pattern of change (startle response) and stability (negative geotaxis) in the heritabilities are strongly affected by the amount of change in environmental variance (V_E) with age (Tables 1 and 2). The more uniform the environmental variance, the more constant is the heritability (negative geotaxis), and, *vice versa* (startle response) (Figure 3). Then, what could account for this differential effect of environmental variance on the pattern of change in heritability with age?

First, beside we used the same isofemale lines for each age category, experimental variance was also very low for each experimental setup. Aging, being a general deterioration in itself both for organismal maintenance and repair, provides highly variable cellular environment and can thus vary the operation of gene expression cascades. Thus, we suggest that the differential response in heritability with age may be due to distinct genotype-by-environment expression contributions from the genome for these traits. Both negative

Figure 3A (left). Change in the environmental variance and heritability of negative geotaxis in response to aging.

Figure 3B (below). Change in the environmental variance and heritability of startle in response to aging.



geotaxis and startle response are components of locomotion, and it is rather interesting that aging should have resulted in two distinct expression patterns in these two traits thought to be mirror images in their contribution to locomotion. This remarkable point should be accounted for, and we are planning experiments in which a mapping population of genomic lines will be used to discriminate the genes contributing to negative geotaxis and startle response at different ages. We hope this further experimentation will clarify the identities and association levels of the genes (hence their functional relatedness to locomotion) contributing to locomotion.

Acknowledgments: We thank Dr. Banu Şebnem Önder for providing the lines.

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Strong reproductive isolation among African species of the *Drosophila montium* subgroup.

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Introduction

The *Drosophila montium* species subgroup is the largest lineage within the *melanogaster* species group, comprising ~90 described species. Although there have been several studies of phylogenetic relationships (Zhang *et al.*, 2003; Yassin *et al.*, 2016; Yang *et al.*, 2012; Da Lage *et al.*, 2007; Miyake and Watada, 2007; Catullo and Oakeshott, 2014), behavior, and reproductive isolation (Kim *et al.*, 1989) in this subgroup, it remains under-studied compared to many other *Drosophila* lineages. At this time, only ~40% of described species are available in live culture.

The *montium* subgroup shows extensive sex-specific variation in the pigmentation of posterior abdominal segments (Yassin *et al.*, 2016; Ohnishi and Watanabe, 1985). In contrast to many other *Drosophila* lineages, female pigmentation in this subgroup evolves more rapidly than male pigmentation (Yassin *et al.*, 2016). Although the females of over 20 different species are polymorphic for light/dark abdominal pigmentation, males of any given species are monomorphic light or monomorphic dark. The only known exception is *D. truncata*, where males as well as females are polymorphic (Ohnishi and Watanabe, 1985). In an effort to map the genetic basis of interspecific variation in male abdominal pigmentation, I have attempted to hybridize closely related African *montium* subgroup species. With the strains I was able to test, these attempts failed.

Materials and Methods

The following species and strains were used in the attempted crosses:

D. nikananu 14028-0601.00 ("nikananu 0")
D. nikananu 14028-0601.01 ("nikananu 1")
D. tsacasi 14028-0701.00
D. bocqueti 14028-0771.00
D. chauvacae 14028-0761.00
D. burlai 14028-0781.00
D. diplacantha 14028-0586.00
D. bakoue Sao Tome

The strain of *D. bakoue* was collected and kindly sent to me by Dr. Jean David. All other strains were obtained from the US *Drosophila* species stock center at the University of California, San Diego.

Crosses were performed between all pairs of species where one parental species had dark males (*D. tsacasi, D. bocqueti, D. chauvacae, D. burlai*, and *D. diplacantha*) and the other had light males (*D. nikananu* and *D. bakoue*) (Table 1). Each cross was performed in both directions. For each reciprocal cross, five mass cultures were set up in vials using at least 20 virgin females and 20 males per vial. Crosses were kept on standard *Drosophila* media at room temperature and ambient light cycle, and transferred to fresh media twice a week until all adults were dead. Vials were inspected regularly for the presence of larvae. All emerging F_1 adults were back-crossed to either the light or the dark parental species in an effort to obtain F_2 progeny. When no F_2 progeny were obtained, a subset of F_1 adults were dissected in insect saline and inspected for the presence of sperm.

Results

Most crosses did not produce any F_1 progeny during the ~2 month life span of the parental adults (Table 1). For these crosses, 5-10 females that were at least 1 month old were dissected per cross, and none were found to carry any sperm, indicating complete pre-mating isolation between the tested strains under these

experimental conditions. The only exception was the cross between *D. tsacasi* and *D. bakoue*, which produced F_1 adults in both directions.

Table 1. Attempted crosses among African montium subgroup species.

	nikananu 0	*nikananu 1*	*bakoue*	*tsacasi*	*bocqueti*	*chauvacae*	*burlai*	*diplacantha*
nikananu 0				-	-	-	-	-
nikananu 1				-	-	-	-	-
bakoue				+	-	-	-	-
tsacasi	-	-	+					
bocqueti	-	-	-					
chauvacae	-	-	-					
burlai	-	-	-					
diplacantha	-	-	-					

Each row indicates the maternal parent species, and each column the paternal parent. "+" indicates that F_1 progeny were obtained, "-" indicates that no F_1 progeny were obtained, and empty cells reflect crosses that were not attempted.

In the cross between *D. bakoue* females and *D. tsacasi* males, several hundred F_1 females and zero males were obtained. Most F_1 females had defective tergite cuticles, suggesting an impairment in the proliferation of abdominal histoblasts. All F_1 females were back-crossed to either *D. tsacasi* or *D. bakoue* males, but did not lay any eggs in either backcross. 10 F_1 females from this cross were dissected, and all were found to have very small degenerate ovaries that did not contain any eggs or recognizable ovarioles.

In the cross between *D. tsacasi* females and *D. bakoue* males, approximately 20 F_1 females and ~15 males were obtained. Most F_1 progeny also had defective tergite cuticles, but not as severe as in the reciprocal cross. All F_1 females were back-crossed to either *D. tsacasi* or *D. bakoue* males, and laid many apparently normal eggs in both backcrosses, but no eggs hatched in either backcross. 8 F_1 females from this cross were dissected, and none were found to carry any sperm, suggesting they were defective in either mating receptivity or attractiveness. F_1 males were crossed to *D. bakoue* virgin females but produced no progeny. 5 males were dissected, and all were found to have small degenerate testes with no sperm. Thus, no F_2 progeny were obtained in either direction, despite the initial success of both crosses.

Discussion

Reproductive isolation among the African *montium* subgroup species appears to be very strong and involve both pre-mating and postzygotic isolating mechanisms. It remains possible, however, that some of these species could be hybridized under different conditions, or using other strains. In this regard, the cross between *D. tsacasi* and *D. bakoue* shows an interesting difference from an earlier report (Rafael, 1984), where no F_1 progeny were obtained. The *D. bakoue* strain used in that report was collected on the African mainland (Cameroon), whereas the strain used here was collected by Dr. Jean David on the island of Sao Tome. The latter strain could represent a divergent population of *D. bakoue*, or a cryptic species closely related to *D. bakoue* (J. David, pers. comm.). These possibilities could not be distinguished due to the lack of any other *D. bakoue* strains. It would be interesting to test other populations and strains of *D. bakoue*, *D. tsacasi*, and related species for the ability to hybridize and produce F_2 progeny. Colleagues who have any such strains are implored to send them to the author.

Acknowledgments: I would like to thank Dr. Jean David for kindly sharing the *D. bakoue* strain, and the US *Drosophila* species stock center for the other strains used in this study. I am also grateful to Dr. Emily Delaney for comments on the manuscript, and to members of the Kopp lab for letting me use their research facilities.

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Electrophoretic variants of Xanthine dehydrogenase enzyme in *Drosophila malerkotliana.*

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Allozymes are electrophoretic variants of an enzyme. A number of enzymes are known to be polymorphic in natural populations of an organism. Xanthine dehydrogenase is an enzyme that belongs to the family of oxido-reductases involved in the oxidative metabolism of purines. This enzyme can be involved in the production of molecules called superoxide radicals. It is found to be polymorphic in a number of species of genus *Drosophila* (Singh *et al.* 1976; Kumar and Singh, 2012, 2016). Presently, we are studying the population genetics of *bipectinata* species complex that comprises four closely related species of *Drosophila* like *D. bipectinata, D. parabipectinata*, *D. malerkotliana*, and *D. pseudoananassae*. *D. malerkotliana* is one of the commonly occurring species in Indian subcontinent and it has been of ample attention to evolutionary geneticists due to its phylogenetic connection with *bipectinata* species complex (Hegde and Krishnamurthy, 1976; Jha *et al*., 1979; Tomimura *et al*., 2005; Singh and Banerjee, 2016). Genetic polymorphism in this species has been carried out by some of the researchers (Yang *et al*., 1972; Bock, 1978; Naseerulla and Hegde, 1993; Parkash *et al*., 1994; Sharma *et al*., 1993; Singh, 2015; Singh and Singh, 2015; Singh and Banerjee, 2016). Genetic polymorphism owing to allozyme variation has very sporadically been studied in this species. In the present study we are reporting the electrophoretic variants of Xanthine dehydrogenase enzyme in natural populations of *D. malerkotliana* by using native polyacrylamide gel electrophoresis.

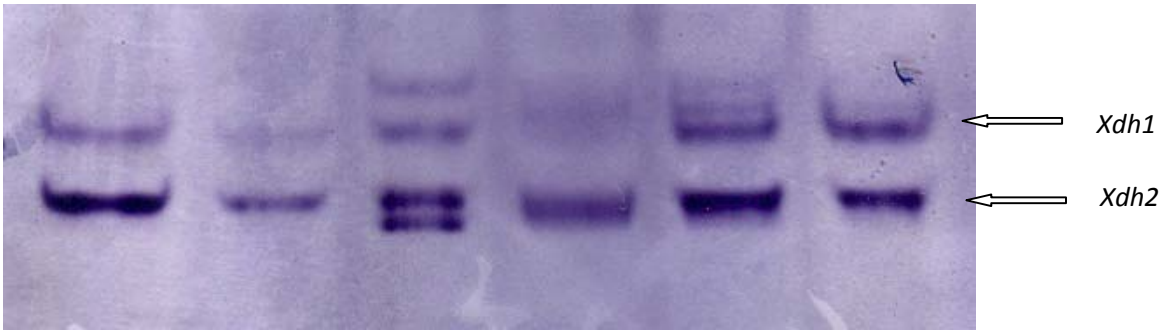

Figure 1. Xanthine dehydrogenase enzyme loci and their electrophoretic variants in *D. malerkotliana.*

For electrophoretic analysis of Xanthine dehydrogenase, single individual fly homogenate was prepared in 30 µl of 20 mM Tris buffer and the homogenate was centrifuged at 12000 rpm at 4°C for 10 minutes. Electrophoresis was performed by using polyacrylamide gel. The electrophoresis was carried out at four degree centigrade to avoid the denaturation of the enzyme. Staining of the gel was performed by using hypoxanthine as a substrate in 0.05M Tris buffer at pH 8.8 for the appearance of enzyme bands. The different

allelic forms (electrophoretic variants) start appearing in about one hour of incubation at room temperature. In this study, we have been able to observe two distinct polymorphic gene loci for this enzyme, *i.e*., *Xdh1* and *Xdh2*. Each locus is further represented by two electrophoretic variants (Fast and Slow). Figure 1 depicts the electrophoretic variants of xanthine dehydrogenase in *D. malerkotliana.* Frequency of these variants in natural populations of this species is being observed and a detailed description in this regard will be documented soon.

Acknowledgment: Financial assistance from CAS (Centre of Advance Study) in Zoology is thankfully acknowleged.

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Drosophila mercatorum (Diptera: Drosophilidae) in Sakhalin Island of Russian Far East.

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During three years, from 2014 to 2016, in the summer, we have performed collections of *Drosophila melanogaster* for population genetic studies on the Sakhalin Island. In 2014 (1-9 August) and 2015 (July 27 - August 8) collections were performed on the west coast of the island, in the city of Tomari (47°46′00″N; 142°04′00″E); in 2016 (July 27 - August 5) in the city of Yuzhno-Sakhalinsk, - the administrative center of the island (46°57′00″N; 142°44′00″E). Since the main object of our interest was a synanthropic and cosmopolitan *D. melanogaster*, we performed collections in residential areas of the cities.

The synanthropic Drosophilidae collected in the course of these measures are represented by five species:

1) *Drosophila (Dorsilopha) busckii* Coquillett, 1901;
2) *Drosophila (Drosophila) mercatorum* Patterson & Wheeler, 1942;
3) *Drosophila (Sophophora) melanogaster* Mg., 1830;
4) *Drosophila (Sophophora) simulans* Sturtevant, 1919;
5) *Lordiphosa magnipectinata* Okada, 1956.

Among these five species an autochthon is only *L. magnipectinata*. This species inhabits the native habitats and is widespread on the islands and mainland of the Russian Far East, in Japan (Hokkaido and Honshu), in Korea, and northeast China (Sidorenko, 2001). Three species, *D. busckii*, *D. melanogaster*, and *D. simulans* are cosmopolitan and their presence may be expected at any point of a Globe populated by people. According to the data presented in the Table, two species, *D. melanogaster* and *D. mercatorum*, are most common and numerous, and the first is clearly dominant in numbers.

D. mercatorum in Sakhalin Island, in 2014, was recorded for the first time, and to this date it is the extreme eastern point of registration of this species in Russia. This fact is the final accord in the history of the installation of the species in the territory of Northern Eurasia.

For the first time in the Old World neotropical species *D. mercatorum* was recorded in 1953, in Spain, in Barcelona (Prevosti, 1953). In the next two decades, the individuals of the species were present in small

amounts in *Drosophilidae* collections in Spain and the Canary Islands (Monclús, 1964, 1976). In the early 80s, according to several authors, the distribution of the species in the Old World remained within the boundaries of the Western Mediterranean (David and Tsacas, 1980; Bächli and Rocha Pite, 1981; Wheeler, 1981; Monclús, 1984).

Table 1. The distribution of species on the collection sites.

Species	Tomari 2014 N (%)	Yuzhno-Sakhalinsk 2015 N (%)	Tomari 2016 N (%)	Total N (%)
D. busckii	0	0	52 (8.5)	52 (4.2)
D. mercatorum	19 (9.1)	86 (20.4)	44 (7.2)	149 (12)
D. simulans	1 (0.5)	3 (0.7)	0	4 (0.3)
D. melanogaster	188 (89.5)	324 (77)	515 (84.3)	1027 (82.7)
L. magnipectinata	2 (0.9)	8 (1.9)	0	10 (0.8)
Total	210	421	611	1242

Since the beginning of the 90s, according to our data and publications, began the spread of the species from the Western Mediterranean to the east and north. In 1990 *D. mercatorum* was first recorded by us in the Soviet Union, in two widely separated locations: in Eastern Europe, in Ukraine, in the city of Uman (Ivannikov *et al*., 1993); and in Western Siberia in Novosibirsk (Ivannikov *et al*., 1998; Ivannikov and Zakharov, 1995). In 1992, *D. mercatorum* was first found in the north of Western Europe, in the Netherlands (Kraaijeveld, 1992). In October 1994, *D. mercatorum* was first found in England (Bennett *et al*., 1995). In November 1994, *D. mercatorum* was first recorded by us in Central Asia - Tajikistan, in Dushanbe (Ivannikov and Zakharov, 1995).

Novosibirsk population of *D. mercatorum*, we found in 1990, in the geographical center of Russia, during the decade of the 90s remained viable, numerous, and stable. However, to find individuals of the species in other cities of Russia we could not until 1998. In 1998, Dr. Yu. Novikov found *D. mercatorum* in Tomsk (200 km north of Novosibirsk). In the summer and autumn of 1999 the species was found in two places in the territory of Russia, - in the Altai Republic (south of Western Siberia, 500 km south of Novosibirsk) and in the Republic of Udmurtia (eastern European part of Russia, 2000 km west of Novosibirsk) (Ivannikov and Zakharov, 2000).

In 2000 *D. mercatorum* was first discovered in the Far East of Russia, in the city of Vladivostok by dipterologist Vasily Sidorenko and was included in the "Key to the Insects of Russian Far East" (Sidorenko, 2001).

In our collections synanthropic fruit flies in the Central Asian republics of the former Soviet Union since the early 2000s to the present time, *D. mercatorum* was found in the big cities: Almaty (Kazakhstan); Bishkek (Kyrgyz Republic); Tashkent (Uzbekistan) (our unpublished data).

Thus, now we can state with confidence that over the past approximately thirty years, *D. mercatorum* fully colonized the Eurasian Continent from Western Europe, including the British Isles, up to the Far East of Russia, including Sakhalin Island.

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Parasitoids of Drosophilidae with potential for parasitism on *Drosophila suzukii* in Brazil.

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Abstract

The spotted wing drosophila is an invading polyphagous species, which causes damage on small stone fruits. In Brazil, the species was detected in 2013. This study registers the occurrence of parasitoids *Leptopilina boulardi* (Barbotin, Carton & Kelner-Pillault) (Hymenoptera: Figitidae) and *Trichopria anastrephae* Lima (Hymenoptera: Diapriidae) on blackberry and strawberry fruits attacked by *Drosophila suzukii*.

Introduction

The spotted wing drosophila, *suzukii* (Matsumura, 1931) (Diptera, Drosophilidae), is a species endemic to Asia, first registered as an invading species in Hawaii in 1980 (Kaneshiro, 1983). It has rapidly spread worldwide in the last few years, arising as one of the main pests of small stone fruits (Asplen *et al*., 2015). In 2008, it was recorded simultaneously in California, in the United States (Bolda *et al*., 2010), and several other localities in Europe (Calabria *et al*., 2012). For South America, records include Brazil (Deprá *et al*., 2014; Schlesener *et al*., 2014; Geisler *et al*., 2015), Uruguay (González *et al*., 2015), and Chile (Medina-Muñhoz *et al*., 2015).

This species presents a short life cycle and high biotic potential (Emiljanowicz *et al*., 2014; Tochen *et al*., 2014), which may lead to a rapid increase in population when environmental conditions are favorable (Wiman *et al*., 2014) possibly resulting in considerable economic losses to commercial crops (Beers *et al*., 2011; Walsh *et al*., 2011). Currently, the main control method applied is chemical (Cancino *et al*., 2015), wherein several insecticide classes – such as pyrethroids, spinosyns and organophosphates – have been proven effective over *D. suzukii* (Bruck *et al*., 2011). Nevertheless, the fly's rapid life cycle, which allows for the occurrence of many generations during a single production cycle, and its occurrence in the fruit's ripening phase, require frequent applications endangering human health and the environment via residue in the fruits, insecticide resistance in insects, as well as the negative effects to pollinators and biological control agents (Cini *et al*., 2012).

Therefore, alternative strategies for the control of *D. suzukii* are demanded. Biological control, particularly by use of parasitoids, may help suppress regional *D. suzukii* population even in crop adjacent habitats (Wang *et al*., 2016). A great diversity of parasitoids associated with the *drosophila* genus has been recorded (Fleury *et al*., 2009). Larvae parasitoids are the most recurring ones, especially those from the

Asobara (Braconidae), *Leptopilina*, and *Ganaspis* (Figitidae) genera and pupal parasitoids from the *Trichopria* (Diapriidae) and *Pachycrepoideus* (Pteromalidae) genera (Rohlfs and Hoffmeister, 2004; Wertheim *et al*., 2006; Mitsui and Kimura, 2010). Regarding *D. suzukii*, numerous field and lab studies performed in Europe and North America have reported that there is an association between the insect and several species belonging to the aforementioned genera, although few have been proven effective in regulating population growth (Mitsui *et al*., 2007; Mitsui and Kimura, 2010; Miller *et al*., 2015; Nomano *et al*., 2015). In its native area, the efficiency of indigenous parasitoids has been evaluated, aiming at exploring a classical biological control strategy, by introducing and establishing natural enemies from the fly`s original area in the invaded areas (Guerrieri *et al*., 2016). Another approach to the biological control of *D. suzukii* would be an increase on control agents already present in the recently invaded areas (Cini *et al.*, 2012).

This study aimed to detect the presence of potential biological control agents in four small-fruit producing areas, in the southern region of Rio Grande do Sul, Brazil. By means of Köppen climate classification, the region has a Cfa climate type, characterized by its humid temperate climate (Kottek, 2006), wherein the temperate climate small-fruit crops stand out, such as strawberry, blueberry, blackberry, and raspberry. Of those, strawberry crops are the most representative fruits (Fachinello *et al*., 2011).

Materials and Methods

The occurrence of potential *D. suzukii* parasitoids has been verified in four small-fruit producing areas during the 2015/2016 crop, places where the presence of the invading species had already been detected via capture traps for adults and insects emerged from previously sampled fruits. The subject areas are located in Pelotas City, Rio Grande do Sul, Brazil, specifically Rincão da Caneleira (31°32’36”S), Cristal (31°35’19”S), Cascatinha (31°38’23”S), and Cerrito Alegre (31°35’12”S). On a weekly basis, blackberry (*Rubus* spp.) fruits were collected from the first three locations and strawberry (*Fragaria* x *ananassa*) fruits from the last location, respectively.

Fruits were weighed and accommodated individually in plastic containers with a fine layer of vermiculite and a screened orifice at the lid. Fruits were kept in an acclimatized room (24 ± 2°C, 70 ± 10% relative humidity and 12h photo phase) until the emergence of flies and/or parasitoids. Emerging insects were aspirated from the containers and placed in Eppendorf tubes containing 70% alcohol for later identification. *D. suzukii* specimens were identified by taxonomical characteristics according to Vlach (2013). Parasitoid identification was performed as established by Costa Lima (1940), Norlander (1980), and Guimarães *et al.* (2003), and voucher species were deposited at the “Oscar Monte” Entomophagous Insect Collection (Biological Institute, Campinas, SP, Brazil; curator: Valmir A. Costa). Parasitoids were transferred to glass tubes (20 mm × 80 mm) containing *D. suzukii* larvae and pupae originated from laboratory breeding, as to confirm parasitoidism and initiate the breeding of such specimens on said host for later studies (Figure 1). Parasitoidism was allowed for 72 hours and, afterwards, specimens were transferred to Eppendorf tubes containing 70% alcohol following identification based on previous species identification.

Figure 1. *Trichopria anastrephae* females over *D. suzukii* pupae.

Figure 2. *Trichopria anastrephae* (a) and *Leptopilina boulardi* (b) females.

Results and Discussion

During the study period, 1,582 fruits were collected, out of which 638 were strawberries and 944 blackberries. The recovered parasitoids were *Leptopilina boulardi* and *Trichopria anastrephae* (Figure 2). In addition to *D. suzukii*, other drosophilid species emerged from the infested fruits, suggesting a secondary attack after the initial damage caused by the species. *L. boulardi* occurred in a small proportion in relation to the *T. anastrephae* (Table 1).

Table 1. *D. suzukii*, *L. boulardi* and *T. anastrephae* specimens emerged from blackberry and strawberry fruits collected in four locations in countryside Pelotas, RS, Brazil, during the 2015/2016 crop.

Location	Infested fruits	*D. suzukii*		*L. boulardi*		*T. anastrephae*	
		♂	♀	♂	♀	♂	♀
Rubus sp.							
Rincão da Caneleira	132	685	730	18	29	55	68
Cascatinha	92	332	378	3	1	6	3
Cristal	479	3,142	3,363	9	12	64	51
Fragaria x *ananassa*							
Cerrito Alegre	169	735	754	7	4	19	23
Total	872	4,894	5,225	37	46	144	145

This fact can be related to the cellular answer presented by *Drosophila* larvae against hymenopterous parasitoids, unchaining an encapsulation process on their eggs, unlike pupae parasitoids, which are less affected (Kacsoh and Schlenke, 2012). In *D. suzukii* this immune response is more potent than in other drosophilids, as can be verified by the higher rate of parasitoid egg encapsulation (Poyet *et al*., 2013).

Leptopilina boulardi is a Drosophilidae larval parasitoid, particularly to species from the *Drosophila* genus (Allemand *et al*., 2002), which lay their eggs individually in second instar larvae (Krzemien, 2008). In the event of eclosion by the hymenopterous larvae, they consume the fly`s internal tissue, and adult parasitoids

emerge from the dipterous pupae (Kacsoh and Schlenke, 2012). Such parasitoids are originally from Africa, but they are currently distributed in tropical and warm temperate climate regions (Seyahooei *et al*., 2011), like Europe, Asia, Africa, the American continent, and Caribbean islands (Allemand *et al*., 2002). Brazil has recorded them in several states, such as São Paulo (Nordlander, 1980), Minas Gerais and Rio de Janeiro (Guimarães *et al*., 2003).

The *T. anastrephae* species was described by Lima (1940), who obtained the specimens from *Anastrephae serpentina* (Wiedemann) and *Anastrephae* spp. (Diptera: Tephritidae) puparia. Species from the *Trichopria* genus are pupae parasitoids, whose females lay their eggs in the *Drosophila*`s hemocele and their larvae consume the pupae`s internal tissues, from where the adult emerges (Kacsoh and Schlenke, 2012). *Trichopria anastrephae* is distributed throughout Minas Gerais (Silva, 2003), Goiás (Marchiori and Penteado-Dias, 2001), Santa Catarina (Garcia and Corseuil, 2004), and Rio Grande do Sul (Cruz *et al*., 2011) states. It has also been recorded in Argentina (Turica and Mallo, 1961) and in Venezuela (Boscán and Godoy, 1996).

The spotted wing drosophila is already present in the agroecosystems of the southern Rio Grande do Sul region, and there have been reports of losses to several crops. Therefore, this drosophilid presents a risk to local fruit production, since the region presents climate characteristics and host plants to allow for its establishment. The results found demonstrate the importance of *L. boulardi* and *T. anastrephae*, species, parasitoids that occur naturally in those areas, and which can help reduce *D. suzukii* population.

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New chromosomic paracentric inversions in *Drosophila cardinoides* (Diptera, Drosophilidae) at Santa Catarina Island, South of Brazil.

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Introduction

Included in the *Cardini* group, which is characterized by drosophila with polymorphisms of abdominal pigmentation and inhabits neotropical region, *Drosophila cardinoides* is a representative species in southern Brazil that can be collected many times in the island of Santa Catarina, mainly on the border of the forest. This species is characterized by a dark abdominal pigmentation, wings and abdomen with bright appearance, but has the body morphology very similar to *D. procardinoides*, forming a monophyletic group. Thus, they are not taxonomically decisive features. Cytogenetically, the chromosomal inversions of *D. cardinoides* are more fixed, following the pattern of the *Cardini* group. According to previous studies, it was expected that the number of inversions of *D. cardinoides* was not so wide, since it is less polymorphic compared to other species, such as *Drosophila polymorpha*, for example. Even with significant advances on the chromosomal map of *D. cardinoides*, there are many gaps and investigations to be made in order to contribute to evolutionary and phylogenetic studies of this species group.

Material and Methods

In the southern part of the island of Florianopolis, in Caieira da Barra do Sul (Figure 1) there is a conserved Atlantic Forest area remaining at the Serra do Tabuleiro State Park, where *Drosophila* were collected in the years of 2015 and 2016 during summer and spring. For this, an entomological network was used over baits with bananas and yeast. From these collections, eleven isolineages of *Drosophila cardinoides* were established, maintained in culture media at a constant temperature of 17ºC. To obtain the polythenic chromosomes, cytological slides were prepared with third stage larvae using the Ashburner technique (1967) with small modifications and for the chromosomal analysis, the Rohde and Valente (1996) and Cordeiro *et al*. (2014) methods.

Figure 1. Point shows the collecting point at Florianópolis Island (S 27º48 '; O 48º56').

Results

In addition, new inversions were detected (Figures 2 and 3) in all *Drosophila cardinoides* chromosome arms. The breaking points of the inversions of Figures 2 and 3 are described in Table 1.

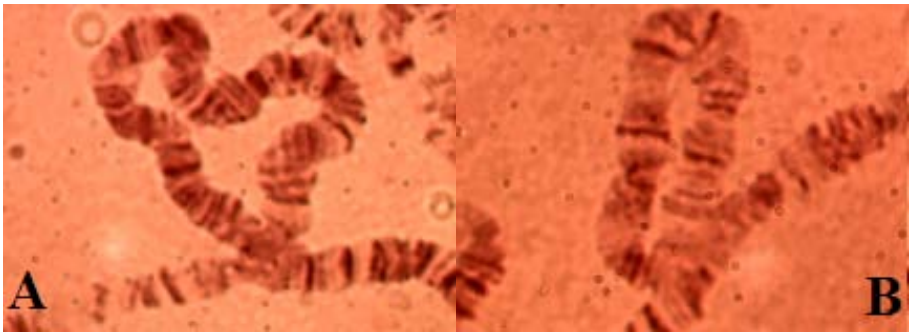

Figure 2. In **A** we have an IIRA inversion, and in B we have an IILC inversion.

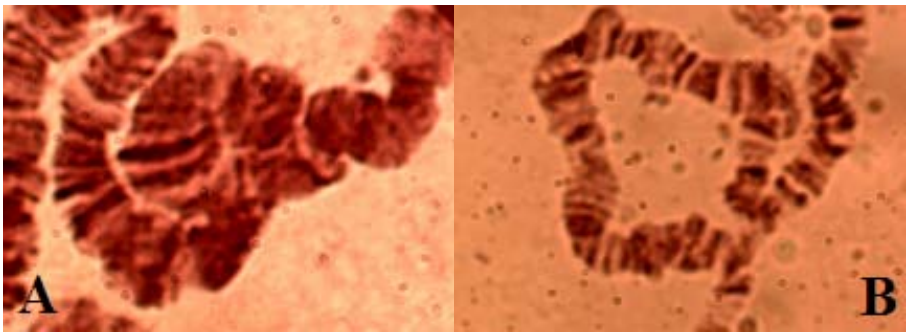

Figure 3. In **A** we have an IIIRA inversion, and in B we have an XA inversion.

Table 1. Break points inversion.

Chromosome arms	Break points
IIRA	56c distal a 49b distal
IILC	31b proximal a 29a proximal
IIIRA	92a proximal a 94c proximal
XA	4c proximal a 7b distal

Conclusions

Studies to date on paracentric heterozygous chromosome inversions in *Drosophila cardinoides* were essential for assembling the chromosomal map of the species and for elucidating the reason of why such high frequencies of this phenomenon are found in nature. Our analyses pointed for a high level of polymorphism in this species besides *D. polymorpha*, as well as the necessity and importance of the continuity of investigations in the area, to help elucidate evolutionary, phylogenetic, and ecological issues of the karyotypic evolution of the *cardini* species group.

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Occurrence of invasive species *Drosophila nasuta* in Atlantic Rainforest, Brazil.

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In March 2015, Vilela and Goñi (2015) reported the occurrence of invasive species *Drosophila nasuta* in the city of São Paulo, Brazil. We have been monitoring shifts in chromosomal inversion frequencies of *D. mediopunctata* from the Parque Nacional do Itatiaia, RJ, Brazil (22°26'S, 44°37'W) in the last 30 years (Ananina *et al*. 2004; Batista *et al*., 2012; Batista and Klaczko, 2013). We had never collected a single specimen of *D. nasuta* in this well preserved National Forest that belongs to the Atlantic Rainforest in Brazil. When sorting collected flies in March 2015, we observed orange colored flies with one row of cuneiform setae on anteroventral side of profemur. We confirmed their identification as *D. nasuta* by examining external morphology and the genitalia of males collected and compared them to the description made by Vilela and Goñi (2015). Thus, for the first time, this species was collected at the Parque Nacional do Itatiaia, RJ, Brazil (N = 60). In our following collection (September 2015), we did not observe *D. nasuta* among the collected flies. However, the species was collected again in our two following field trips: November 2015 (N = 14) and March 2016 (N = 19).

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High abundance of exotic drosophilids in a gallery forest of the Brazilian savanna.

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Introduction

Among the terrestrial environments, forests shelter the highest biodiversity because of their environmental heterogeneity. The Brazilian savanna holds a complex of very rich vegetal formations, located mainly in central Brazil. Forests occupy only 5% of this biome, but contain the highest biodiversity of this region, because they harbor most of the unique and rare species, as well as common species of its different environments (Mittermeier *et al*., 2005; Tidon, 2006). Nevertheless, countless gallery forests of the Brazilian savanna have not yet been studied and so are extremely threatened by anthropogenic pressures (fragmentation,

deforestation, and fire). These areas deserve, therefore, local studies toward obtaining critical subsidies necessary for their preservation and conservation.

Taxonomic surveys focusing on drosophilid of the Brazilian savanna revealed the existence of 129 species (Roque *et al*., 2015). Most of this biodiversity is native to the Neotropical region and is found mainly in the rainy season in preserved forests (Tidon, 2006; Mata *et al*., 2008a; Roque *et al*., 2013). This distribution pattern has been associated with greater availability of resources (fruits, fungi, flowers, etc.) in forest environments during the rainy season (Leão and Tidon, 2004; Roque *et al*., 2009; Valadão *et al*., 2010).

This research is part of a broader project aimed at describing the fauna and flora of the *Campus Planaltina* of the *Instituto Federal de Brasília* to support the creation of an integral protection area for its biodiversity. Thus, to improve the knowledge about the richness and the distribution of drosophilids in the Brazilian savanna, we made collections of adult drosophilids in a gallery forest of Planaltina-DF to (1) identify the local drosophilid fauna, and (2) evaluate the existence of temporal patterns of this fauna.

Materials and Methods

We performed eight collections of adult drosophilids from September 2015 to April 2016 in a gallery forest situated in the vicinity of the *Campus Planaltina* of the *Instituto Federal de Brasília* (15°38'44.2"S; 47°41'44.9"W). In each collection, we sampled the forest by exposing five retention traps (Roque *et al*., 2011) separated by at least 30 m. The traps were baited with fermented bananas and left inside the forest for three consecutive days.

We preserved captured flies in ethanol 70% and identified all of them using external morphology (Freire and Pavan, 1949; Magalhães, 1962; Poppe *et al*., 2015). In cases of cryptic species, we conducted analysis of male terminalia according to Bächli *et al*. (2004). Individual-based rarefaction curves for all drosophilids were compiled to assess the completeness of the samples (Sest; EstimateS 9.1; Cowell, 2013). To evaluate temporal fluctuation of this fauna, we quantified the total amount of native (NEO) and exotic (EXO) species. After removing November 2015 (N = 0) from our data, statistically significant differences in the proportional values of NEO and EXO among months were assessed using the chi-squared statistical test (Contingency Table) in Past 2.16 (Hammer *et al*., 2001). We deposited vouchers at the Collection of the *Laboratório de Biologia Animal* of the *Instituto Federal de Brasília* (*Campus Planaltina*) for comparisons.

Results and Discussion

Overall, we captured 1,876 drosophilids representing 22 species of the genera *Drosophila*, *Scaptodrosophila*, and *Zaprionus*. *Drosophila* was the most specious genus (20 species) and *D. simulans* the most abundant species (N = 466) (Table 1). The rarefaction curves showed a tendency to reach an asymptote (Figure 1), but additional collections will be required for a full description of this assemblage. Although the biodiversity of drosophilids recognized for the Brazilian savanna (Roque *et al*., 2015) is an underestimate, studies carried out in this biome have shown that gallery forests are the biodiversity centers for this taxon (Tidon, 2006; Mata *et al*., 2008a; Roque and Tidon, 2013). Unfortunately, our data do not follow such a pattern, because we captured only about 17% of the total Drosophilidae fauna of the Cerrado. A possible reason for this difference is that the studies cited above were performed within protected areas of the Cerrado with high sampling efforts. Therefore, a complete description of the local species richness may be achieved through additional taxonomic surveys across a larger temporal scale and using different strategies to collect drosophilids.

Fifteen drosophilids were considered NEO, but seven EXO accounted for 77.6% of the total abundance (Table 1). Except in October 2015, the proportions of EXO were higher than NEO across the temporal scale (Chi-squared = 396.53; df = 6; $p = 0.0001$) (Figure 2). Studies have shown that the frequency of exotic drosophilids is related to the degree of disturbance in the environment, that is, altered areas (deforested, fragmented, burned, etc.) tend to harbor more individuals of such species (Ferreira and Tidon, 2005; Mata *et al*., 2008b). As agropastoral areas are found in the surroundings of the researched forest, and the presence of grasses, clearings, and a river bed in advanced silting process were verified in its interior, we conclude that this forest is extremely modified. Thus, our data support the idea that exotic species are more

common in disturbed environments. Such a combination of introduced species and an altered environment compromises the stability of the ecosystem which, in turn, tends to decrease its native biodiversity.

Table 1. Drosophilids collected in a gallery forest in the vicinity of *Campus Planaltina* of the *Instituto Federal de Brasilia*. Data collected from September 2015 to April 2016.

Drosophilids	Collections								Total
	Sep-15	Oct-15	Nov-15	Dec-15	Jan-16	Feb-16	Mar-16	Apr-16	
*Drosophila simulans**	36	1	0	414	2	4	5	4	466
*D. malerkotliana**	0	0	0	14	54	185	122	80	455
*Zaprionus indianus**	8	1	0	209	3	74	2	0	297
*D. nasuta**	0	0	0	8	73	66	33	18	198
D. sturtevanti	0	0	0	1	6	107	50	2	166
D. willistoni	0	0	0	3	3	12	86	7	111
D. nebulosa	1	2	0	10	0	3	4	49	69
D. cardini	8	3	0	6	0	2	15	2	36
*Scaptodrosophila latifasciaeformis**	0	0	0	0	0	20	2	0	22
D. saltans	0	1	0	1	1	1	12	1	17
*D. immigrans**	2	0	0	2	9	2	0	0	15
D. mediostriata	0	0	0	0	0	0	0	5	5
D. fumipennis	0	0	0	0	0	0	0	4	4
D. mercatorum	1	0	0	1	0	2	0	0	4
D. cuaso	0	0	0	1	0	0	0	2	3
*D. melanogaster**	2	0	0	0	0	0	0	0	2
D. arauna	0	1	0	0	0	0	0	0	1
D. paraguayensis	0	0	0	0	0	0	0	1	1
D. sp1	0	0	0	1	0	0	0	0	1
D. sp2	1	0	0	0	0	0	0	0	1
D. sp3	0	0	0	0	0	0	0	1	1
D. sp4	0	0	0	0	0	0	0	1	1
Total	59	9	0	671	151	478	331	177	1,876

**Exotic species*

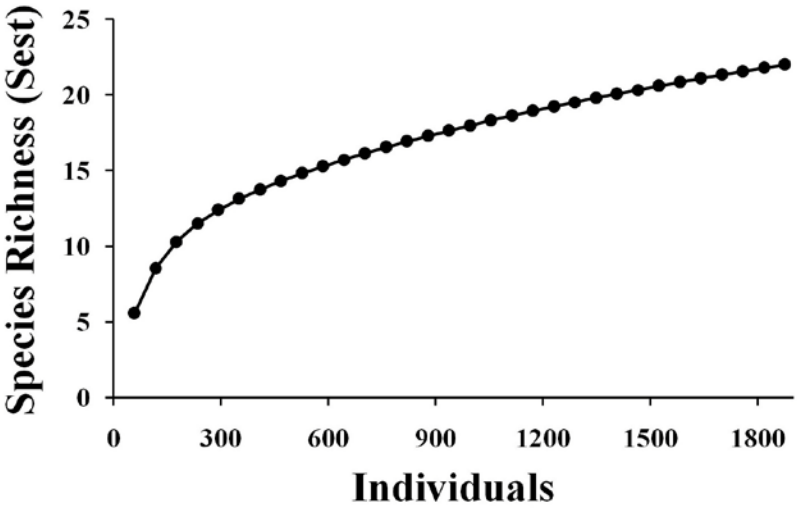

Figure 1. Individual-based species rarefaction curves (Sest) of the drosophilid assemblages associated with a gallery forest near to *Campus Planaltina* of the *Instituto Federal de Brasília*. Data collected from September 2015 to April 2016.

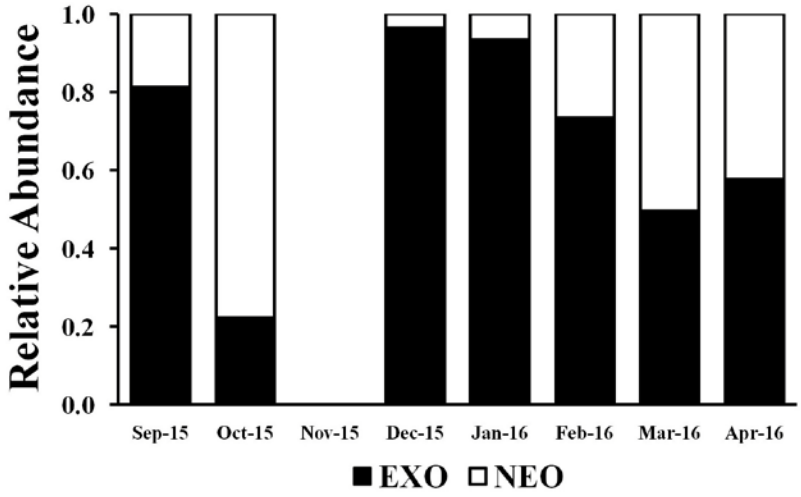

Figure 2. Relative abundance of exotic (**EXO**) and neotropical (**NEO**) drosophilids collected in a gallery forest in the vicinity of *Campus Planaltina* of the *Instituto Federal de Brasília*. Data collected from September 2015 to April 2016.

In summary, this study conducted in an unprotected gallery forest of the Brazilian savanna revealed a smaller number of drosophilid species compared to those studies performed in protected areas of this biome, and a significant reduction in abundance of native species. Controlling anthropogenic actions that degrade nature and facilitate biological invasions is the greatest challenge of humanity, and such actions are essential not only for the maintenance of wildlife but also for the survival of humans. Thus, we recommend the immediate legal protection of biological resources in still unprotected forest environments, the loss or alteration of which may contribute to an additional reduction of the heterogeneity and biodiversity of the neotropics.

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Three new records of drosophilids for the Brazilian Savanna.

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The Brazilian Savanna, locally known as Cerrado, covers most of the interior of Brazil and includes a few small contiguous areas in Bolivia and Paraguay (Oliveira and Marquis, 2002). This extremely biodiverse savanna is the second largest South American biome and one of 34 biodiversity hotspots around the world, due to its high endemism and the extreme threats that it faces (Mittermeier *et al*., 2005). Thus, documenting biodiversity in this area is a significant step toward obtaining critical subsidies for its preservation and conservation.

Taxonomic inventories of drosophilids in the Brazilian savanna have revealed 129 species representatives of the genera *Amiota*, *Drosophila* (the richest genus, with 90 species), *Diathoneura*, *Hirtodrosophila*, *Leucophenga*, *Mycodrosophila*, *Neotanygastrella*, *Rhinoleucophenga*, *Scaptomyza*, and *Zygothrica* (Roque *et al*., 2015). Most species are endemic to the Neotropical Region and are distributed unevenly in this biome probably because of its temporal and spatial heterogeneity. The data produced by these inventories also suggest that biodiversity of drosophilids of the Brazilian Savanna is poorly explored, because of the high number of morphotypes and new records continuously registered for this biome. In this paper, we document the first record of three drosophilid species in the Brazilian Savanna and update the total number of drosophilids known for this biome. From October 2013 to April 2016, we monitored drosophilid assemblages in the IBGE Ecological Reserve (15º56' S; 47º53' W), using retention traps (Roque *et al*., 2011) with fermented banana as bait. Twelve specimens of *Drosophila piratininga* Ratcov and Vilela were collected in gallery forests (several collections), and one male of *D. aldrichi* Patterson and Crow was recorded in *cerrado sensu stricto* (February 2014). In the *Campus Planaltina* of the *Instituto Federal de Brasília* (15°38' S, 47°41' W), distant about 60 km from IBGE, we captured two specimens of *Scaptomyza vittata* Coquillet in February 2016 from bean plants, using drop cloths. All drosophilids were maintained in ethanol 70% and identified using taxonomic keys and descriptions (Brncic, 1955; Hackman, 1959; Wheeler and Takada, 1966; Vilela, 1983; Vilela and Ratcov, 2007). Voucher specimens were deposited in the Collection of the *Laboratório de Biologia Evolutiva* of the *Universidade de Brasília* and *Laboratório de Biologia Animal* of the *Instituto Federal de Brasília* (*Campus Planaltina*).

Drosophila (Drosophila) piratininga belongs to the *canalinea* group, which includes cryptic neotropical species easily recognized by being mainly dark brown flies with two brown rings on each yellowish tibia and a mesonotum exhibiting an intricate pattern of diffuse longitudinal stripes (Ratcov and Vilela, 2007). *D. piratininga* was previously registered in Southern and South-eastern Brazil, in the Pampas (RS) and Atlantic Forest (RS, SC and SP) biomes (Ratcov and Vilela, 2007; Döge *et al*., 2008; García *et al*., 2012; Valer *et al*., 2013; Poppe *et al*., 2014). Consequently, this is the northernmost record for *D. piratininga.*

Drosophila (*Drosophila*) *aldrichi* is a cryptic species of the *mulleri* subgroup of the *repleta* group. It was recorded in USA (TX), from Mexico to Brazil, and in Australia (Brake and Bächli, 2008). In Brazil, this species has been previously registered in the Amazon Forest (AM), Pantanal (MS), and Atlantic Forest (PR) (Vilela, 1983).

Scaptomyza (*Mesoscaptomyza*) *vittata* is a yellowish species characterized by two acrostical rows between three pairs of dorsocentral bristles, and it is probably a leaf miner in its larval stages. This fly is a neartic-neotropical widely distributed species (Brncic, 1955; Hackman, 1959; Wheeler and Takada, 1966), previously recorded in North and Central America, Colombia, Ecuador, Peru, and Bolivia. This is the first record of *S. vittata* in Brazil, extending its easternmost distribution in the Neotropical Region.

In sum, considering the absence of *Drosophila saltans* (a species recorded in Roque and Tidon, 2013) in the list published by Roque *et al*. (2015) and the three new records of drosophilids reported here, we extend the total number of drosophilid recognized for the Brazilian Savanna to 133 species (117 Drosophilinae and 16

Steganinae).

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Chromosomal polymorphisms in natural populations of *Drosophila malerkotliana.*

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Structural aberrations in the chromosomes of *Drosophila* can be distinctly observed due to presence of polytene chromosomes. Inversions, particularly paracentric inversions, are very common in *Drosophila* (Dobzhansky,1950; da Cunha, 1960; Sperlich and Pfriem, 1986). Presently, we are studying genetic polymorphisms at all the three levels, *i.e.*, chromosomal, protein, and nucleotide, in different natural populations of *bipectinata* species complex. *Drosophila bipectinata* species complex is a group of four closely related species that includes *D. bipectinata, D. parabipectinata, D. malerkotliana*, and *D. pseudoananassae*. Phylogenetic relationships among these four species have been documented by earlier researchers (Bock, 1971; Singh and Banerjee, 2012; Singh and Banerjee, 2016; Tomimura, 2005). Chromosomal polymorphisms in *D. malerkotliana* has been reported by some of the population geneticists, and their study has revealed that this species is chromosomally polymorphic (Jha and Rahman, 1972; Naserulla and Hegde,1993; Singh and Singh, 2015). In this report we are describing about four new paracentric inversions, which have been observed in two distantly located natural populations of *D. malerkotliana*.

Isofemale lines established from two natural populations of *D. malerkotliana* collected from Varanasi (Uttar Pradesh) and Bilaspur (Chhattisgarh) were analyzed for chromosomal polymorphisms. These two places are separated from each other by a distance of about 530 km. Third instar larvae randomly selected from isofemale lines were dissected in insect saline to isolate salivary glands, and the glands were then transferred onto cleaned glass slides. The glands were stained in lacto-aceto-orcein and squashed in mountant (60 percent acetic acid + lactic acid in 1:1 ratio) for polytene chromosomes preparation. Hundreds of larvae subjected to this study from the two natural populations enabled us to identify eleven different types of paracentric inversions. Out of these, four new inversions were observed for the first time in this species. Among the four new inversions, three were located on autosomal chromosomes and one in the X-chromosome. Two inversions, *i.e.*, median and basal, were found to be present in 3L and a single basal inversion was present in 2R. A single X chromosome inversion was median in position in the left arm of X chromosome. Figure 1a-d depicts the microphotographs of these inversions in different chromosome arms of

D. malerkotliana. Figure 2 shows line diagrams indicating the break points of these inversions, and the inversion breakpoints have been identified by following the salivary gland chromosomal map of *D. malerkotliana* constructed by Jha and Rahman (1972).

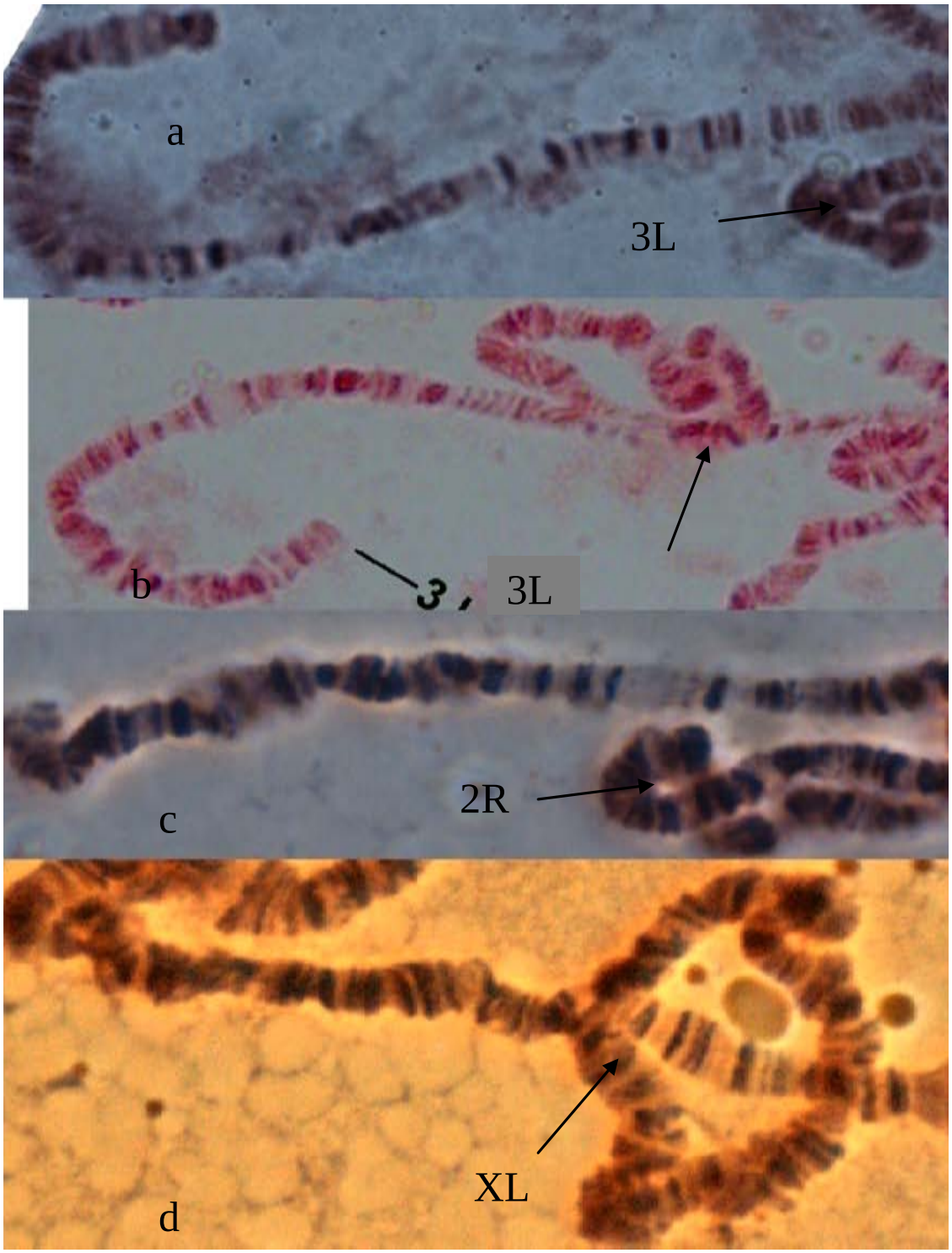
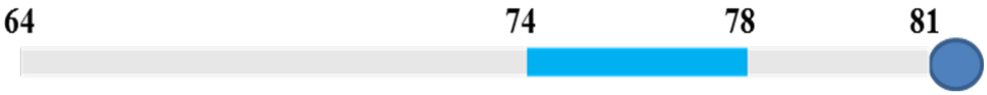


Figure 1. a-d: Microphotographs showing four new inversions in *D. malerkotliana.*

- Basal inversion of 3L (Figure 1a): Observed from Bilaspur population. It extends between the regions 81 to 77 occupying nearly 20% of the chromosome arm.

- Median inversion of 3L (Figure 1b): Detected in Bilaspur population and it extends between the regions 78 to 74. It involves about 20% length of the chromosome arm.



- Basal inversion in 2R (Figure 1c): Observed from both populations and occupies about 30% of the chromosome arm.

- Median inversion in XL (Figure 1d): Observed in Varanasi population, extends approximately 30% of the chromosome arm.

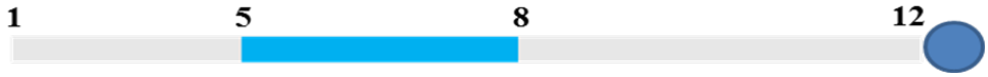


Figure 2. Line diagrams indicating the break points of inversions.

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Study of inversion heterozygosity in three Indian populations of *Drosophila ananassae.*

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Abstract

Changing environmental conditions play a major role in bringing mutational modifications in the genetic makeup of the species. On encountering any stress in the surroundings, the organisms may undergo several behavioral changes to adapt to the new environment. Inversion is one such chromosomal aberration wherein the sequence on one or both of the chromosomes gets inverted. Inversion heterozygosity is linked to formation of loops on account of which recombination frequency in the inverted region decreases and the underlying genes escape themselves to a certain amount. Literature suggests that inversions play a significant role in adaptation, speciation, and affects life-history traits of an organism. Inversion polymorphisms are found to be species-specific, therefore often used for phylogenetic analysis and species identification. Inversions are also found to be population specific, thus help to determine the eco-geographic distribution of a species population. *Drosophila* is an ideal model system for studying inversion polymorphism as it possesses polytene chromosomes in its larval stage, which is extensively studied for banding patterns and differential gene expression. The present work was carried out to study inversion heterozygosity in *Drosophila ananassae* belonging to Northern (Ahmedabad, Ghaziabad) and Southern (Kochi) India. The study was further extended to examine the reproductive traits, *e.g.*, ovariole number and fecundity in the Kochi population, and a correlation was established between inversion heterozygosity and reproductive success. The results of the current study revealed that the Kochi population had the maximum inversion heterozygosity. Keywords: Inversion-heterozygotes, *D. ananassae*, India, Reproductive –fitness

Introduction

Inversions, a kind of chromosomal rearrangement, are classified into different categories on the basis of (i) position, *i.e*., paracentric or pericentric depending on the inclusion of centromere (Griffith *et al*., 2000); (ii) demography, *i.e*., common cosmopolitan (frequency > 5%), rare cosmopolitan (< 5%), recurrent endemics (occur in a few individuals), and unique endemics (found only once) (Mettler *et al*., 1977); (iii) chromosomal arrangement, *i.e*., homozygous (Inv/Inv) or heterozygous (ST/Inv). The significant role of inversion polymorphism in different evolutionary processes and disease pathogenesis in human beings has been well documented in earlier studies (Feus *et al.,* 2005; Kehrer *et al.,* 2005; Broman *et al*., 2003). *Drosophila* has been an attractive genetic model since the time of Morgan and contributed immensely to understanding both the classical and molecular aspect of complex biological systems (Beckingham *et al.,* 2007). Various mutations are observed in its natural populations. Successful establishing and maintenance of mutant lines in the laboratory conditions makes it more instrumental in conducting inheritance experiments. The presence of polytene chromosomes in the third instar larval stage of this organism allows its use in cytogenetic studies. The polytene chromosome helps the organism to undergo development and growth at a fast rate and hence is present at the larval stage wherein an organism requires maximum growth. The banding pattern present in the polytene chromosome shows the expression profile of genes and also helps in determining inversion polymorphism. The importance of inversions in *Drosophila*, their population-specific and species-specific occurrence and adaptive nature has been reported in earlier studies (Singh, 2008; Rezende *et al*., 2010; Singh *et al*., 2012; Kenig *et al*., 2015). Inversions are known to help in the process of adaptation (Dolgova *et al*., 2010), speciation (Noor *et al*., 2001) and play an important role in species identification (Feder *et al*., 2014) and also in understanding evolution of sex chromosomes (Ming and Moore, 2007).

Drosophila ananassae is a common cosmopolitan species having a world-wide distribution and that has become a model for various genetic and evolutionary studies due to its unique characteristics, *e.g*. spontaneous male recombination, segregation distortion, mutation (Mukherjee and Das, 1971), occurrence of genetic mosaics for autosomal genes (Singh and Mohanty, 1992), high mutability (Tobari, 1993), and extra-chromosomal inheritance (Sturtevant, 1942). *D.ananassae* is known to harbor a large number of paracentric and pericentric inversions in its four chromosomes (Singh, 1989). The present work was conducted to find the frequency of inversion polymorphism in Indian *D. ananassae* collected from three different regions of Northern (Ghaziabad, Ahmedabad) and Southern (Kochi) India. The study was further extended in one of the populations, *i.e*., Kochi, to find whether there is any correlation between inversion heterozygosity and the variations in life-history traits, *e.g*., fecundity and ovariole number.

Material and Methods

Drosophila *strains used*

D. ananassae flies were collected from Ahmedabad, Kochi, and Ghaziabad between July-October 2015. The flies were collected from fruit orchards by net sweeping method from shaded areas. Each single naturally caught female fly was kept in separate vials to establish iso-female lines. The progeny from each iso-female line were routinely sub-cultured to fresh media vials and a subsequent generation record was maintained for each line. Fly stocks were kept in incubator at 25°C and were transferred to fresh food vials on a weekly basis. Eight iso-female lines from each of these regions were used in the present study.

Setting up of experiment

20 male and female flies were kept in vials and allowed to mate for 4-5 days after which the parental flies were discarded or transferred. To ensure healthy growth of the larvae, yeast paste was added after the 5th day, and the vials were observed on a daily basis for emergence of third instar larvae.

Cytological analysis

Healthy third instar larvae (~20 from each iso-female lines) were taken and the salivary gland was isolated for preparation of polytene chromosome slides by the squashing method. Polytene chromosomes were observed under a microscope. The chromosomal arms with inversion polymorphism were identified by comparing them with the chromosomal maps from FlyBase. Inversions identified on each chromosomal arm were categorised into different types as mentioned above.

Fecundity and ovariole number

Both fecundity and ovariole number were calculated in 4 iso-female lines of the Kochi population. 20 virgin males and females each from 4 days old age were kept in a mating chamber for 48 hours, and the eggs laid during this period were counted to find the fecundity rate. For ovariole number, ~ 8-10 days aged females were dissected for counting of their ovarioles present in both the ovaries.

Results and Discussion

1. Inversion Heterozygosity with respect to different chromosomal arms:

In all studied populations, the 2L chromosome arm was found to be highly polymorphic for inversions as carrying the maximum number of different inversions. Alpha (Figure 1.a.i), a common cosmopolitan inversion, was found to be present at a frequency of 12% in Kochi and Ghaziabad and 4% in Ahmedabad populations (Figure 3). Also, four more inversions were found on the 2L arm out of which one of them (Figure 1.a.ii) was common to both Kochi and Ahmedabad, whereas the third one (Figure 1.a.iii) was common to Kochi and Ghaziabad. The rest of the two were specific for their regional population (Figure 1.a.iv, v). Alpha covers around 60% of the total chromosomal arm whereas the other four inversions cover around 20% of the area. All of the inversions were found to be sub-terminal. The right arm of the second chromosome harbored 2 different inversions, one of them being cosmopolitan, *i.e*., Zeta as shown in Figure 1.b.i (Singh and Mohanty, 1990), was found in all three regional populations, whereas the second one was specific for Kochi

(Figure 1.b.ii). Zeta spans around 50% of the chromosomal arm and the new inversion takes around 20% of the region. The 3L arm of the chromosome has one cosmopolitan inversion, Delta (Figure 2.a.i), which spans around 50% of the arm and was found to be present in Kochi, Ghaziabad, and Ahmedabad populations at a frequency of 41%, 25%, and 17%, respectively (Figure 3). Also a new sub-terminal inversion, which occupies 15% of the region on the 3L arm, was found only in the Kochi population (Figure 2.a.ii). Eta is a cosmopolitan inversion found on 3R, which is located very close to the centromere and occurred at 23%, 49%, and 12% in Kochi, Ghaziabad, and Ahmedabad, respectively (Figure 2.b.i; Figure 3). It spans around 20% of the total chromosomal area and was reported in all three populations. A second inversion, 3R1 (Figure 2.b.ii), found on this arm spans around 20% of the area and was specific for the Ahmedabad region.

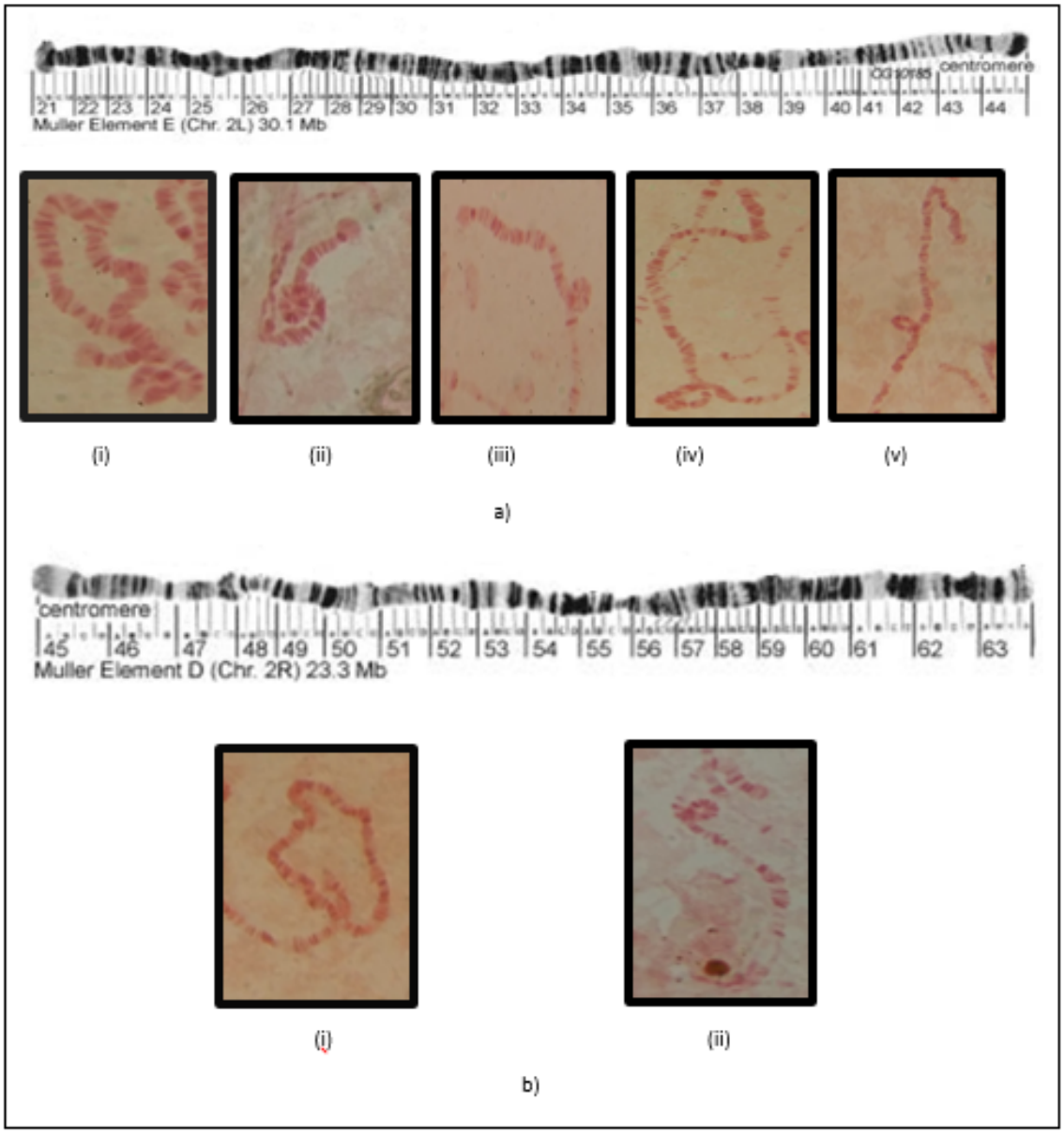


Figure 1. Cytological map and inversions found on the second arm of the chromosome.

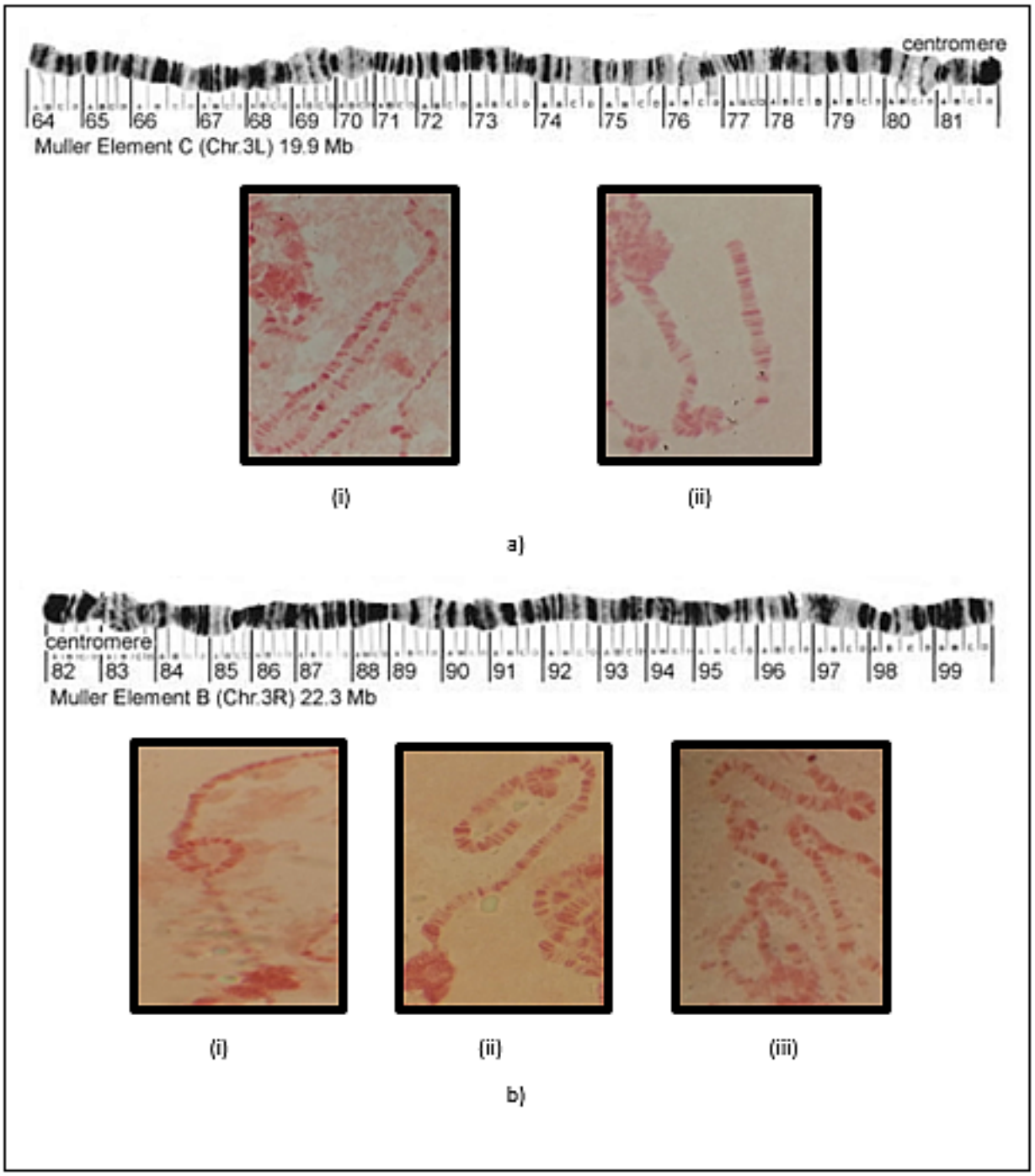


Figure 2. Cytological map and inversions found on the third arm of the chromosome.

2. Clinal variation in inversion heterozygosity

Overall, all three populations reported the presence of cosmopolitan inversions; however, some of the inversions were found to be only region specific. Also by calculating the mean number of inversion heterozygosity, it was found that the Kochi population has higher mean heterozygosity (0.95), followed by Ghaziabad (0.83), and Ahmedabad (0.55) showing the minimum mean heterozygosity. Such a variation in the frequency of inversion heterozygotes prompted us to dig the literature and find if this frequency distribution

shows any clinal pattern. One of the works on the Nagaland population of *D. ananassae* by Bovito (2015) reported the frequency distribution of three cosmopolitan inversions (2L, 3L, 3R). Thus, we have analysed the data obtained for these three inversions in the present study and compared it to the data from literature (Figure 3). All these regions show a similar pattern with respect to frequency distribution by showing higher percentage of inversion heterozygotes on the third arm of chromosome (Delta-3L, Eta-3R), which can be contrasted from the one obtained in the Nagaland population, which harbors a higher number of Alpha heterozygotes (Bovito, 2015). Such a variation clearly shows that inversion is an adaptive trait, and it also shows clinal pattern of distribution which was also well established through earlier studies (Anderson *et al*., 2005; Umina *et al*., 2005).

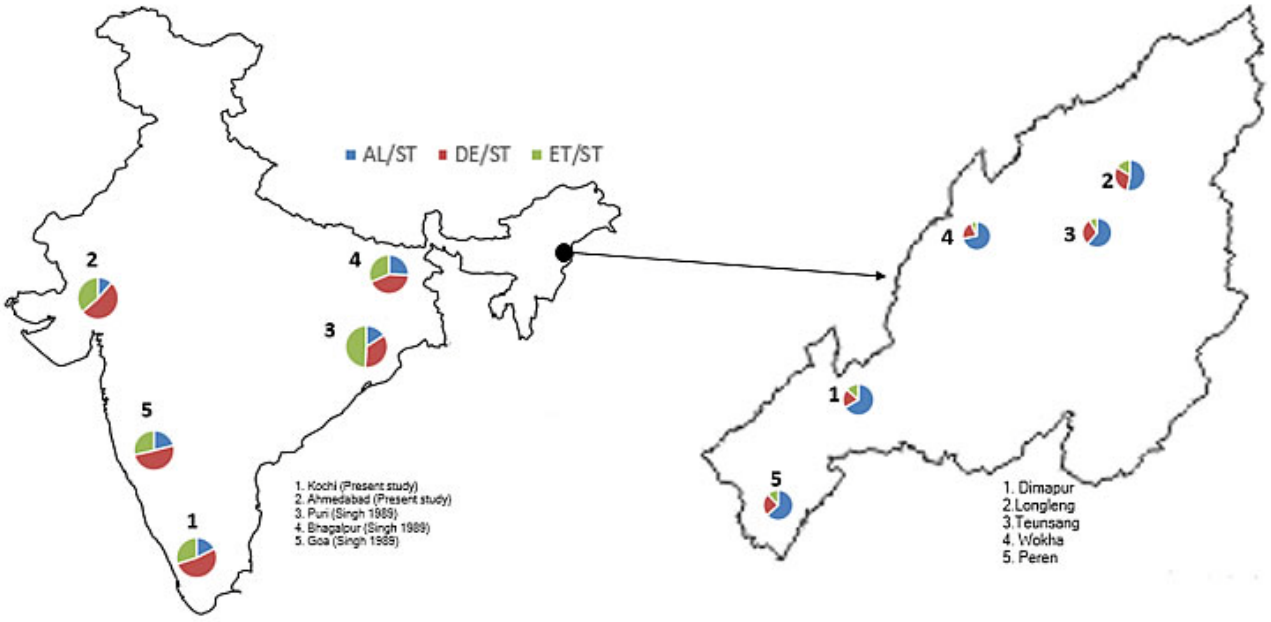


Figure 3. Frequency distribution of cosmopolitan inversions in different regions of India and comparison with Nagaland population frequency distribution (The data for Puri, Bhagalpur, and Nagaland have been taken from literature.).

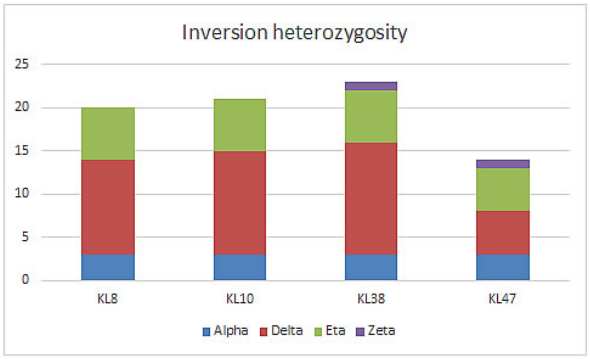
3. Life-history trait analysis in Kochi population

The extended study in the Kochi population revealed that a correlation exists between inversion heterozygosity and rate of fecundity. However, a slight variation was observed with respect to the number of ovarioles when compared with the inversion heterozygosity. Amongst the four iso-female lines of Kochi, KL 38 shows a higher number of inversions and maximum number of ovarioles (average 22 per ovary) and high fecundity values (70 per 20 females in 48 hours) as shown in Figure 4. In contrast, KL 47 has a lower number of inversion heterozygotes, least number of ovarioles, and also less fecundity. Such an occurrence can help us to establish a hypothesis that inversion heterozygosity is linked to reproductive success of the females.

Conclusion

Inversion heterozygosity shows a variation on the basis of population and is an adaptive trait. The results clearly show that the Kochi population shows more inversions as compared to Ahmedabad or Ghaziabad populations. The third chromosome inversions are found to be fixed for all three populations. From the overall comparison between inversion frequency in these different regions of India including

Nagaland, it can be further concluded that inversions are regional-specific and are known to show latitudinal clines. The study involving life-history trait variation along with the inversion heterozygosity in Kochi population evidences that heterozygous inversion can influence the reproductive success of an organism. To come up with a strong inference, the study needs to be extended with an increased sample size. Earlier studies have reported that not only the inversion frequency but the total chromosomal region included in the inversion heterozygote loops also affects the recombination in other chromosomes. Breakpoint analysis of inversions and further digging into the essential genes present in the inversion area may help us to determine the significant role of these inversions on the functional genes and to understand more about the process of mutation and adaptation.



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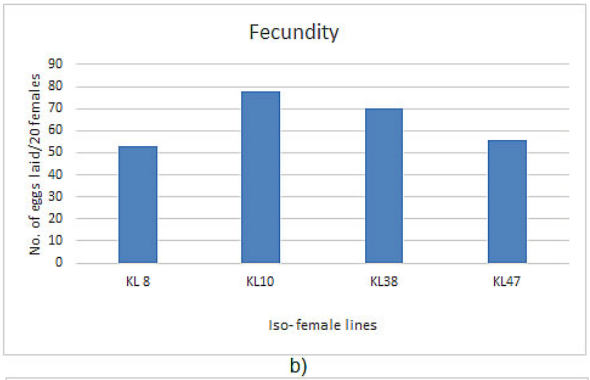

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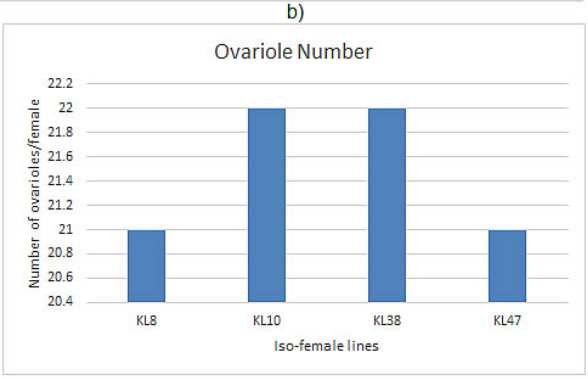

c)

Figure 4. Shows variation in a) inversion heterozygosity, b) fecundity, and c) ovariole number in four iso-female lines of Kochi.

Acknowledgment: The authors thank the Vice Chancellor, JIIT for extending facilities for carrying out the present work.

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Magnesium supplementation reduces seizure and paralysis in *technical knockout* and *easily shocked* bang-sensitive mutants.

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Introduction

The idea that diet and nutrition play a role in health disorders is not a new one. Nutritional factors have been shown to be involved in the regulation of electrical activity in the brain (Gaby, 2007). In particular, maintenance of proper electrolyte concentrations are important, since their disruption commonly results in seizure and convulsions (Castilla-Guerra *et al.*, 2006). Epilepsy is a common seizure disorder that is treated by anticonvulsants; however, these drugs have limited efficacy in many patients. Because drugs have been somewhat ineffective, other treatments have been sought including diets. For example, the ketogenic diet, which is high in fat and low in carbohydrates and protein, has been utilized in controlling epilepsy in children (Lutas and Yellen, 2013).

Magnesium (Mg) has been used as an anticonvulsant for the condition of pre-eclampsia (Castilla-Guerra *et al.*, 2006). However, the electrolyte has not been used as a treatment for general epilepsy. There are many properties of Mg that may make it a potentially effective anticonvulsant. Mg is the fourth most common mineral found in the human body and is required for over 300 enzyme systems and as a cofactor for mitochondrial energy production. Magnesium also inhibits NMDA receptors, increases prostaglandin synthesis, stabilizes neuronal membranes, and acts as a calcium channel blocker (Castilla-Guerra *et al.*, 2006). Mg deficiency also has been shown to cause seizures or increase susceptibility to seizures. Sufferers of grand mal seizures have been found to maintain lower serum concentrations of magnesium than controls (Gaby, 2007). In a study of hippocampal seizures in rats, injection of $MgSO_4$ proved to increase the rats' seizure thresholds (Hallak *et al.*, 1992). In addition, Mg content in food has been in decline over the past sixty years and up to 75% of Americans do not take in the recommended dietary allowance of the mineral (Yuen and Sander, 2012).

Our study examines if magnesium supplementation could alter seizure behaviors in bang-sensitive mutants that are models of mitochondrial disease and epilepsy. Bang-sensitive (BS) paralytics exhibit seizures and paralysis in response to mechanical stimulation (Reynolds *et al.*, 2004). They also show a reduced threshold for seizures compared to wild-type in electrophysiology experiments (Kuebler and Tanouye, 2000). Bang-sensitive mutants include *bangsenseless* (*bss*), *bang-sensitive* (*bas*), *easily shocked* (*eas*), and *technical knockout* (*tko*), among others that express the bang-sensitive phenotype, but with differing biological underpinnings. We found that *eas* and *tko* mutants raised on diets supplemented with Mg would experience decreased seizures and shorter recovery times when exposed to stress conditions.

Methods and Procedures

Flies

Laboratory stock cultures of *tko* and *eas* flies as well as the CS wild-type background were used for the experiments. Flies were cultured at 25°C under 12 hr dark/light conditions on standard molasses/yeast/cornmeal (MYC) food with and without 30 mM $MgSO_4$.

Testing

After eclosion, flies were anesthetized with carbon dioxide and distributed to holding vials for 24 hrs in groups of approximately 10. Flies were then moved to test vials and rested for 15 min. Each vial was vortexed on the highest power setting for ten seconds and was only tested once. The total number of flies in each vial was recorded as well as the number that were paralyzed or seizing on their backs after the ten seconds and the recovery time to standing for each individual fly.

Analysis

A percent paralysis was calculated for each vial and the average percentage was computed for each condition. Average recovery time was assessed for each condition by averaging the recovery times for each individual fly that was paralyzed. Statistical comparisons were made using T tests assuming equal variance (all samples showed equal variance) and using a p value of 0.05 as significant.

Results

The results of the experiments are presented in Table 1. CS flies showed no evidence of seizure or paralysis in either the control or experimental condition. The bang-sensitive mutants *eas* and *tko* both showed a significant improvement in the percentage of flies that seized/paralyzed (91% *vs*. 74% and 95% *vs*. 40%, respectively). The recovery time for both mutants was also reduced by the Mg supplementation. The mutant *tko* was more greatly impacted in terms of percent paralysis, but less impacted in terms of recovery time.

Table 1. The impact of 30 mM magnesium supplementation on bang-sensitive paralysis and recovery.

Strain	Condition	Replicates (N recovery)	% paralysis	T test P value	Recovery in sec	T test P value
CS	No Mg	11	0		--	
	Mg	11	0		--	
eas	No Mg	11 (107)	0.91 ± 0.09	< 0.001	99 ± 40	< 0.0002
	Mg	11 (99)	0.74 ± 0.09		76 ± 41	
tko	No Mg	11 (105)	0.95 ± 0.09	< 0.0001	82 ± 41	0.017
	Mg	11 (51)	0.40 ± 0.11		68 ± 40	

Discussion

Mg supplementation appears to reduce seizure and paralysis in bang-sensitive flies. The amount of Mg increased by supplementation in the food in our experiments is substantial. MYC food is normally about 3 mM for Mg and so the supplemented food is about 10× the normal amount. The results are striking since both mutants were impacted by the supplementation, and to a similar extent observed in feeding antiepileptic drugs such as phenytoin to flies (Reynolds *et al.*, 2004). The gene associated with *eas* encodes ethanolamine kinase, a lipid biosynthetic gene, and the mutant is known to impact lipid composition in the nervous system (Pavlidis *et al*., 1994; Nyako *et al.*, 2001). The gene associated with *tko* encodes a mitochondrial ribosomal protein, and the mutant strain appears to have reduced cytochrome oxidase activity (Royden *et al.*, 1987). Both mutants may interact with Mg through deficits in mitochondrial function or perhaps by stabilizing neuronal membranes. It is possible that Mg deficiency contributes to poor efficacy of anti-epileptic drugs or the prevalence of seizure disorders. While this result is preliminary, it suggests that the interaction between Mg supplementation and epilepsy be further explored.

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Drosophilid assemblages in burned and unburned vegetation in the Brazilian Savanna.

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Introduction

The Brazilian Savanna, locally known as Cerrado, is a unique savanna hotspot covering about 2,000,000 km² of South America. It contains savanna vegetation of highly variable structure on the well-drained interfluves, with gallery forests or other wetland vegetation following the watercourses (Oliveira and Marquis, 2002). This biome is highly seasonal, characterized by a distinct dry season from May to September. Wildfire events have been occurring for a long time in this region, as evidenced by the unique array of fire-adapted plant species.

Natural populations of drosophilids have been investigated in this biome, particularly over the past two decades, revealing that the strong environmental heterogeneity impacts the structure of drosophilid assemblages. Communities and populations of these insects decrease through the dry seasons, expand during the rainy seasons, and differ remarkably among vegetation types (Tidon, 2006; Mata *et al.*, 2015). Anthropogenic disturbances, such as urbanization and forests in successional stages, also play a role in drosophilid communities (Ferreira and Tidon, 2005; Mata and Tidon, 2013). Although fire is recognized as a significant form of disturbance due to its potential to influence global ecosystem patterns and processes (Bowman *et al.*, 2009), little is known about the post-fire effects on these flies' assemblages.

In this study, we investigated drosophilid assemblages from forest and savanna patches two years after they had been affected by a severe fire, as well as nearby long-unburned patches used as control sites.

Material and Methods

This research was conducted in the Ecological Reserve of IBGE, located 35 km south of Brasília, the capital of Brazil. This reserve is part of a continuous Environmental Protection Area of 10,000 ha, which has been considered since 1993 by UNESCO to be a core area of the Cerrado Biosphere Reserve. In September 2011, two years before our collections, an accidental and severe fire affected about 90% of the IBGE Reserve and changed its landscape harshly.

Drosophilid samples were carried out bimonthly, from October 2013 to August 2015. On each sampling occasion, four sites representing different habitat types were sampled: (1) burned forest, (2) unburned forest, (3) burned savanna, and (4) unburned savanna. To control for the high heterogeneity of the savanna vegetation, our collections (3 and 4) were made in *cerrado sensu stricto*, a type of savanna very common in the biome. In each site, three sampling units (SU) were established at least 30 m apart. Each SU contained three retention traps (Roque *et al.*, 2011) arranged 10 meters apart. Thus, we came to a standardized sampling effort of three replicates in each one of the four sampled habitats, corresponding to 36 traps per sampling occasion.

The traps were baited with bananas fermented with *Saccharomyces cerevisiae* for 24 hours, and then they were left in the field for four consecutive days. The drosophilids were identified, whenever possible, to the species level. Vouchers of the captured species were deposited at the Collection of the *Laboratório de Biologia Evolutiva da Universidade de Brasilia.*

The diversity between burned and unburned patches was evaluated by rarefaction curves ($S_{est,}$ plus 95% confidence intervals) (Colwell *et al*., 2012). The comparison of species richness was done by rescaling the expected *sample-based* species rarefaction curves by *individuals*, instead of leaving them scaled by samples, as recommended by Gotelli and Colwell (2001). Presenting the curves scaled by samples, however, allowed evaluating the suitability of the sampling. The data for the curves were generated in the software EstimateS 9.1 (Colwell, 2013).

Results and Discussion

In this study, we collected 34,968 drosophilids representing 45 nominal species in the genera *Drosophila*, *Rhinoleucophenga*, *Scaptodrosophila*, or *Zaprionus*. The abundance of flies declined in the burned areas (unburned forest: n = 11,952 and mean = 332.00; burned forest: n = 8,510 and mean = 236.39; unburned savanna: n = 9,444 and mean = 262.33; burned savanna: n = 5,062 and mean = 140.61). As the sampling effort was standardized among burned and unburned sites, this difference probably reflects biologically meaningful patterns of resource availability and growth conditions. High severity fires have previously been shown to reduce the abundance of many insect groups, because niche diversity and availability is lower in recently burned habitats (Swengel, 2001; Buckingham *et al*., 2015). After being affected by fire, forests and savannas may become drier and lack feeding and breeding sites for drosophilids.

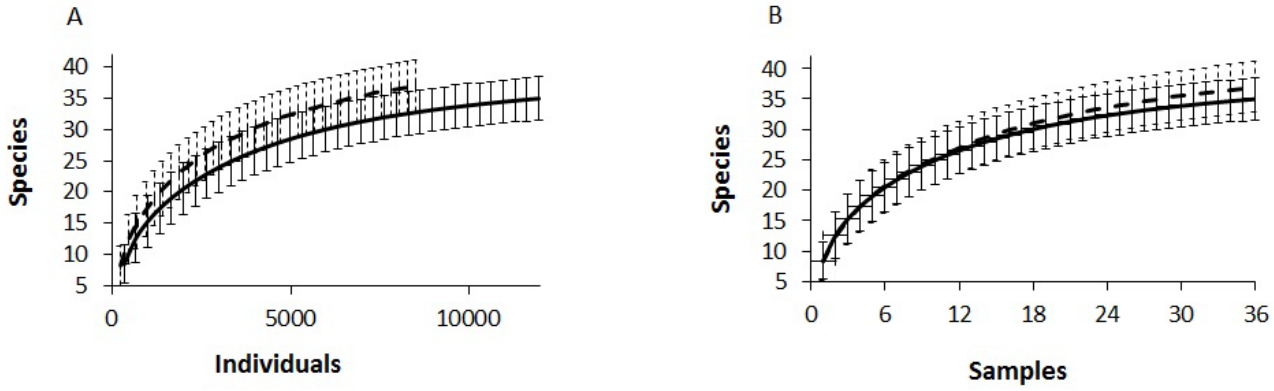

Figure 1. Rarefaction curve of drosophilid species (S_{stat}; ± 95% IC) recorded at burned (dashed line) and unburned (bold line) forests in the Ecological Reserve of IBGE between October 2013 and August 2015 scaled by A) individuals and B) samples.

Species richness and distribution of abundances did not differ between burned and unburned forest patches (Figures 1 and 2). In the savanna, on the other hand, the burned curve tends to stabilize at around 20 species, whereas the unburned curve is still rising (Figure 3). Moreover, the burned savanna patch has lost rare species and is strongly dominated by *Zaprionus indianus* (Figure 4). These results contrast with those obtained by Mata and Tidon (2013), who found differences between disturbed and undisturbed forests but not between disturbed and undisturbed savannas. This apparent incongruence, nonetheless, may be explained by two factors. First, it can be due to methodological inadequacies in the sampling design adopted by Mata and Tidon (op cit.). These authors compared undisturbed habitats with a group of habitats under different types and levels of disturbance such as fire, anthropic buildings, and dusty roads. The different effect of each type of disturbance could puzzle the results. Alternatively, the inconsistency pointed here can be due to sampling insufficiency of the present study. For each vegetation type (forest or savanna), we compared only one burned

site with its unburned control. Although each site sampled here had three replicates, we are aware that if we had searched a wider geographical area the result would have been different.

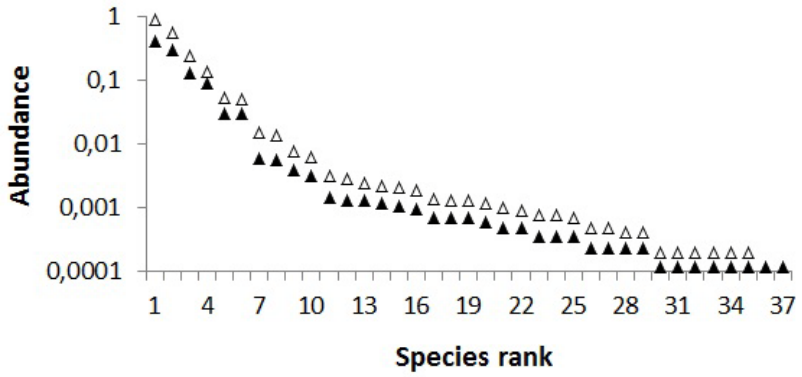

Figure 2. Rank-abundance plot of the drosophilid species collected at burned (black triangles) and unburned (empty triangles) forests in the Ecological Reserve of IBGE between October 2013 and August 2015. The relative abundance of each species is shown on a log 10 scale.

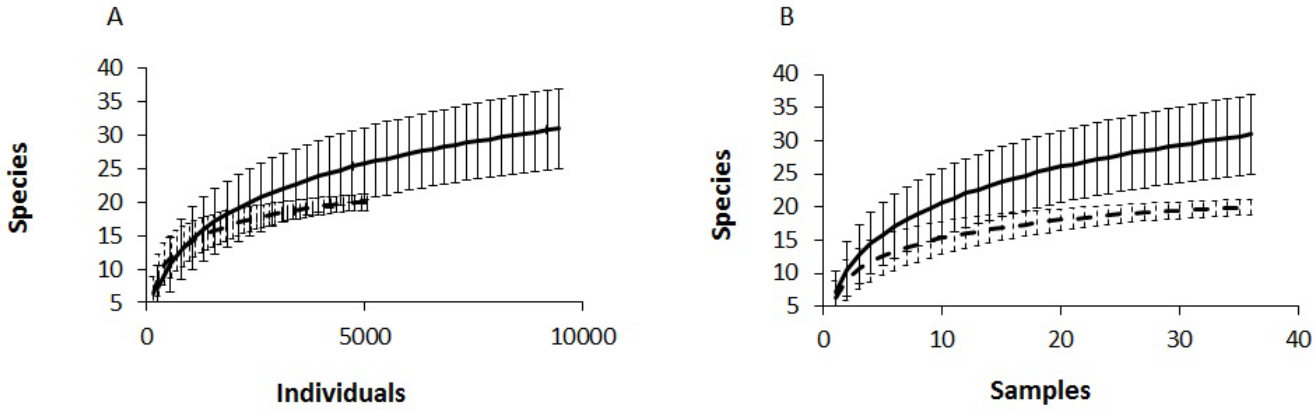

Figure 3. Rarefaction curve of drosophilid species (S_{stat}; ± 95% IC) in burned (dashed line) and unburned (bold line) savannas in the Ecological Reserve of IBGE between October 2013 and August 2015 A) scaled by individuals, and B) scaled by samples.

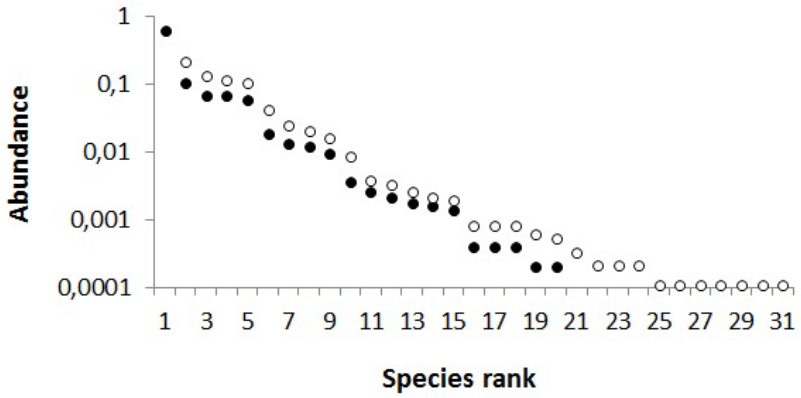

Figure 4. Rank-abundance plot of the drosophilid species collected at burned (black circles) and unburned (empty circles) savanna in the Ecological Reserve of IBGE between October 2013 and August 2015. The relative abundance of each species is shown on a log 10 scale.

The effects of fire on insect assemblages have been investigated in a few insect orders, including Hymenoptera, Lepidoptera, Collembola, Hemiptera, and Coleoptera (New, 2014). Nonetheless, different groups have been shown to become variously poorer and less abundant or richer and more abundant in burned than unburned 'control' patches. Such trends are difficult to predict because insect responses are affected by the geographic region, climatic conditions, fire regime, and intensity. Clearly, more research is needed to predict population and community dynamics, as well as ecosystem and global changes (Showalter, 2012). Drosophilids are good models for investigating the effects of disturbance on natural populations, because they are diverse, easily sampled, and sensitive to environmental variations. Here, we showed differences in assemblages between burned and unburned habitats based on abundance and richness of drosophilids. Future research should refine these results by increasing the geographical scale and including community analyses that consider not only abundance and richness but also species composition.

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Drosophilids of the Lake Urmia National Park, Iran (Dip.: Drosophilidae).

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Abstract

Drosophilid fauna of the Lake Urmia National Park was studied for the first time in the framework of an ongoing survey on the economically and veterinarily important flies of the Iranian national parks. A total of 10 species have been identified, of which the genus *Lordiphosa* Basden and the three species *Drosophila hydei* Sturtevant, *Lordiphosa andalusiaca* (Strobl), and *Scaptodrosophila lebanonensis* (Wheeler) are found to be new records for the Iranian fauna. The species *D. melanogaster* Meigen, *D. phalerata* Meigen, *D. simulans* Sturtevant, *D. subobscura* Collin, *Scaptomyza flava* (Fallén), *Scaptomyza pallida* (Zetterstedt), and *Zaprionus indianus* Gupta are first recorded from the park. A key to the drosophilids of the Lake Urmia National Park and images of the genitalia of each species are presented.

Introduction

The drosophilid fauna of Iran is poorly studied, being represented by only 25 species, none of which is recorded from the western provinces (Bächli, 2016). The northwestern province of West Azarbaijan borders

Turkey and Iraq to the west and Nakhchivan to the northeast and covers the west coast of the Lake Urmia. Except for a handful of recorded species (Asem *et al.*, 2016), the insect fauna of the Lake Urmia National Park and its islands have remained largely unknown. Due to mismanagement and habitat loss, Lake Urmia has been devastatingly shrunk over the past decades risking the lives of the local people and endangering the lake's fauna and flora. The insect fauna of the Lake Urmia National Park deserves a thorough investigation through which the fragility of the park's ecosystem and the need for sound and effective remedial measures can be underlined.

1

2

Figures 1–2. General views of the two collecting sites at the Lake Urmia National Park. 1, Rashakan and the dry bed of the Lake Urmia; 2, Kaboudan Island.

Material and Methods

Using Malaise traps and pan traps, we have assembled a sizable collection of flies including drosophilids at two locations in the province of West Azarbaijan. The traps were set up in the vicinity of Rashakan village (Figure 1) and Kaboudan Island (Figure 2). The fly specimens are preserved at the Hayk Mirzayans Insect Museum (HMIM), Tehran, Iran.

Localities

Rashakan: Rashakan is a village located 35 km south of the provincial capital city of Urmia. Malaise traps were run from 8 July to 6 September 2016 through the grounds of the Research Station for the Lake Urmia National Park, seated at an elevation of 1315 meters above sea level, on the fringe of the village, in a mixed-fruit orchard close to the coast of the lake (37º20´38.8″N 045º17´37.4″E).

Kaboudan Island: Kaboudan is the largest island of the Lake Urmia National Park. The area of the island is about 3175 hectares (7846 acres) and tightly protected for being home to a number of vulnerable wild bird and animal species. Malaise traps were used from 19-24 June 2016 at an altitude of 1322 meters (37º29´42.7″N 045º38´13.9″E).

Results

We here record 10 drosophilid species, which, except *D. melanogaster*, are all new to the west of Iran including the province of West Azarbaijan and the Lake Urmia National Park. The genus *Lordiphosa* Basden is newly recorded from Iran. The species *Drosophila hydei* Sturtevant, *Lordiphosa andalusiaca* (Strobl), and *Scaptodrosophila lebanonensis* (Wheeler) are recorded from Iran for the first time. The remaining species are as follows: *Drosophila phalerata* Meigen, *Drosophila simulans* Sturtevant, *Drosophila subobscura* Collin, *Scaptomyza flava* (Fallén), *Scaptomyza pallida* (Zetterstedt), and *Zaprionus indianus* Gupta.

Key to the drosophilids of the Lake Urmia National Park

The following key is primarily based on the key in Bächli *et al.* (2004) and includes all recorded drosophilid species of the park. Images of genitalic characters have been provided to enhance the accuracy of the identifications.

1 Mesonotum, scutellum and orbital plates with longitudinal stripes surrounded by black lines; fore femur with a row of setae combined with basal spines (4♂♂, 5♀♀ Rashakan) (Figures 3–5)
……***Zaprionus indianus*** Gupta
[*a polyphagous fruit pest attacking almost 80 plant species that has been spreading rapidly throughout the country since its arrival in 2008 (Parchami-Araghi & Mohammadi-Khorramabadi, 2009)*]

1' Mesonotum, scutellum and orbital plates without longitudinal stripes; fore femur without composite spines
……..….2


3


4


5

Figures 3–5. Male genitalia of *Zaprionus indianus*. 3, lateral view; 4, ventral view; 5. posterior view.

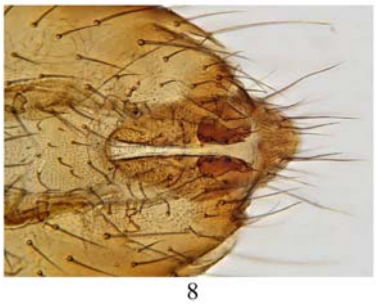
2 2–4 rows of acrostichal setae; 2 katepisternal setae……...3
……..…..…..…..…...*Scaptomyza* Hardy

2' 4–8 rows of acrostichal setae; 3 katepisternal setae…….……..4

3 2 rows of acrostichal setae (1♂, 1♀ Rashakan; 1♀ Kaboudan Island) (Figures 6–8)
…….…….……...….***Scaptomyza pallida*** (Zetterstedt)
[*a saprophagous fly that serves as a secondary pest in canola fields in the Caspian Sea provinces of Iran (Parchami-Araghi et al., 2015, in press)*]


6


7


8

Figures 6–8. *Scaptomyza pallida*. 6, male genitalia, lateral view; 7, male genitalia, ventral view; 8, female postabdomen including sclerotized oviscaps, ventral view.

9

10

11

Figures 9–11. Male genitalia of *Scaptomyza flava*. 9, lateral view; 10, ventral view; 11. posterior view.

3' 4 rows of acrostichal setae (1♂, Rashakan) (Figures 9–11)
……..…***Scaptomyza flava*** (Fallén)
[*a leaf-miner of gypsophila and brassicas* (*Martin, 2004)*]

4 Median katepisternal seta at most half as long as anterior one; carina developed; prescutellar acrostichal setae absent…….....5
…….*Drosophila* Fallén

4' Median katepisternal seta at least as long as anterior one; carina absent or present; prescutellar acrostichal setae absent or present…….....9

5 Marginal bands on abdominal tergites uniform and complete……...6

5' Marginal bands on abdominal tergites medially narrowed or interrupted..……...8

12

13

14

15

Figures 12–15. *Drosophila subobscura*. 12, male genitalia, lateral view; 13, male genitalia, ventral view; 14, male genitalia, posterior view; 15, sex combs on protarsomeres 1-2 of male.

6 Both protarsomeres 1 and 2 in male with distinct sex combs; wing slightly darkened along vein Costa in apical half (2♂♂, Rashakan) (Figures 12–15)
……..…….***Drosophila subobscura*** Collin
[*Its larvae cause problems in wineries, breweries, and distilleries by feeding on grapes, but at the same time transfer wild yeasts that give rich bouquet (Bächli, 2004).*]

6' Only protarsomere 1 in male with a distinct sex comb; wing hyaline...……7

16

17

18

Figures 16–18. Male genitalia of *Drosophila melanogaster*. 16, lateral view; 17, ventral view; 18, posterior view.

7 Gena about 1/10 of large eye diameter; male dorsal branch of epandrial ventral lobe triangular and pale in lateral view (12♂♂, 18♀♀ Rashakan) (Figures 16–18) ……..…..……***Drosophila melanogaster*** Meigen
[*a saprophagous, cosmopolitan species*]

7' Gena about 1/20 of large eye diameter; male dorsal branch of epandrial ventral lobe quite roundish and amber in lateral view (3♂♂ Rashakan) (Figures 19–21) ……..***Drosophila simulans*** Sturtevant
[*closely related to D. melanogaster in both external morphology and habitat*]

19

20

21

Figures 19–21. Male genitalia of *Drosophila simulans*. 19, lateral view; 20, ventral view; 21, posterior view.

8 Wing crossveins R-M and dM-Cu brown and narrowly shadowed; abdominal tergites 2–4 each with 4 partially isolated or narrowly connected brown, more or less triangular spots (3♂♂ Rashakan) (Figures 22–24) .....***Drosophila phalerata*** Meigen
[*Its larvae breed in fungus (Bächli 2004).*]

8' Wing crossveins hyaline, abdominal tergite bands medially interrupted, laterally broadened; male with elongated setae on inner side of protarsus (12♂♂, 11♀♀ Rashakan) (Figures 25–27) ……..……..***Drosophila hydei*** Sturtevant
[*a new species record for Iran. Its larvae breed in spoiled fruit and decaying potatoes and adults invade houses, farmhouses, and grocery stores to become nuisances to the inhabitants (Bächli, 2004)*]

22

23

24

Figures 22–24. Male genitalia of *Drosophila phalerata*. 22, lateral view; 23, ventral view; 24, posterior view.

25

26

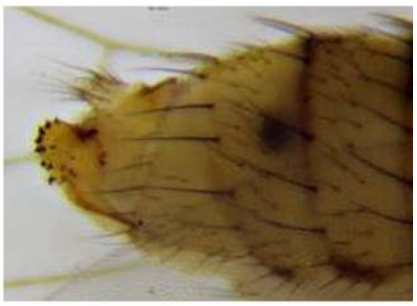
Figures 25–27. Male genitalia of *Drosophila hydei*. 25, lateral view; 26, ventral view; 27, posterior view.

27

9 Median katepisternal seta longer than anterior one; face flat, carina almost absent; prescutellar acrostichal setae absent; abdominal tergites 2–4 each with a pair of posterolateral dark bands (1♀ Kaboudan Island) (Figures 28–29)
……***Lordiphosa andalusiaca*** (Strobl)
[*new genus and species records for Iran*]

9' Median katepisternal seta almost as long as anterior one; face with a bulbous carina; prescutellar acrostichal setae short; abdomen dark brown (2♂♂ Rashakan) (Figures 30–32)
……..…….....***Scaptodrosophila lebanonensis*** (Wheeler)
[*a new species record for Iran*]

28



29

Figures 28–29. Female of *Lordiphosa andalusiaca*. 28, habitus, lateral view; 29, postabdomen, including oviscap, lateral view.

30

31

32

Figures 30–32. Male genitalia of *Scaptodrosophila lebanonensis*. 30, lateral view; 31, ventral view; 32, posterior view.

Conclusion

The existing work is considered a preliminary report on the family Drosophilidae of the Lake Urmia National Park as our current findings suggest the high diversity of the flies in the park that requires further faunistic studies in the future. We are planning to set up traps in other areas of the park including Ashk, Espir, and Arezoo islands in addition to Rashakan and Kaboudan Island.

Acknowledgments: The Department of Environment of the province of West Azarbaijan and the park rangers at Rashakan and Kaboudan Island are thanked for facilitating our expedition throughout the protected area.

References: Asem, A., A. Eimanifar, and M. Wink 2016, Diversity 8(1): 6, doi:10.3390/d8010006; Bächli, G., 2016, TaxoDros. The database on Taxonomy of Drosophilidae. http://taxodros.uzh.ch/; Bächli, G., C. Vilela, S. Andersson Escher, and A. Saura 2004, Fauna Entomologica Scandinavica, vol. 39. Brill, Leiden. 362 pp; Martin, N.A., 2004, New Zealand Journal of Zoology 31: 27-32; Parchami-Araghi, M., E. Gilasian, and A.-A. Keyhanian 2015, Studia dipterologica (in press); Parchami-Araghi, M., and A. Mohammadi-Khorramabadi 2009, Studia dipterologica 16(1/2): 243-244.

Guide to Authors

Drosophila Information Service prints short research, technique, and teaching articles, descriptions of new mutations, and other material of general interest to *Drosophila* researchers. The current publication schedule for regular issues is annually, with the official publication date being December. The annual issue will include material submitted during the calendar year. The submission deadline for each annual issue will be 31 December of the publication year, but articles are accepted at any time. Submissions should be in MS Word. .

Submission: Proofs will not be sent to authors unless there is some question that needs to be clarified. The editor reserves the right to make minor grammatical, spelling, and stylistic changes if necessary to conform to DIS format and common English usage. Tables may be slightly reformatted to conform to DIS style. Color illustrations will appear black and white in the printed version but will be in color on our web site (www.ou.edu/journals/dis).

Citation of References: Citation should be by name and date in the text of an article (Smith, 1989; Jin and Brown, 1990; Waters *et al*., 1990). At the end of the article, references should be listed **alphabetically** by senior author, listing all authors with initials, date, journal, volume and page numbers. Titles will not be included except for books, unpublished theses, and articles in press. An example format is:

Green, R.L., 1998, Heredity 121: 430-442.

Waters, R.L., J.T. Smith, and R.R. Brown 1990, J. Genet. 47: 123-134.

Note the initials are before each name except for the senior author.

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Technique Notes

A highly nutritive medium for rearing *Drosophila.*

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There is already available a plentiful number of medium recipes in specialised literature and lab manuals for rearing *Drosophila* (*e.g.*, Markow and O'Grady, 2006). The choice of the medium, however, is an important decision since there is not a single medium recipe that will be adequate for all the species for a researcher aiming to study diversity, neither for all the wide variety of research purposes. Different species have different ecologies and dietary requisitions or preferences, and some of them simply will not rear adequately in some recipes, or it will be impossible to obtain good material needed to accomplish the objectives of the study. Therefore, especially for studies not dealing with *D. melanogaster* or other common and generalist species, normally several recipes might be tested and adjusted to obtain better results.

The recipe described below was developed and tested in order to raise some species difficult to grow in the standard corn flour medium (Marques *et al.*, 1966) in the *Drosophila* laboratory of *Universidade Federal do Rio Grande do Sul* (Porto Alegre, Brasil). In comparison to the Marques *et al.* (1966)'s medium, it proved to be more successful for raising several species, such as *D. bromeliae*, *D. bromelioides* (*D. bromeliae* species group), and *D. fumipennnis* (*D. willistoni* species group).

After that, this recipe is also being successfully adopted in the same laboratory for studies on cytogenetics, especially those on polytene chromosomes. These studies need well fed third instar larvae to obtain good squashes from salivary glands cells, for description of karyotypes and chromosomal polymorphisms, for obtaining high quality photomicrographs to elaborate reference photomaps, and to be used in other techniques such as hybridization *in situ*. The recipe described here was successful for obtaining good polytene chromosomes preparations for species as *D. willistoni* and *D. nebulosa* (*D. willistoni* species group). This medium was chosen from a range of attempts with other recipes for establishing crosses between females and males of *D. nebulosa*. This methodology, in species little generalist and prolific, tends to be extremely laborious since the majority of crosses render few larvae for cytogenetic analysis. Thus, this culture medium showed better success in encouraging females to oviposit, and also to ensure good nutrition for offspring.

The recipe uses common and accessible ingredients and equipment, is cheap, and of easy preparation and handling.

Mixture 1:

640 mL of distilled water
24 g of rye flour
22 g of dry yeast
6 g of agar
2 g of methylparaben

Mixture 2:

160 mL of distilled water
110 g of banana
38 g of corn syrup

Cooking:

- Measure all ingredients and prepare mixtures 1 and 2.

- Heat mixture 1 in a stove or a microwave oven, stirring some times to prevent clumping. Let it boil, stir the mixture, and boil again.
- Separately, prepare mixture 2 in a blender.
- After boiling mixture 1 twice, add the blended mixture 2 and heat again, stirring some times. Let it boil three times.
- Remove it from the heat source and transfer it to clean vials.
- Let the medium cool for some hours, protected from dust and other contaminants.

After the medium is cool, it is advisable to scratch the surface with a clean spatula to stimulate oviposition and add a previously sterilised folded piece of filter paper, to control excessive moisture and provide a perching and pupation site.

The mixture of simple and complex carbohydrate sources results in a highly nutritive medium, fulfilling dietary requisitions for more exigent species and allowing the development of well-fed third instar larvae for salivary glands preparations. Karo® and Yoki® were successfully used as corn syrup. Methylparaben (Nipagin®) is a mold inhibitor. In this recipe ethanol is not used (as some recipes advise to improve mold inhibition) to avoid high concentrations of ethanol in the medium, which some species may not tolerate. In spite of this, as the recipe is boiled several times, proliferation of mold has not been a problem. The prepared vials with medium can be stored for a few days.

Acknowledgments: The author thanks Vera L.S. Valente, Carolina F. Garcia, and Paula D.S. Berrutti for the stimulus to publish this note, after reporting the success of this recipe in their studies.

References: Marques, E.K., M. Napp, H. Winge, and A.R. Cordeiro 1966, Dros. Inf. Serv. 41: 187; Markow, T.A., and P.M. O'Grady 2006, Drosophila – *A Guide to Species and Identification Use*, Elsevier.

A comparison of feeding rate methods in *Drosophila melanogaster* indicates that consumption is influenced by body size.

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Abstract

Dietary restriction, a decrease in nutrient intake without malnutrition, has been shown to increase life span in many species and is highly linked to feeding behavior. Although *Drosophila melanogaster* is an excellent model organism to study the effects of dietary restriction on life span and associated traits, measuring feeding rate in this organism is particularly challenging. Several methods have been used to estimate feeding rate in *Drosophila melanogaster*, but it remains unclear which method is most precise. We examined the effectiveness of two popular methods that label media with blue dye or radioactive isotopes to quantify food uptake. We found that the radioactive label assay was more precise than the blue dye assay and likely most useful for comparing the effects of different treatments (genotypes, diets) on feeding rates. We found that the relationship between feeding rate and dietary treatment depends on the size of the fly, so we also suggest incorporating body size as a covariate in data analysis to improve the accuracy of feeding rate estimates.

Introduction

Dietary restriction (DR), reducing nutrient intake or specific components of the diet without malnutrition, is known to increase life span in a diverse range of organisms (reviewed in Katewa and Kapahi,

2010). *Drosophila melanogaster* has been an important model in many of these studies (Chippindale *et al*., 1993; Chapman and Partridge, 1996; Pletcher *et al*., 2002; Mair *et al*., 2003). One drawback of using flies in DR experiments is that it is difficult to control for the degree of DR achieved. Unlike vertebrate studies where the amount of food available can be controlled and the amount consumed easily measured, most *Drosophila* experiments are carried out on solid media where the amount of food available is unlimited. Traditional DR treatments using flies dilute or otherwise manipulate the concentration of nutrients in the medium. A critical assumption of this approach is that there is uniform consumption across treatments (*e.g*., different diets or by different genotypes). However, flies may manipulate their feeding behavior when faced with different diets. Uncoupling the effects of DR and feeding rate on focal phenotypes is important for interpreting the results of such studies. Several experimental approaches have been developed to quantify feeding rate in *Drosophila* to determine if flies partially or fully compensate for lowered nutrient levels with increased consumption (reviewed in Tatar, 2011). Some studies find common results of compensatory feeding in diet-restricted flies compared to unrestricted flies (Carvalho *et al*., 2005; Mair *et al*., 2005; Ja *et al*., 2007; Wong *et al*., 2009); however, other studies report conflicting results of no compensatory feeding (Wong *et al*., 2008, 2009), which may be confounded by differences in the type of assay used. As a result, the relationship between feeding rate and the effects of DR remain unclear. In this study we attempt to evaluate two existing methodologies that measure feeding rate of *Drosophila melanogaster* on solid medium and suggest an additional step in analysis to improve upon the precision of one method.

Several feeding assays have been developed, but each one has several limitations. One method estimates feeding rate as the frequency of flies observed with their proboscis extended and inserted into the medium for a given amount of time (Mair *et al*., 2005). Although this method allows data collection without disturbing the flies with vial transfers, it does not account for the total volume of food ingested, and not all proboscis extensions result in uptake (Carvalho *et al*., 2005). Another method utilizes a capillary feeder (CAFE) to accurately quantify the volume of food ingested by flies (Ja *et al*., 2007). This method has many benefits, including its accuracy and the fact that it permits repeated measurements throughout a fly's lifetime. A major criticism is that CAFE requires a liquid diet (Wong *et al*., 2009), which differs from the typical solid medium used in many DR studies.

Two other commonly used approaches measure food intake by labeling the food with either a visible dye or a radioactive isotope tracer to quantify consumption. The dye assay requires transferring flies to new vials and measuring feeding rate for 30 minutes, which separates ingestion from egestion (Wong *et al*., 2008). One drawback of this approach is the physical disturbance of transferring flies and the exposure to fresh food may alter the short-term feeding rate (Mair *et al*., 2005; Wong *et al*., 2009). Additionally, feeding rate data are obtained only in a "snapshot in time". As such, the consumption estimates are influenced by the fly's condition at the time of measurement and likely vary with the time of day. Likewise, the data may be influenced by behavioral differences in daily feeding patterns, especially if different genotypes are being compared that differ in this trait.

An advantage of the radioactive label assay is that it allows flies to consume labeled media for 24 hours (Carvalho *et al*., 2005) and so minimizes the "snapshot in time" problem. However, measuring consumption over the longer term is criticized for confounding absorption and elimination rates (Wong *et al*., 2008). Additionally, the amount of isotope incorporated in a fly depends on its body's capacity to retain the label (Wong *et al*., 2008), which could be particularly concerning in DR studies, because flies on restricted diet treatments may have larger gut capacities (Wong *et al*., 2008). However, when used in feeding rate studies, radiolabels like ^{32}P and ^{14}C in dietary media were found to accumulate in fly tissues in a nearly linear fashion for up to 72 hours when flies are fed on labeled medium. Because this assay ceases at 24 hours, long before a saturation plateau would occur, it is not likely that body capacity limits the rate of label uptake (Carvalho *et al*., 2005; and see Carvalho *et al*.'s reply in Wong *et al*., 2008).

In this study, we compare feeding rate results of the blue dye and radioactive labeling assays to determine which test is a more precise feeding rate method to complement *Drosophila* DR studies. We compared the results of these assays using flies maintained on either a high or low yeast diet which had previously been shown to produce differences in life span and reproduction (Skorupa *et al*., 2008). We carried out the blue dye assay at two different times of day to test the hypothesis that diurnal patterns of feeding rate influence the results of the blue dye assay. We also used two different genotypes of flies from the Genetic

Reference Panel (DGRP) (Mackay *et al*., 2012) to compare genotype specific responses to these treatments. Finally, we attempted to improve the precision of the radioactive label assay by accounting for the effect of body size on feeding rate.

Methods

Stocks

Newly eclosed virgin females of two genotypes from the *Drosophila melanogaster* Genetic Reference Panel (DGRP) (Mackay *et al*., 2012): DGRP_161 and DGRP_774 were collected and placed with age-matched males from line DGRP_774. Individual pairs were maintained in vials containing either high yeast (HY) or low yeast (LY) media. The media consisted of 200 g (HY) or 50 g (LY) baker's yeast (Lesaffre Yeast Corporation, Milwaukee, WI), 50 g sucrose (Domino, Baltimore, MD), 15 g agar (Moorhead & Co, Inc., Rocklin, CA), 3 ml propionic acid (Fisher, USA), and 15 ml 20% methyl paraben (in 95% EtOH) (Mallinckrodt Baker, Inc., Phillipsburg, NJ) for every 1000 ml distilled water. The diets used in this study, modeled after Skorupa *et al*. 2008, differed only in yeast concentration (20% HY and 5% LY). As the primary protein source for the flies, yeast is a major dietary component influencing life history traits like life span and fecundity (Skorupa *et al*., 2008). Flies were maintained on these diets for one week before they were used to evaluate the effectiveness of the two different feeding rate assays.

Blue Dye Assay

The blue-dye assay used standard 5% (wt/vol) blue food coloring (McCormick, Hunt Valley, MD) to label the HY or LY medium in a manner similar to reference (Wong *et al*., 2009). Five females from either line DGRP_161 or DGRP_774 were transferred into vials containing 2% agar to starve for two hours to stimulate feeding. They were then placed on either HY or LY medium and allowed to feed for 30 minutes starting from the first observed proboscis extension. These five flies were then homogenized in a microcentrifuge tube with 0.5 ml distilled water. For each line and diet combination, this method was repeated using both dyed food and non-dyed food to control for genetic variation in fly pigment and variation in medium color (HY is darker than LY) that may influence color intensity measurements. The supernatant of flies fed the non-dyed medium was then used as a blank for spectrophotometric analysis of the corresponding genotype/diet category of flies fed the dyed medium. The blue color intensity of each tube was quantified using a spectrophotometer (SmartSpec Plus Spectrophotometer, BioRad, USA at OD 600 nm) to estimate the average feeding rate of the five homogenized females in each of two replicate tubes for each category. This assay was carried out over four consecutive days at 10 am and 2 pm to detect variation in circadian patterns that may affect the results of experiments using this assay. In total this assay used 80 flies to create 16 samples allowing 2 replicate absorbance measurements for each genotype and diet combination. These were compared to 80 additional flies that were used as spectrophotometric blanks.

Radioactive Label Assay

The radioactive labeling assay was modeled after Carvalho *et al*. (2005), and used the radioactive isotope [α-^{32}P]dATP (Perkin Elmer, Boston, MA, catalog # BLU512H25OUC) to label HY and LY food. Because variation in maximum gut capacity between flies may influence their absolute feeding rate, we measured the thorax length of each female prior to experimentation to estimate body size. This was then used as a covariate in the statistical analysis of feeding rate. Both diets were labeled with 20% radioactive isotopes. We heated 20 mls of each diet to a smooth liquid state before adding 40 μCi of [α-^{32}P]dATP. While still in a liquid state, 1 ml of the mixture was pipetted into individual vials and 1 ml of each diet was pipetted into scintillation vials for initial isotope counts. The initial isotope level in each scintillation vial was then measured in an LS 6500 Multi-purpose scintillation counter (Beckman Coulter, Inc., USA). Once the mixture in the vials solidified, individual females were transferred into each vial for a 24 hour period. Flies were transferred onto 2% agar for thirty minutes to allow them to clean their exterior of any radioactively labeled food (Carvalho *et al*., 2005), and then frozen on dry ice for five minutes before being placed into individual scintillation vials with 15 ml scintillation fluid to enhance the isotope measurement. Isotope levels within each individual fly were quantified as above using a scintillation counter. The volume of food ingested by each fly

(in µl) was then calculated by first converting the initial food isotope count from CPM/ml to CPM/µl and then dividing the CPM isotope count in each individual fly the following day by this value. To ensure that no radioactivity was present in the atmosphere or in the food prior to labeling it, isotope levels of individual females maintained on unlabeled food for 24 hours in the same location as the experimental flies were measured in a similar fashion. Radioactivity in these control flies was found to be negligible (*i.e*., under 30 CPM per fly), so they were not considered in any further analyses. In total, five replicates were conducted per line for each diet over two days yielding 40 values from 40 flies. An additional four control flies were used to assess background radioactivity, one reared on HY and one on LY for both days.

Statistical Analysis

All statistical analyses were completed using the PROC GLM procedure in SAS version 9.2 (SAS Institute, Cary, NC). Analysis of variance (ANOVA) was used to evaluate the influence of genotype, diet, and time of day on feeding rate for the blue dye assay using the model:

$$y = \mu + l + d + t + l*d + l*t + l*d*t + \varepsilon$$

where y is the volume of food ingested in ml, μ is the mean, l is the effect of genotype (line), d is the effect of diet, t is the effect of the time of day, $l*t$ is the effect of the line*diet interaction , $l*t$ is the interaction between line and time of day, $l*d*t$ is the three-way interaction term, and ε is the effect of error.

Analysis of covariance (ANCOVA) was used to evaluate the data from the radioactive label assay in a similar fashion. The full model was run and non-significant interaction terms were dropped. The final model used for ANCOVA was:

$$y = \mu + bs + g + d + g*d + bs*d + \varepsilon$$

where bs is the covariate body size as estimated by thorax length in mm, $bs*d$ is the interaction term between body size and diet, and all other variables are defined as above.

Results

Differences in feeding behavior are evident when using radioactive labels but not evident when using blue dye method

When the blue dye assay was used we found no significant differences in feeding rate between genotypes ($p = 0.4650$), diets ($p = 0.4292$), time of day ($p = 0.6836$), or any interactions between main effect terms, but some general trends were apparent. Females of both genotypes tended to eat more on HY (mean ± 1SE) (3.5869 ± 0.4909 absorbance units AU / 5 flies) than LY (2.9245 ± 0.5830 AU / 5 flies) medium (Figure 1 a and b), and line DGRP_774 (3.5609 ± 0.5037 AU / 5 flies) ate more food overall than line DGRP_161 (2.9505 ± 0.5762 AU / 5 flies). Although females of line DGRP_161 showed a difference in feeding rate between the diets of 0.25 AU and 0.04 AU at 10 am and 2 pm, respectively, our statistical power was too small to detect the difference ($1 - B = 0.118$). Likewise, line DGRP_774 showed a 1.95 AU difference in feeding rate between the diets at 10 am and a 0.4 AU difference at 2 pm; however, the power was also too small to detect this difference ($1 - B = 0.142$). We also observed differences in circadian patterns of feeding rate between the genotypes, but this was not significant using this assay ($p = 0.1127$). Females of line DGRP_774 ate more in the afternoon (4.3000 ± 0.3000 AU / 5 flies *vs*. 4.0 ± 0 AU / 5 flies) on both diets, but the power was too low to detect this difference ($1 - B = 0.076$). Line DGRP_161 ate more in the morning (3.9510 ± 0.0490 AU / 5 flies *vs*. 2.0965 ± 1.9035 AU / 5 flies), and again the power was too low to detect this difference ($1 - B = 0.102$). The lack of significance in this analysis is due to a large amount of variation in the data. A power calculation reveals a minimum of 11 samples per diet and time of day would have been needed to detect differences in feeding rates between the diets at a power of 0.80 with an alpha value of 0.05. This equates to a minimum of 220 and 180 flies for lines DGRP_161 and DGRP_774, respectively, for feeding rate measurements using the blue dye assay. In addition, doubling this sample size would be required to obtain the flies used as spectrophotometric blanks in absorbance analysis. The demands of sample size and number of flies required to obtain sufficient power using this method is a major drawback in the use of this methodology.

The radioactive assay identified the same general trends in feeding rate as the blue dye assay, but was able to detect significant differences in feeding rates between the dietary treatments. This difference is likely a reflection of the larger sample size and statistical power achieved by the ability to measure individual flies

using the radioactive label method. Flies ate significantly more on HY (9.3745 ± 0.5917 µl/fly) than LY (5.9437 ± 0.4400 µl/fly) ($p = 0.0134$), and this pattern did not differ by genotype ($p = 0.5845$) (Figure 1c). Line DGRP_774 showed a higher but non-significant ($p = 0.5556$) feeding rate (8.1723 ± 0.5795 µl/fly) than line DGRP_161 (7.1459 ± 0.6999 µl/fly) (Figure 1c). There is no need to examine differences in circadian feeding patterns in this assay, because it encompasses a 24 hour sampling window. Although statistical analysis using ANCOVA revealed no significant effect of body size as a main effect ($p = 0.9697$), we did see a significant interaction between diet and body size ($p = 0.0088$). Our data indicate that smaller flies did not alter feeding rates between diets, whereas larger flies fed more on HY than on LY (Figure 2).

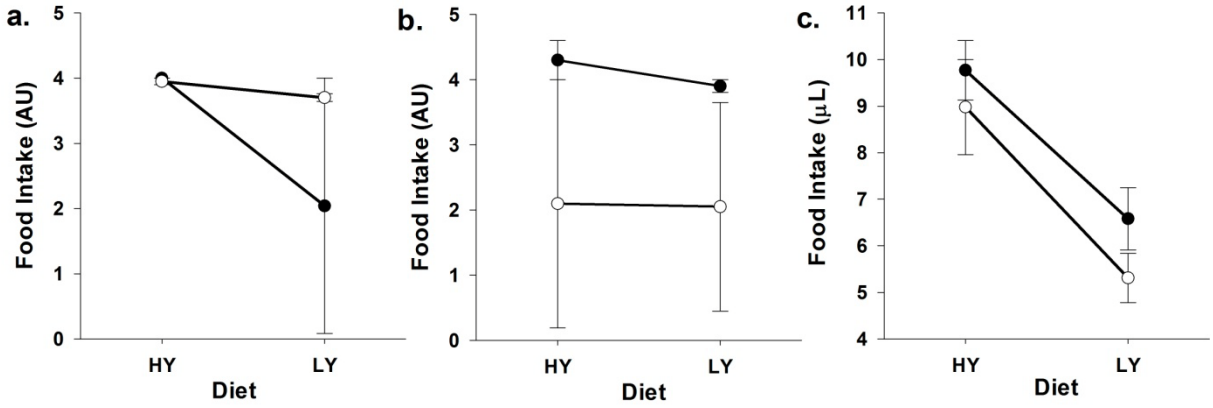

Figure 1. Radioactive label assay is more precise than the blue dye assay, even before correcting for body size. Both graphs show the effect of diet on feeding rate for each genotype using (a and b) the blue dye assay (AU = absorbance units for 5 flies total over 30 minutes) and (c) the radioactive labeling assay (µl food consumed in 24 hours per fly). Open circles represent line DGRP_161 and closed circles represent line DGRP_774. Blue dye assay reveals no significant relationship at (a) 10 am ($p = 0.4319$) nor (b) 2 pm ($p = 0.8951$), whereas (c) the radioactive label assay indicates a significant difference in feeding rate between diets, averaged over both lines ($p = 0.0003$). Data points indicate mean food intake ± 1 SE. The blue dye assay yields much higher error rates than the radioactive assay. Note that blue dye data include 5 flies total in each sample ($n = 2$ per diet and line combination) and radioactive label data indicate individual flies ($n = 10$ per diet and line combination).

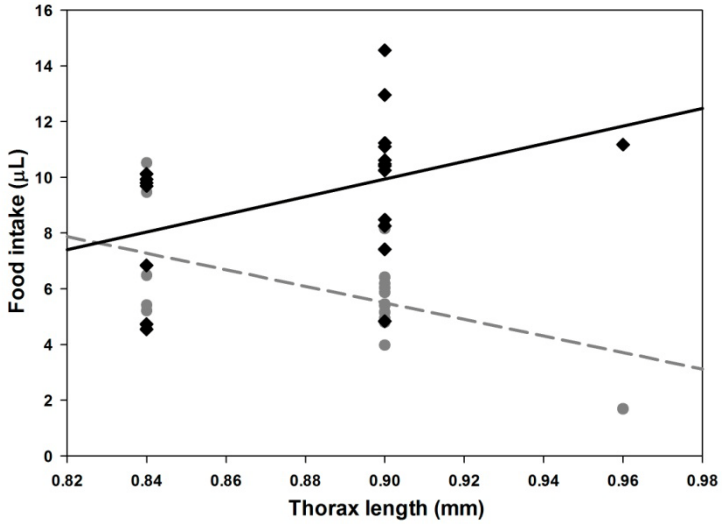

Figure 2. Effect of diet on feeding rate depends on body size. Points indicate feeding rate of individual flies as measured by [α-^{32}P]dATP tracer levels converted to µl of food consumed. Gray circles and dashed line indicate LY and black diamonds and solid line indicate HY. Linear regression shows that small flies showed little to no difference in consumption between diets whereas larger flies ate more on a HY diet than a LY diet ($p = 0.0339$).

Discussion

High degree of error leads to difficulty detecting variation in feeding behavior using the blue dye assay

While both methods used in this study are useful for estimating feeding rates, the radioactive assay was superior to the blue dye assay for detecting significant differences between treatments. The blue dye assay is advantageous in that it is a more cost and time effective method. Compared with the radioactive label assay, food coloring is inexpensive. Additionally this assay is complete in less than three hours while it takes two days to complete the radioactive isotope assay. However, the blue dye assay was more labor intensive and led to high experimental variance that could be influenced by procedural sources such as vial transfers, human error, and aggregated sampling. Vial transfers at the start of each trial cause physical disturbances to the flies that may alter normal feeding patterns (Wong *et al*., 2009), and the short length of this assay may not be enough time for flies to recover. Human error is also inevitable, because careful observation is required to detect the first proboscis extension from any of 5 flies in each vial to mark the start of the 30 minute feeding period. This usually took 10-30 minutes to occur, and the difficulty of tracking 5 flies in a vial limited the sample size possible for each experimental session to only four vials per person each time the assay is carried out. Additionally, this assay required a large number of flies for each data point, because the level of blue dye in the fly homogenate is not adequately detected with fewer than five flies in each sample. Each sample also required a control homogenate to serve as a spectrophotometric blank, so a total of 10 flies are required to create one data point, and each data point potentially produces a high degree of measurement error, because it assumes relatively equal feeding rates among flies within a vial.

Several other factors may also contribute to the high experimental variance of the blue day assay. Using proboscis extensions as a starting point to measuring food intake is likely inaccurate, because every extension does not result in food intake (Carvalho *et al*., 2005). Including proboscis extensions in the assay may bias the sample toward inflated feeding rates, and removing this step of the assay would be a less laborious methodology that could allow a larger sample size collection; however, it would also lower averaged feeding rate measurements by including flies that did not attempt to feed at all (having a zero feeding rate). Another pitfall of this method is that flies were required to starve for two hours before the assay, which may alter normal feeding rate if flies "binge" after starving (Farhadian *et al*., 2012). Additionally, feeding rate must be measured after just thirty minutes of feeding, because the food ingested by the fly could be excreted after this time frame and it would be impossible to separate ingestion from egestion (Wong *et al*., 2008). Finally, in accordance with previous research (Wong *et al*., 2009), our results indicate that flies vary feeding rate with circadian patterns, and this behavioral pattern varies with genotype. Because the blue dye assay is brief, it is susceptible to increased experimental variance due to differences in circadian behavior patterns.

Improved radioactive label assay yields precise, repeatable results in detecting differences in feeding rates

Although the radioactive label assay is more costly and time consuming for each replicate, its benefits as a method to measure feeding rate outweigh the pitfalls. Human error is reduced by eliminating the proboscis extension observations, and circadian behaviors are controlled for by allowing flies to feed undisturbed for 24 hours. This 24 hour feeding session is acceptable for this assay because labeled food is not only ingested, but also metabolically processed and the radioactive label maintained in the tissues of the fly rather than being excreted (Carvalho *et al*., 2005). Another benefit of the radioactive label assay is that it evaluates the feeding rate of individual flies rather than groups of five flies at a time. This translates to larger sample sizes within each replicate block. These factors allow for a much more accurate sampling of the population and more efficient data acquisition.

Previously used radioactive labeling methods have been criticized in that the amount of isotope detected may vary by the body capacity of the flies, so results may actually indicate the volume of food a fly is capable of housing rather than the rate of food intake (Carvalho *et al*., 2005; Wong *et al*., 2009). We accounted for this problem by estimating the body size of each fly with a thorax length measurement. We used thorax length as a proxy to estimate gut size, because it is relatively easy to measure on live flies. Other measures, such as crop size, are more accurate estimates of gut size, but require the flies to be sacrificed, and so would have to be completed after the flies feed on the radioactive food. This is problematic because of the

amount of time required to dissect and measure the crop of each individual fly before isotope measurements and the potential loss of isotopes to any surfaces used in the dissection process.

Using body size as a covariate in our statistical analysis revealed that, although body size itself did not significantly influence feeding rate as a main effect, it did show a significant interaction with diet, meaning that the relationship between feeding rate and diet depends on the size of the fly (Figure 2). Including a body size estimate would likely increase the precision of the blue dye assay as well (Wong *et al*., 2008), but would have to be aggregated for the number of flies used in each spectrophotometric measurement, thus introducing further variation compared to the radioactive label assay that measures individual flies.

This study was conducted to identify a feeding rate assay that would best detect differences in feeding rate between samples of flies. Thus, our goal was to determine which method yielded the most precise estimates of feeding rate. While our results suggest that the radioactive label assay best satisfies our goal of precision, the blue dye and other assays may be more ideal than the radioactive label assay for other research goals such as high-throughput capabilities, or cost-effectiveness. It is also important to note that many aspects of these two assays are procedurally different. For example, the blue dye assay measures only a thirty minute sample of feeding and requires starvation before the assay, whereas the radioactive label assay measures a 24 hour sample with no starvation. For this reason, we cannot compare absolute feeding rates across assays, but rather, we compare the ability of each assay to detect differences in feeding rates, if one exists.

Conclusions

Overall, the radioactive assay was a more effective and precise method of measuring feeding rate in *Drosophila melanogaster* than the blue dye assay. In addition, this study indicates that body size is an important variable to consider in feeding rate measurements and other assays involving dietary treatments because consumption appears to be dependent on body size. We suggest that future experiments either are limited to size-matched individuals or that body size be measured to account for the effects of body size variation on feeding rates.

Acknowledgments: We thank Pankaj Kapahi for technical guidance and for sharing the radioactive isotope methodology. We thank Varsha Rao and Charles Bieberich for technical assistance and the use of their laboratory and equipment in performing the radioactive assay. We thank Trudy Mackay for providing the DGRP lines used in this work. This research was supported by a grant from the National Institutes of Health 5R01 DK084219-02 and an Undergraduate Research Award from the University of Maryland Baltimore County awarded to P.D.D. 2010-2011.

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Inexpensive automated *Drosophila* geotaxis & phototaxis assay with real-time data analysis.

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Introduction

Traditionally the simplest and most common *Drosophila* assay involves geotaxis and phototaxis. This assay is used to determine *Drosophila's* ability to climb up a surface toward a light source. The conventional method uses a capped graduated cylinder, a flashlight, and a stop watch. In a darkened room the cylinder is tapped to ensure the *Drosophila* are dislodged to the bottom of the cylinder. The flashlight is then turned on, and a timer is started. The user determines the time points and number of flies able to climb past a specific threshold distance. Issues with this method are numerous. For example, conventional flashlights do not provide a consistent light source as battery power decreases overtime, and the amount of ambient light in a room is not consistent from assay to assay. As the cylinder is tapped down by hand at the beginning of each assay, there is a large degree of variation from user to user, which could result in variations in fly recovery time. Most notably as the assay relies on visual observation, measurement accuracy is highly variable from person to person.

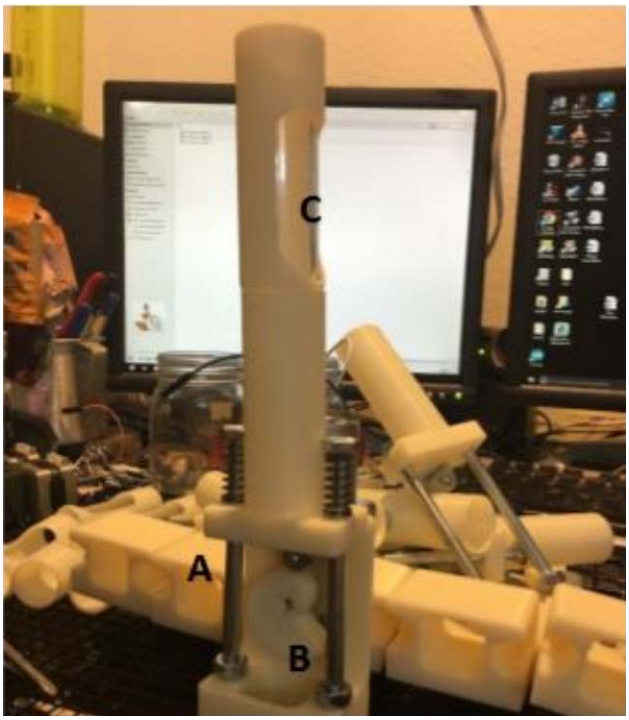

Figure 1. Motor cam and cylinder holder. (A) 4" × ¼" spring loaded bolts guide rails. (B) Snail cam with cabinet door roller. (C) Cylinder holder and viewing port.

Methods

An inexpensive automated method was developed after several design iterations to counter these issues found in the manual method geotaxis and phototaxis assay. The automated assay uses a full enclosure to ensure consistent ambient lighting levels. The plastic enclosure consists of 1/8th inch high strength ABS plastic, heat formed and later 3D printed to fully enclose the automated assay.

An RGB LED replaced the flashlight as the light source used to enable phototaxis (Figure 3 C). RGB LEDs can provide red, blue, or green light as well as true white or any shade between to allow for color specific phototaxis assays. Unlike the flashlight, the LED light is of constant intensity due to direct wiring to a consistent power source. The fly cylinder is tapped down via a motor cam mechanism to ensure consistent force for each trial. Earlier iterations of this system used a vibration mechanism to dislodge the flies, but this resulted in increased fly recovery time (Figure 1 A). The cam mechanism is made up of 4" × ¼" bolts with ¼" springs and nuts. The roller is a cabinet door roller (Figure 1 B). These components can be found at any hardware store. The cam mechanism frame and sample holder are made of 3D printed ABS

plastic. An inexpensive Logitech HD C270 webcam was stripped of its casing allowing for adjustment of its focal length and removal of its infrared filter (Figure 4 A). This allows the system to record high definition close range images in low light of the flies as they climb beyond a 10 cm threshold mark. The camera was then mounted to a 3D printed L bracket in front of a viewing port on the cylinder holder (Figure 1 C). An Arduino UNO was used as the controller for the RGB LED and cam motor (Figure 3 B). A solid-state switching circuit was created to allow for interfacing the UNO controller to the cam motor. The UNO and C270 were routed to a USB 3.0 hub (Figure 3 A). This allowed for additional bandwidth for peripherals such as a vacuum optical fly counter to be piggybacked through a single USB 3.0 port for PC control. The USB hub also allowed for wiring of power switches to act as manual resets to the C270 and UNO components. Additional power was supplied via a 9v DC power supply.

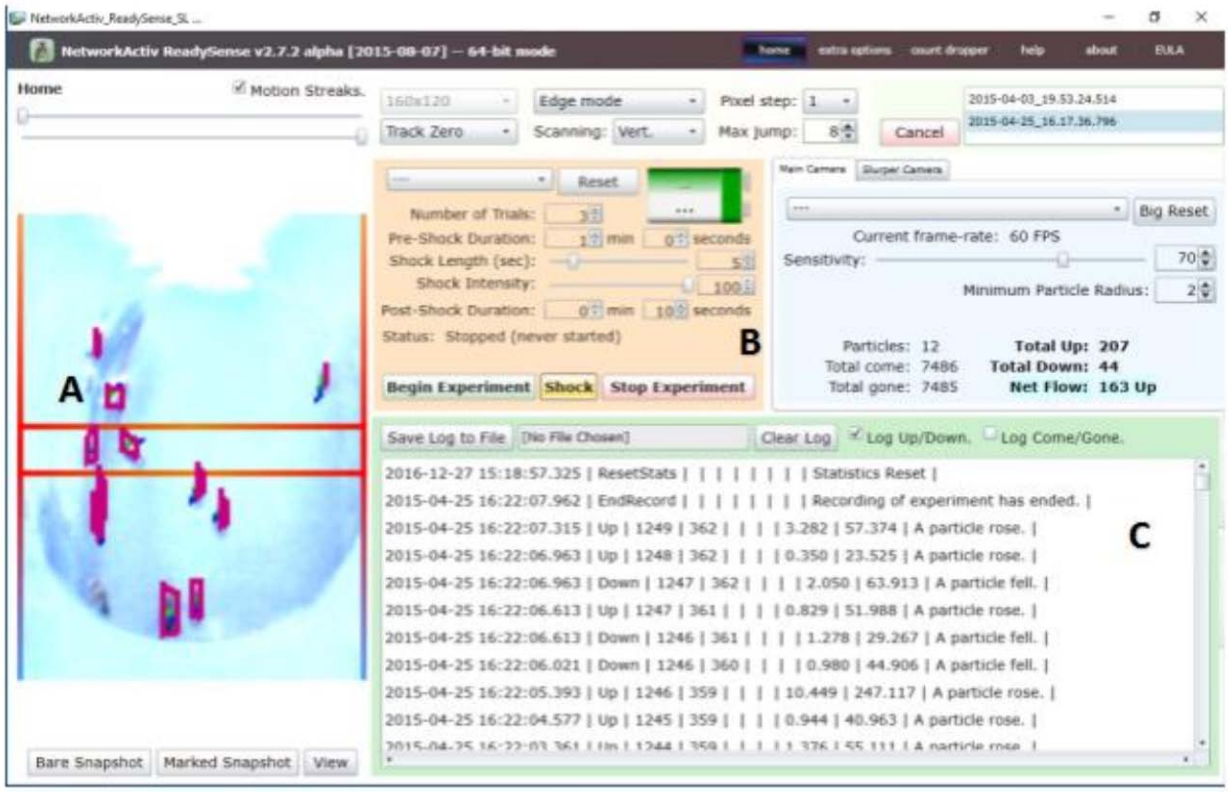

Figure 2. ReadySense user interface. (A) Video feed with object tracking and 10 cm threshold marker. (B) Assay settings menu. (C) Raw data log area.

Software called ReadySense was developed by NetworkActiv to control this system (Figure 2). The software interfaced with the Arduino UNO controller allowing for computerized control of RGB LED spotlight intensity and color balance. The motor cam mechanism is controlled as well allowing the experimenter to set the duration of tapping (Figure 2 B). The software allows the experimenter to set the number of assays to run, as well as the duration and timing of each assay. The software interface with the C270 webcam allows for control of camera settings as well as video capture. A user defined detection region and threshold level can be set to allow for bidirectional counting of flies passing the 10 cm mark in either direction (Figure 2 A).

The software analyzes video capture frames in real-time, or optionally post-experiment. Two modes of object detection are provided: Mass Mode (pixel cluster density) or Edge Mode (contiguous region), to accommodate different lighting, resolution, and processing power circumstances. Detected objects (flies) are assigned identifier values in order of appearance, and their telemetry (location, speed, and direction) is tracked, allowing flies temporarily to obstruct one another while crossing paths without interfering with motion

tracking. Detection thresholds can also be controlled by the experimenter to allow the software to track objects within a specific size range, and automatic debris management ignores unmoving anomalous spots or regions in the image. The software can process telemetry data both in real-time and from prerecorded video files allowing for rapid and high throughput analysis (Figure 2 C). All data are then outputted to an Excel file for easy analysis. Assay settings are saved automatically to allow quicker experiment replication with less chance of misconfiguration.

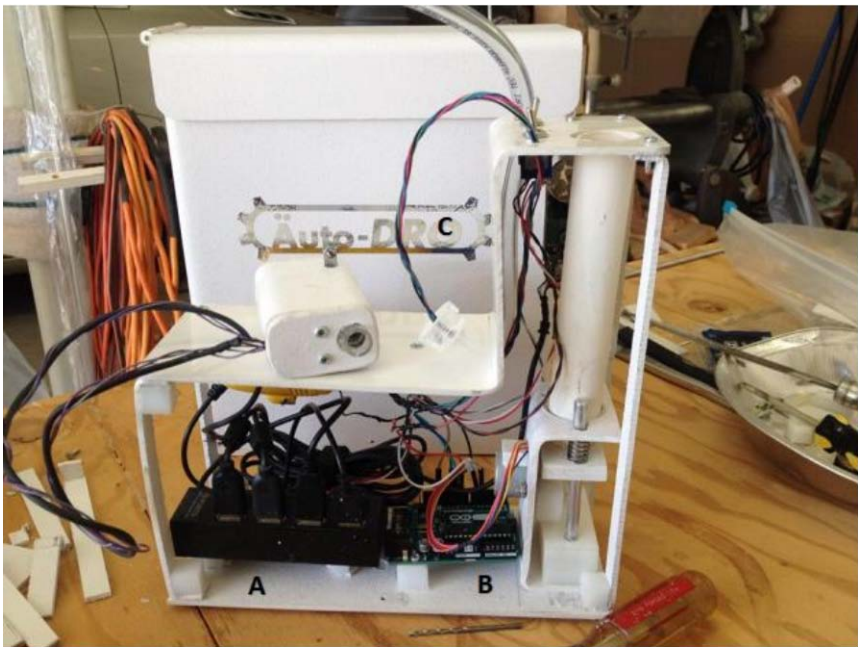

Figure 3. "AutoDRO" assay internal configuration. (A) USB 3.0 hub modified with manual switches. (B) Arduino UNO controller. (C) RGB LED spotlight.

Figure 4. C270 HD webcam on L bracket. (A) Logitech C270 HD webcam with modified focus and removed IR filter.

The software also provides additional modules to interface with systems for vacuum optical fly counting and sugar preference assay image analysis.

The automated *Drosophila* geotaxis & phototaxis assay nicknamed “AutoDro” in conjunction with the custom software ReadySense allows for consistent reproducibility regardless of the experimenter. The ABS outer casing allows for consistent control of ambient lighting. RGB LEDs allow for consistent control of light source intensity and color balance. The motorized cam mechanism allows

for mechanical tapping of the cylinder. The C270 HD webcam allows for precise recording of *Drosophila* movement. The software enables real-time or post-processing of video allowing not only for counting and recording of threshold crossing time points, but also for other data such as speed. Without the need for a human observer, trials may span extended lengths while maintaining repeatability. All assay data are automatically outputted, further reducing the possibility of human error.

Mutation Notes

Drosophila melanogaster P{GAL4-Hsp70.PB} transposon insertion on 3rd chromosome creates mutations in *mth* and *Ptpmeg* genes.

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The third chromosome P element insertion P{w^{+mC}=GAL4-Hsp70.PB}89-2-1 (Bloomington stock number 1799) (Brand, Manoukian, and Perrimon, 1994), hereafter referred to as P{*hs-GAL4*}89, is being used as a heat-inducible GAL4 driver for the ubiquitous, conditional activation of UAS responders (Armstrong *et al*., 2002; Chanut *et al*., 2002; Kozlova and Thummel, 2002; Liu and Lehmann, 2008; Roman, He, and Davis, 2000; Seong, Ogashiwa, Matsuo, Fuyama, and Aigaki, 2001). To determine whether the P insertion in the P{*hs-GAL4*}89 line may potentially interfere with the expression of a gene or genes located at the genomic integration site, we amplified flanking genomic DNA by inverse PCR. Genomic DNA isolated from P{*hs-GAL4*}89 flies was digested with *Hpa*I, which cuts in the polylinker of the pCaSpeR3 transformation vector from which the P{*hs-GAL4*}89 element was derived (Brand *et al*., 1994) and in flanking genomic DNA. Restriction fragments were ligated under dilute conditions to favor circularization of DNAs. The portion of the polylinker that remained attached to genomic DNA after *Hpa*I digestion was then cut by digestion with *Sal*I to generate linearized DNAs with P-element-derived ends. Linearized DNA was then amplified by PCR and sequenced using primers 5'-GGATCCCCGGGCGAG-3' and 5'-CCTGCAGCCCAAGCTT-3'.

PCR yielded a single product of about 0.8 kb. Sequencing of the PCR product indicated that the transposon resides in the *Ptpmeg* gene, located 543 bp upstream of the transcription start of transcripts RI and RJ, and within a large intron that is specific for transcripts RH and RK (Figure 1). This intron harbors three other genes, *methuselah* (*mth*) and *methuselah-like 9* and *10* (*mthl9*, *mthl10*), that are transcribed in the opposite direction to *Ptpmeg*. The P{*hs-GAL4*}89 element is inserted after position +38 downstream of the putative transcription start site of the *mth* gene (Figure 1B). Consistent with this observation, microarray data indicate that *mth* RNA was 30-fold reduced in salivary glands of homozygous P{*hs-GAL4*}89 animals compared to control animals without the element (Liu and Lehmann, 2008). These findings are surprising, because *mth* is considered to be an essential gene. Homozygous *mth* null mutants die during the embryonic stage (Ja, Carvalho, Madrigal, Roberts, and Benzer, 2009). Despite the strong reduction, *mth* RNA was still detectable in homozygous animals (Liu and Lehmann, 2008), suggesting that basal expression of *mth* does not require sequences located immediately upstream of the transcription start site. It appears that a minimal amount of *mth* RNA sufficient for survival can be produced from an internal promoter. This interpretation is supported by the report of a positive transcriptional control element located +28 to +217 relative to the transcription start site of *mth*. A reporter gene that carries this element, but entirely lacks upstream sequences of *mth*, indeed exhibits basal transcriptional activity (H. Kim, Kim, Lee, Yang, and Han, 2006).

Finally, we wondered whether the GAL4 gene of the transposon is transcribed in the same direction as *mth* or *Ptpmeg*. The inverse PCR results had indicated that the P transposon is inserted in the same 5'-3' orientation as Ptpmeg. We determined the orientation of hs-GAL4 within the transposon by PCR with the help of primer 5'-CAGACACTTGGCGCACTTCGGT-3', which hybridizes within the GAL4 gene, and primers Casper1 (5'-GATCCCCGGGCGAGCTCGAAT-3') and Casper2 (5'-AACGCTACAAACGGTGGCGA-3'), which hybridize with vector sequences. Only Casper2, which was designed to give a PCR product if hs-GAL4 was oriented the same way as vector and Ptpmeg, but not Casper1, yielded a product. Thus, hs-GAL4 is transcribed in the same direction as Ptpmeg (Figure 1B).

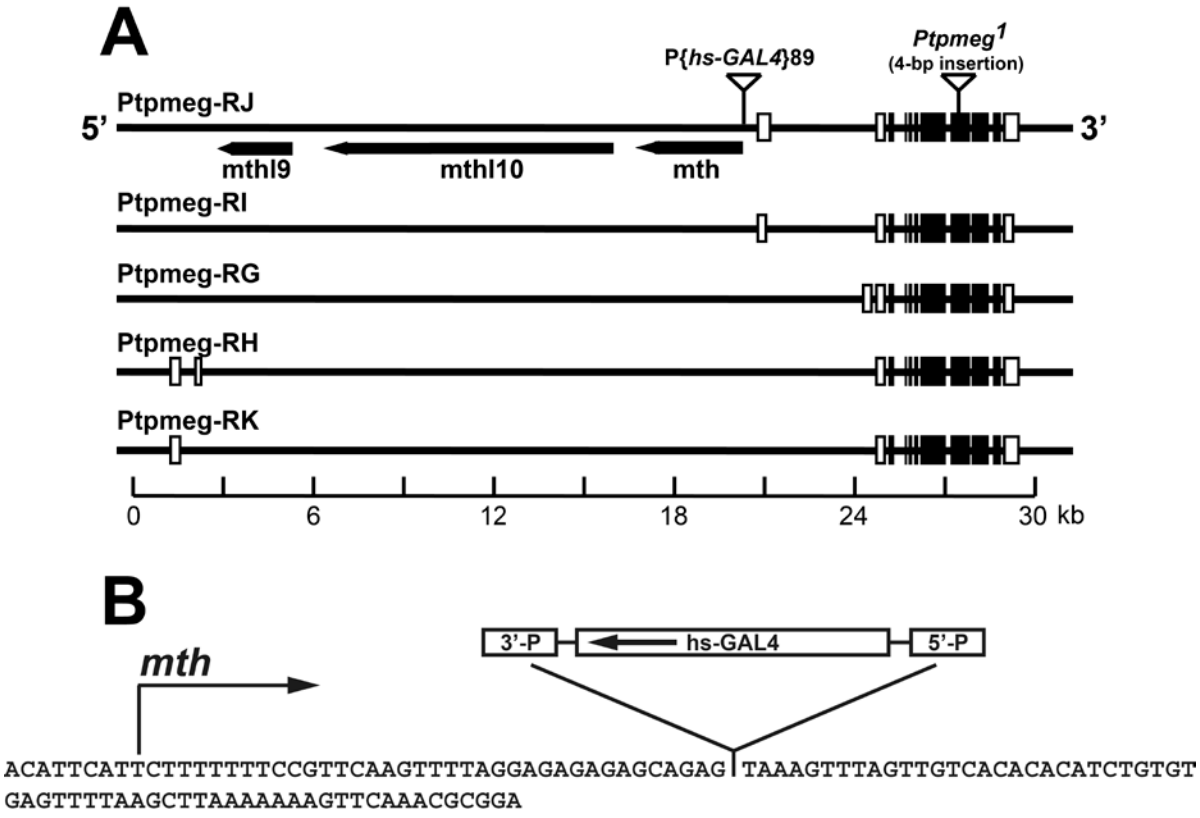

Figure 1. Genomic location and orientation of hs-GAL4 in the P{*hs-GAL4*}89 line. (A) Genomic organization at the P(*hs-GAL4*}89 integration site. *Ptpmeg* is transcribed from left to right, whereas the intronic genes, *mth*, *mthl10*, and *mthl9*, are transcribed from right to left. (B) P element and hs-GAL4 transgene are located in the indicated orientations within the non-coding 5'-exon of *mth* close to the putative transcription start site predicted by Flybase.

Acknowledgments: This work was supported by NIH grant 1R15GM104836-01.

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Teaching Notes

Reduction in fitness and possible population extinctions due to the accumulation of deleterious mutations on non-recombining X chromosomes in *Drosophila melanogaster* males.

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Background

H.J. Muller (1964) predicted that organisms that do not undergo recombination (for example, asexual organisms) will accumulate deleterious mutations over time and will not be able to eliminate them by crossing over leading to recombinant chromosomes with fewer or no mutations. If the least-loaded chromosome (the chromosomes with the fewest mutations) is lost by drift, a "ratchet" occurs and the population has a reduction in fitness. Felsenstein (1974) called this drop in fitness "Muller's ratchet" (see reviews of this topic in Maynard Smith, 1978; Charlesworth and Charlesworth, 1997; Arjan *et al.*, 2007; Loewe and Hill, 2010; Charlesworth, 2012, 2013). This ratchet process could also lead over time to the extinction of some populations or species with low numbers. There is theory on how fast Muller's ratchet clicks and on the expected time to extinctions, which is mainly based on population size, deleterious mutation rate, and selection against deleterious mutations (Felsentein, 1974; Haigh, 1978; Maynard Smith, 1978; Wagner and Gabriel, 1990; Gabriel *et al.*, 1993; Stephan *et al.*, 1993; Butcher, 1995; Gessler, 1995; Gessler and Xu, 1999; Gordo and Charlesworth, 2000a,b, 2001; Bachtrog and Gordo, 2004; Jain, 2008; Loewe and Cutter, 2008; Neher and Shraiman, 2012). Yet, no direct measures of Muller's ratchet that include extinctions in multicellular organisms have been performed, leaving many unanswered questions on the role of the ratchet in metazoans. Hence, this proposed study is an attempt to identify reductions in the fitness of males due to the accumulation of X-linked mutations and possible extinctions of *D. melanogaster* populations.

Mutational accumulation studies with higher organisms, including *Daphnia pulex*, *Caenorhabditis elegans*, and *D. melanogaster*, have shown consistent decreases in fitness over time and occasional extinctions of lines (see discussions in Garcia-Dorado *et al.*, 1998; Vassilieva *et al.*, 2000; Estes *et al.*, 2004; Gong *et al.*, 2005; Baer *et al.*, 2007; Arjan *et al.*, 2007; Halligan and Keightley, 2009; Schaack *et al.*, 2010; Mallet *et al.*, 2011). These mutational accumulation experiments, however, were not set up to identify reductions in fitness and extinctions of lines in the same experiment. In this study, changes in fitness will be measured by comparisons of male to total progeny ratios over generations, and extinctions will be identified by the elimination of males in lines that contain sibling females.

Hypotheses

This proposed study will allow for the testing of two hypotheses regarding the role of mutation accumulation of X-linked recessive mutations on male fitness and population extinctions.

Hypothesis 1: The fitness of males is expected to decrease over time due to the accumulation of recessive deleterious mutations on X chromosomes that remain only in males, where they cannot be removed by recombination (there is no pairing partner for the single X chromosome in males). Tests of this hypothesis will provide experimental insights into the importance of recombination on the accumulation of deleterious mutations and its potential fitness consequences in small populations or species.

Hypothesis 2: In addition, mutation accumulation over time may cause some lines to go extinct toward the end of the experiment because of the loss of males with low fitness due to the expression of

recessive, X-linked, deleterious mutations. On the other hand, their female siblings with two X chromosomes survive because they do not express recessive, X-linked, deleterious mutations. This proposed protocol will reduce the chance that extinctions occur by handling accidents or poor fly husbandry.

Experimental Plan

The accumulation of recessive deleterious mutations on the X chromosomes of males in small populations of one male and one female over four generations was measured in each of 17 coded vials (Figure 1). In these crosses, w^{1118} is a white-eyed mutation that marks the X chromosome in w^{1118} / Y males, and C(1)DX, *y f* / Y are females that contain two X chromosomes attached to a single centromere, with the markers *y* for yellow body color and *f* for small, forked bristles (Lindsley and Zimm, 1992). Hence, from these crosses patroclinous male progeny receive their w^{1118} X chromosome from their fathers (where there is no recombination) and female progeny receive the attached-X chromosome from their mothers. Recessive deleterious mutations on the X will be expressed in the hemizygous males, but not in the diplo-X females. One male and one female sibling were used in each cross each generation to increase the probability of population (vial) extinctions.

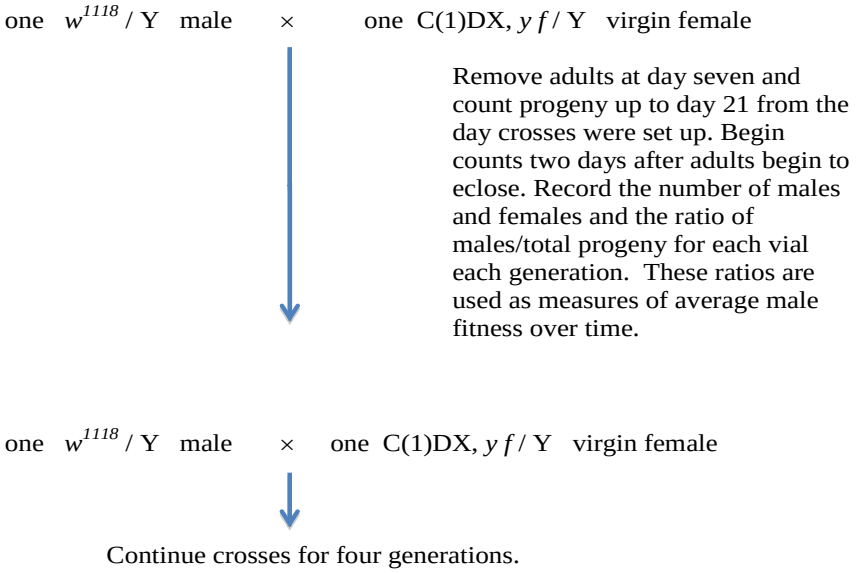

Figure 1. Crossing scheme to accumulate deleterious, X-linked, recessive mutations.

Hypothesis 1 was tested by measuring changes in the ratios of males/total progeny per line over generations as a measure of male fitness. The accumulation of new recessive deleterious mutations on the X chromosome in males will cause the ratio of males/total progeny to decrease with time, because diplo-X females will not express new recessive deleterious X-linked mutations.

Hypothesis 2 was tested by measuring for extinctions of vial populations, which will be identified by the absence of w^{1118} / Y males in lines that contain sibling females. It is expected that line extinctions will occur in latter generations of this proposed study.

Since Haag-Liautard, *et al.* (2007) observed 1.2 deleterious mutations per diploid genome each generation in *D. melanogaster*, and the X is about 15 percent of the genome (National Center for Biotechnology Information: http://www.ncbi.nlm.nih.gov/genome/47), we expect about 0.18 (0.15 × 1.2) deleterious mutations per fly. Hence, about one new X-linked deleterious mutation is expected in three of the 17 total vials each generation (0.18 × 17).

Results

Although we expected about one new deleterious mutation per line, there was a non-significant reduction in the fitness of males over four generations due to the accumulation of mutations on their X chromosomes (Figure 2). In addition, no extinctions of lines due to the absence of males were observed. These results indicate that for fitness decreases and population extinctions to be observed using this experimental design, a much larger number of crosses must be initiated and the experiment run for more generations. For example, with the expected reduction in fitness of about three percent for each new mutation (Simmons and Crow, 1977) and an expected X-chromosome mutation rate of 0.18 each generation (Haag-Liautard *et al.* (2007), after 20 generations of a larger experiment the expected average drop in male fitness per line would be about 10 percent, with some lines showing a larger reduction in fitness. This expected drop in fitness from the accumulations of recessive deleterious mutations should allow for the identification of population extinctions.

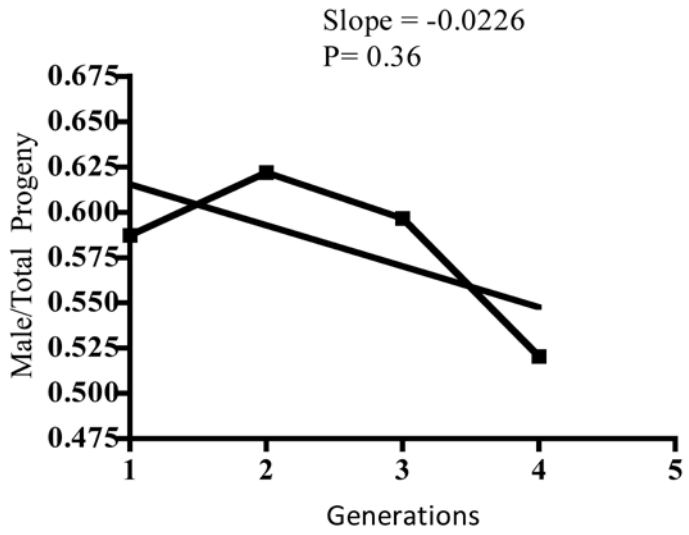

Figure 2. Mean Ratio of Male to Total Progeny. Each point represents the mean of 17 vials from that generation, showing a non-significant decrease in male fitness over four generations.

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New species of *Drosophila* or not.

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Three undergraduate students (Honigford, Rochester, and Schimmoeller) were given a stock of *Drosophila* that had white eyes and were told that it was sent to the Department of Biological Sciences at Bowling Green State University as a possible new species of *Drosophila*. The students were asked to determine if this were true, or was the white-eyed stock just *Drosophila melanogaster* with a new mutation. The students were told to determine from the literature mechanisms of speciation and the definition of a species (Coyne and Orr, 2004; Price, 2008) and to use this information to compare the white-eyed stock with the Canton-S wild type stock of *D. melanogaster*.

The students decided to test the hypothesis that the white-eyed stock was a new species based on the biological species concept: groups of interbreeding natural populations that are reproductively isolated from other groups (Mayr, 1966, 1982). Accepting the biological species concept, species are defined by reproductive isolation, including premating isolation mechanisms (for example, the prevention of the formation of hybrid offspring due to ecological or habitat isolation, seasonal or temporal isolation, sexual isolation, and mechanical isolation), or by postmating isolation mechanisms (for example, reduced viability or fertility of hybrid offspring) (Dobzhansky, 1937; Klug *et al.*, 2013). In his famous 1859 book *On the Origin of Species by Means of Natural Selection*, Darwin said: "Nor shall I here discuss the various definitions which have been given of the term species. No one definition has as yet satisfied all naturalists." (Darwin, 1859). Yet, Darwin did anticipate the biological species concept where species are defined by reproductive isolation: "This view generally entertained by naturalists is that species when intercrossed, have been specially endowed with the quality of sterility." (Darwin, 1859).

At first the students in this study looked for any morphological differences between the white-eyed and Canton-S stocks. Other than the color to the eyes, they observed no obvious differences in the two stocks. Hence, these two stocks did not fit the morphological species concept, where different species are different in appearance (Coyne and Orr, 2004; Herron and Freeman, 2014). They did, however, note that the number of progeny in vials of the Canton-S stock was greater than in vials of the white-eyed stock and that development time to adult was slower in the white-eyed stock.

To determine if the two stocks were different species based on the biological species concept, the students next mated white-eyed virgin females with Canton-S males and white-eyed males with virgin Canton-S females. Flies from the white-eyed stock were observed to freely mate with flies from the Canton-S stock in both crosses. Therefore, there was no premating isolation between these two stocks.

In the first cross (white-eyed females with Canton-S males), some progeny were produced in one vial, but the progeny had white eyes, suggesting that non-virgin females were used in the cross. In additional crosses, no adult progeny were observed and the progeny were observed to die as pupae.

After reading articles on stocks of *D. melanogaster* with rearranged chromosomes (Ashburner, 1989; Holm *et al.*, 1980; Boulton and Woodruff, 2010), the students were told that the white-eyed stock was *D. melanogaster* and it had two attached 2L chromosomes and two free 2R chromosomes; C(2L), *dp*; F(2R), *cn c bw*, giving the flies white eyes due to the interaction of the *cn* and *bw* mutant genes (Grell, 1970; Ashburner, 1989; Lindsley and Zimm, 1992); *D. melanogaster* with a normal karyotype have two 2L.2R chromosomes (the period represents the centromere). The students also determined that the reason for the low progeny number in the white-eyed stock was because one-half of the progeny have four 2L chromosomes or no 2L chromosomes

(Boulton and Woodruff, 2010, see their Figure 5). In addition, the reason why no adult progeny were recovered from crosses of the white-eyed stock with Canton-S was because the progeny either had three 2L chromosomes or one 2L chromosome, leading to chromosomal imbalance (Ashburner, 1989; Boulton and Woodruff, 2010, see their Figure 4).

In summary, the students concluded that the white-eyed stock was not a new species, but was a *D. melanogaster* stock with rearranged chromosomes. It is known that chromosome rearrangements can lead to reproductive isolation and speciation (White, 1978; King, 1993). We also observed that flies with three 2L chromosomes or only one 2L chromosome lived until the pupal stage. Holm *et al.* (1980) stated that monosomic 2L flies die during early embryogenesis while the trisomic 2L flies survive to late pupae. It seems, therefore, that flies with an extra 2L chromosome in this study were able to develop up to the pupal stage, but were not able to eclose as adults. The reason for this is unknown.

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Chemical stress and recombination in *Drosophila melanogaster*.

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It has been reported that environmental stresses can influence frequencies of recombination. For example, temperature, nutrition, bacterial infections, and wasp predation can increase the frequency of recombination in *Drosophila melanogaster* (Gowen, 1919; Stern, 1926; Bergner, 1928; Neel, 1941; Singh *et al*., 2015; for a review of this topic, see Parsons, 1988). These observations suggest that organisms can respond to stresses by increasing the frequency of recombination, producing a quick increase in genetic variation that may allow for adult survival (Badyaev, 2005).

It is important, therefore, to determine if exposure to environmental chemical stressors can also influence recombination frequencies. Hence, in this proposed study we tested if copper sulfate in the food of *D. melanogaster* can alter the recombination frequency for X-chromosome linked markers. Copper sulfate is known to be toxic to *D. melanogaster* at moderate to high concentrations (Egli *et al*., 2006).

The following crosses resulted in F1 females that were heterozygous for X-linked visible mutant markers *w* (white eyes; map position 1.5) and sn^3 (singed, small bristles; map position 21) and F1 males that have these two markers on their single X chromosome.

P *w* sn^3 / *w* sn^3 females × + + / Y males

F1 + + / *w* sn^3 females × *w* sn^3 / Y males

The F1 females and males were treated with four concentrations of copper sulfate (0.1, 0.2, 0.3, or 2.5 mM) mixed in instant *Drosophila* food along side untreated controls (water only). The F2 progeny were scored as non-recombinants (+ + / *w* sn^3 females and + + / Y males, which have red eyes and long bristles, or *w* sn^3 / *w* sn^3 females and *w* sn^3 / Y males, which have white eyes and singed bristles) and as recombinants (+ sn^3 / *w* sn^3 females and + sn^3 / Y males, which have red eyes and singed bristles; or *w* + / *w* sn^3 females and *w* + / Y males, which have white eyes and long bristles). As a positive control, F1 flies were also raised at high temperature (30°C) that is known to increase recombination in some regions of the genome, and their frequency of recombination was compared to the recombination frequency in flies raised at room temperature (21°C to 22°C) (Plough, 1917, 1921; Stern, 1926; Smith, 1936; Mather, 1939; Grell, 1966, 1978; Grell and Chandley, 1965; Ashburner, 1989).

The frequency of recombination for the *w* and sn^3 interval was compared between the control crosses and the crosses treated with high temperature and copper sulfate by using the Fisher exact test (Whitlock and Schluter, 2009). The expected frequency of recombination between the white locus and the singed locus in untreated flies is 19.5 percent (Lindsley and Zimm, 1992).

It is our hypothesis that high temperature and copper sulfate will increase the frequency of recombination in *D. melanogaster*, suggesting that organisms can respond to an environmental temperature or chemical stressor and produce new genetic variation by recombination. In fact, we observed that recombination frequencies between the white gene and the singed gene were not significantly changed with exposure to high temperature (P = 0.30) or copper sulfate (P = 0.07 for 0.1 mM; P = 0.19 for 0.2 mM; P = 0.51 for 0.3 mM; and P = 0.44 for 2.5 mM) (see Figures 1 and 2).

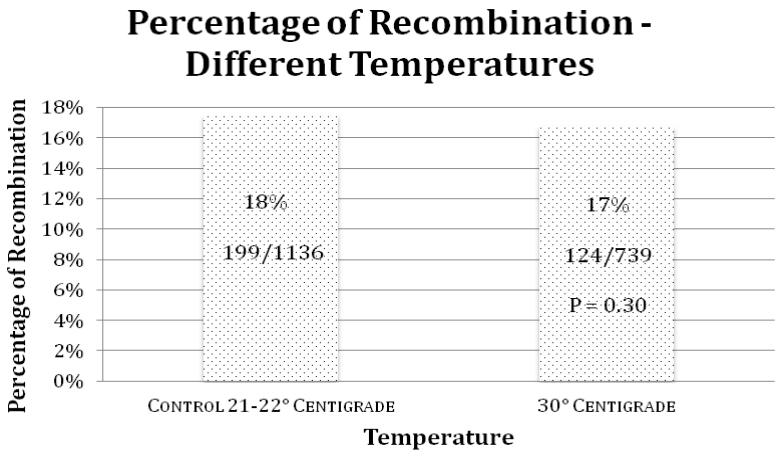

Figure 1. Comparison of the percentage of recombination between the white and singed genes of *D. melanogaster* raised at room temperature (21°C to 22°C) and at high temperature (30°C).

The reason for the lack of an increase in recombination with exposure to high temperature, which has been reported to increase recombination, is not known. Plough (1917), however, reported that high temperature increased the recombination frequency between the black and prune genes on the second chromosome, but did not increase the frequency between the prune and curved genes (see a discussion of this topic in Ashburner, 1989, p. 461). In addition, the influence of temperature on recombination is less for the X

chromosome than for the autosomes (see discussion in Stern, 1926). Hence, the influence of high temperature on recombination may not be uniform over the whole genome (Ashburner, 1989).

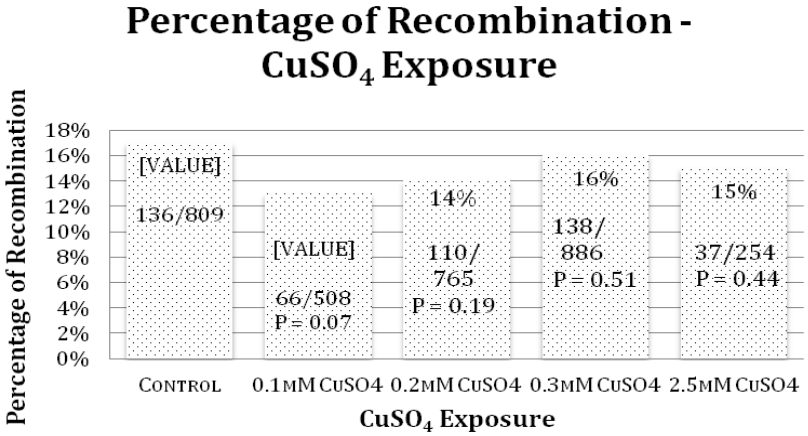

Figure 2. Comparison of the percentage of recombination between the white and singed genes of *D. melanogaster* raised on a control (water in instant *Drosophila* medium) and in 0.1, 0.2, 0.3, or 2.5 mM copper sulfate.

The recombination frequency was also not increased between the X-chromosome markers *w* and *sn*^{3} by copper sulfate in this study. The highest concentration tested, 2.5 mM, did reduce the number of progeny (254 *vs* 508-886). Hence, this concentration did reduce the viability of the treated flies. This result may be related to observations that nutrition can influence recombination frequencies (Neel, 1941). For example, it has been observed that treatment of mice with endocrine disrupting chemicals reduces recombination (Vrooman *et al*., 2015).

What was also surprising in this study was that the recombination frequencies were decreased, but not significantly, in the four copper sulfate concentrations compared to the control (Figure 2). It would be of interest to repeat these treatments at a higher progeny count to see if copper sulfate treatments do decrease recombination. It could be, for example, that some recombinants are sensitive to copper sulfate.

A class discussion of the results of this study might include the possible reasons why organisms, including humans, have evolved sexual reproduction that allows for recombination, instead of reproducing asexually. These reasons include the faster incorporation of favorable mutations that are on separate chromosomes by recombination, the elimination of harmful mutations by recombining chromosome segments with fewer or no deleterious mutations, allowing for the normal segregation of homologous chromosomes at meiosis, and allowing for the repair of double-strand chromosome breakage events by recombination repair (see discussions of this topic in Maynard Smith, 1978; Michod and Levin, 1988; Crow, 1994; Michod, 1995; Charlesworth and Charlesworth, 1998; McDonald, Rice and Desai, 2016).

References: Ashburner, M., 1989, Drosophila: *A Laboratory Handbook*. Cold Spring Harbor Laboratory Press; Badyaev, A.V., 2005, Proc. R. Soc. B 272: 877-886; Bergner, A.D., 1928, J. Exp. Zool. 50: 107-163; Charlesworth, B., and D. Charlesworth 1998, Genetica 102/103: 3-19; Crow, J.F., 1994, Dev. Genet. 15: 205-213; Egli *et al*., 2006, Cellular Biol. 26: 2286-2296; Gowen, J.W., 1919, Genetics 4: 205-250; Grell, R.F., 1966, Genetics 54: 411-421; Grell, R.F., 1978, Genetics 89: 65-77; Grell, R.F., and A.C. Chandley 1965, Proc. Natl. Acad. Sci. USA 53: 1340-1346; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster. Academic Press, New York; Mather, K., 1939, Genetics 24: 413-435; McDonald, M.J., D.P. Rice, and M.M. Desai 2016, Nature 531: 233-236; Maynard Smith, J., 1978, *The Evolution of Sex*. Cambridge University Press, Cambridge; Michod, R.E., 1995, *Eros and Evolution*. Addison-Wesley Publishing Company, Reading, MA; Michod, R.E., and B.R. Levin 1988, *The Evolution of Sex*. Sinauer Associates, Inc., Sunderland, MA; Neel, J.V., 1941, Genetics 26: 506-516; Parsons, P.A., 1988, Biol. J. Linnean Soc. 35: 49-68; Plough, H.H., 1917, J. Exp. Zool. 24: 147-209; Plough, H.H., 1921, J. Exp. Zool. 32: 187-202; Singh *et al*., 2015, Science 349: 747-750; Smith, H.F., 1936, Nature 138: 329-330; Stern, C., 1926, Proc. Nat. Acad. Sci. USA 12: 530-532; Vrooman *et al*., 2015, PLoS Genetics

DOI:10.1371/journal.pgen.1004949; Whitlock, M.C., and D. Schluter 2009, *The Analysis of Biological Data*. Roberts and Company Publishers, Greenwood Village, CO.

Heritability for bristle number in *Drosophila melanogaster.*

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For a quantitative trait to respond to human (experimental) selection or to evolve by natural selection it must be at least partially under genetic control, *i.e.*, have heritability. Heritability is defined either as the fraction of the total variation in a trait that is due to variation in genes, or the proportion of phenotypic variance that parents can pass to offspring. Environmental factors can also influence quantitative traits (see a discussion of this topic in Falconer and Mackay, 1996; Frankham, Ballou, and Briscoe, 2002).

Heritability in the narrow sense (h^2) is the fraction of the total variation in a trait that is due to the additive effects of genes. There can also be dominant effects and interactions between genes and the environment. Narrow sense heritability is the best measure of whether a trait will evolve or respond to selection (Allendorf and Luikart, 2007). How can one estimate h^2? One way is to examine the slope of the regression line of trait values between parents and their offspring. For example, in Figure 1 an estimation of h^2 of 0.69 is determined from the slope of the regression between the height of students and their mid-parent values (Woodruff, unpublished). Comparisons where the regression slope is zero would have a h^2 of zero, whereas a slope of one would have a h^2 of one.

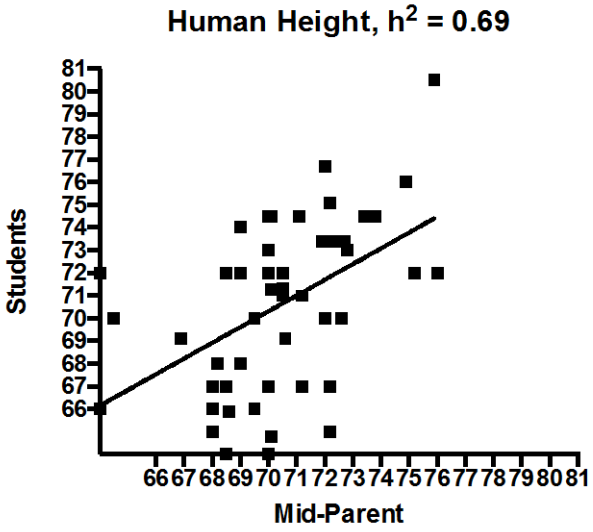

Figure 1. Student height heritability estimate.

In this study, we measured h^2 for sternopleural bristle numbers in *D. melanogaster* by the slope of the regression line of midparent bristle number and the offspring bristle number. Sternopleural bristles are shown in Figure 2 (Woodruff and Thompson, 2005). Estimations of h^2 for sternopleural bristle number in *Drosophila*, which are based on selection experiments and parent offspring regression analysis, ranged from 0.01 to 0.75 (Roff and Mousseau, 1987; Falconer and Mackay, 1996; Woodruff and Thompson, 2005; van Heerwaarden *et al*., 2008).

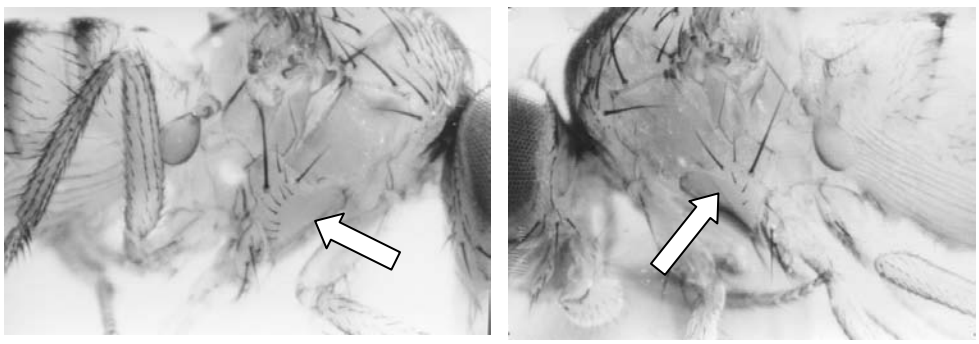

Figure 2. Sternopleural "heart-shaped" section of two *Drosophila* marked with white arrows. Sternopleural bristle numbers are: left, 11; right, 6 (Woodruff and Thompson, 2005).

To measure h^2 for bristle numbers we performed the following crosses (OBL1&2 is a wild-type stock that was established from natural population flies in 2010 (Carr *et al.*, 2010); bristles were counted for both sides; and a total of 55 crosses were scored for sternopleural bristle numbers).

one OBL1&2 virgin female × one OBL1&2 male

↓

one progeny female and one progeny male

Narrow sense heritability was determined by the slope of regression line for female and male progeny *vs* the mid-parent values (Figures 3, 4), using the Prism program.

The mean (± standard error) number of bristles for female (18.22 ± 0.22) and male (17.45 ± 0.23) progeny are significantly different (P = 0.02; Figure 5).

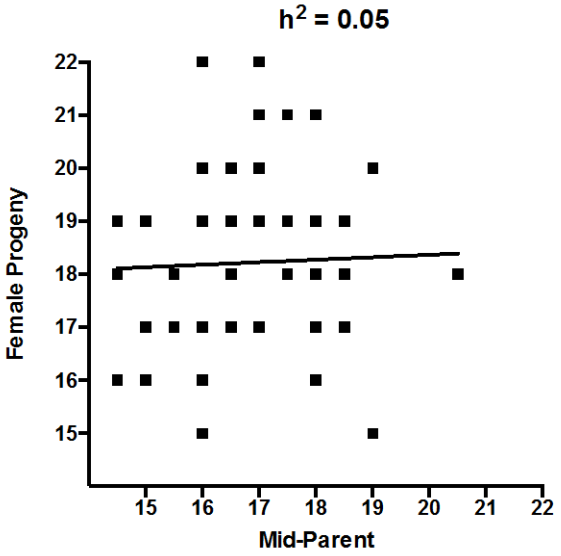

Figure 3. Estimation of narrow sense heritability (h^2) for sternopleural bristle numbers using female progeny.

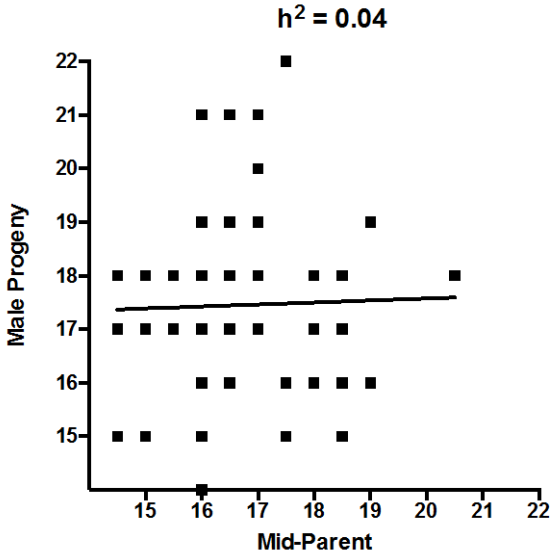

Figure 4. Estimation of narrow sense heritability (h^2) for sternopleural bristle numbers using male progeny.

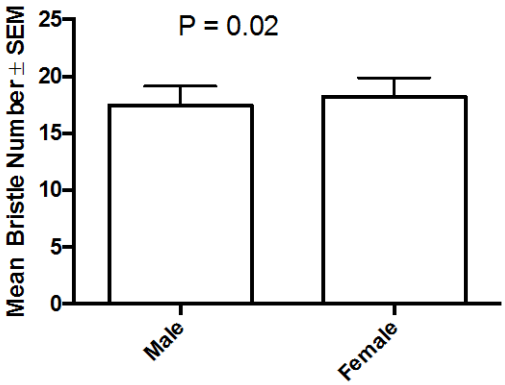

Figure 5. Mean (± standard error) of the sternopleural bristle numbers in males and females (P = 0.02, paired t test).

The observed h^2 values of 0.05 using female progeny and 0.04 using male progeny for sternopleural bristle numbers are at the low end of those reported in the literature (Falconer and Mackay, 1996; van Heerwaarden *et al*., 2008). Woodruff and Thompson (2005), however, reported h^2 values of 0.01 for females and males based on a selection response experiment in lines that were inbred by brother-sister matings for 41 generations and possessed low levels of genetic variation, whereas flies with greater levels of genetic variation had h^2 values of 0.11 for males and 0.15 for female. Since the OBL1&2 wild-type stock used in this study had been maintained in the laboratory for six years before this study (Carr *et al*., 2014), it may contain a reduced amount of genetic variation due to partial inbreeding, resulting in the observed low h^2 values.

A class discussion of the results of this study could include: 1) Ask students to estimate the number of quantitative trait genes controlling bristle number in *D. melanogaster*. It is about eight (Gurganus *et al*., 1999). 2) Ask students to estimate the narrow sense heritability for height in humans by determining the slope of the regression line for their heights *vs*. their mid-parent heights. The height of female parents and students should be multiplied by 1.08, because of the difference in height of men and women.

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Description of a double mutant strain of *Drosophila melanogaster* useful for genetic laboratory courses.

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Many years ago, individuals showing drastically reduced eyes arose in our laboratory *e* (*ebony*) strain (Bridges and Morgan, 1923). We selected those flies presenting both traits and constituted a new double mutant strain *e su* (*e*, ebony; *su*, '*sense ulls*', eyes drastically reduced). Both mutations were linked and located in the chromosome III. We used this strain in linkage analyses with our undergraduate students. We then proceeded to assess which described gene was allelic to our *su* mutation. With a recombination experiment we deduced that *su* was located at 36.7 m.u. from the *e* gene. Consulting the genetic map of chromosome III we hypothesized that *su* could be the *eyg* (*eyegone*) gene (Ives 1940), whose phenotype is also eye reduction. We carried out a pseudodominance study using a deletion that covered the *eyg* region (ED2015, http://flybase.org/), and we observed that the individuals not showing the dominant marker (*Sb*, *Stubble*, Dobzhansky 1930) of the balancer chromosome (TM6C, Chyb and Gompel 2013) presented drastically reduced eyes. Finally, we wanted to confirm that *su* was actually *eyg* carrying out a complementation test crossing both strains. We obtained *eyg* strain from a stock center and the result of complementation test confirmed that *su* was a mutation of *eyg* gene.

This double mutant strain *e su* can be used for different genetic laboratory courses and we can send it upon request.

Acknowledgments: This research was financed by grant 2015PID-UB/010 from Universitat de Barcelona.

References: Bridges, C.B., and T.H. Morgan 1923, Carnegie Inst. Washington Pub. 327: 1-251; Chyb, S., and N. Gompel 2013, *Atlas of* Drosophila *Morphology*. Academic Press, London, U.K.; Dobzhansky, Th,, 1930, Z. Indukt. Abstamm. Vererbungsl. 54: 427-457; Ives, P.T., 1942, Dros. Inf. Serv. 16: 48-49.

A hands-on genetics teaching approach at university level.

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Teaching general Genetics is a cornerstone of a large number of university degrees. Being a scientific topic, laboratory classes are an essential element in student-centered learning. Here, we present our experience in implementing new material for teaching hands-on genetics, a subject of interest for other academic professionals in the field of Genetics. Our students carry out a genetic analysis of the *su* (*sense ulls*) mutation of *Drosophila melanogaster*, which produces a drastic eye reduction. The complete strain description can be found in Mestres *et al.* (2016a). The aim of the course is to give students the appropriate genetics tools to answer the three following questions: 1) Is the *su* mutation dominant or recessive? 2) In which chromosome is *su* located? 3) Can we identify in which gene the *su* mutation is?

To answer the first two questions we designed a pattern of genetic crosses taking advantage of a double mutant strain *e su*, being *ebony* a recessive mutant producing black body color (Lindsley and Zimm, 1992; Chyb and Gomple, 2013). *Drosophila melanogaster* presents a karyotype composed by two large metacentric autosomes (II and III), a punctual autosome (IV), and the sexual chromosomes (I = X and Y). For chromosome location we first inform our students that the *su* mutation could be either inherited as a sex-linked or autosomal trait and discard other genetic patterns such as partial sex-linked inheritance, uniparental inheritance, maternal effect, and others. The genetics crosses proposed to the students are:

e su females (virgin) × *vg* males

and the reciprocal cross:

vg females (virgin) × *e su* males

The recessive mutation *vg* (*vestigial*, wings extremely reduced and held at right angles to the body) is located in chromosome II (Lindsley and Zimm, 1992; Chyb and Gomple, 2013), whereas *e* is in chromosome III. In both reciprocal crosses, all F_1 individuals show wild type phenotype, and thus students should conclude that *su* mutation is autosomal recessive. Later, analyzing the F_2 offspring it is possible to observe that *su* presents an independent inheritance with regard to *vg*, but is linked to *e*. Therefore, it is logical to deduce that *su* is located in chromosome III.

In past years, we finished the laboratory experiments at this level (solving only questions 1 and 2), but last year we decided to go further and try to answer question 3. To do so, we estimated the recombination between *su* and *e*. The value obtained was 36.65 m.u. from the location of *e* gene (70.7). We searched in the genetic map of the species (Lindsley and Zimm, 1992) which genes were located to the right (70.7 + 36.65 = 107.34) and left (70.7 – 36.65 = 34.05) of *e*. At 37.5 is *eyg* (*eyegone*), whose phenotypic description fits well with that of *su*. To confirm whether *su* mutation belongs to the *eyg* gene, we designed a pseudodominance experiment choosing the deletion Df(3L)ED215 from the DrosDel deletion collection (Ryder *et al.*, 2007) that spans the *eyg* gene. To study the pseudodominance the students carried out the cross between *e su* and

Df(3L)ED215 flies. Approximately half of the offspring flies presented eyes drastically reduced, indicating that most probably *su* was an *eyg* gene mutation:

e su × Df(3L)ED215

↓

≈1/2 normal eyes + ≈1/2 reduced eyes

Furthermore, to confirm that the *su* mutation maps to the *eyg* gene, the students performed a complementation test crossing *e su* with *eyg* flies. All offspring individuals showed drastic eye reduction, and thus confirmed our hypothesis:

e su × *eyg*

↓

100% reduced eyes

We complemented the study with a couple of computer sessions using the *Drosophila* database Flybase (http://flybase.org/). In the first one, the students analyzed possible candidate genes presenting mutations that produced a similar phenotype to *su*. We selected *lz* (*lozenge*), *eya* (*eyes absent*), *eyg* (*eyegone*), and *ey* (*eyeless*) located in chromosomes I, II, III, and IV, respectively. The second computer session was programmed at the end of the laboratory course to present and comment the Df(3L)ED215 deletion and the balancer chromosome used to maintain it (TM6C).

The answer to question 3 implied a lot of work for our students and we had logistical problems to implement the whole experimental design (a restricted number of laboratory sessions, too many vials needed, *etc*.). For these reasons, we decided to simplify the crosses to be carried out by the students. They all worked in teams of four members: two of them carried out the initial reciprocal crosses, the third member performed the pseudodominance cross, and the fourth the complementation test. Particular details on the organization of the work can be found in Mestres *et al.* (2016b).

We introduced this new pattern of laboratory classes during the 2015-2016 academic year to 320 undergraduate students divided in 16 laboratory groups. The result of the experience was excellent. Students understood much better the genetic concepts of pseudodominance and complementation, obtaining better qualifications in the corresponding questions of the final exam. Additionally, they were satisfied to carry out a complete genetic study being able to properly answer the three proposed questions on *su* mutation. We also obtained an additional indirect benefit, which is the active study of a balancer chromosome is needed to maintain the Df(3L)ED215. For all these reasons we encourage other colleagues to use this pattern of laboratory classes in general courses of Genetics. We can send the necessary strains (*e su*, *vg*, Df(3L)ED215, and *eyg*) upon request to those interested in this experimental design.

Acknowledgements: This study was supported by grant number 2015PID-UB/010 from Programa de Millora i Innovació Docent (Universitat de Barcelona, Spain).

References: Chyb, S., and N. Gompel 2013, *Atlas of* Drosophila *Morphology*. Academic Press, London, U.K.; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster. Academic Press, N.Y.; Mestres, F., T. Adell, S. Araujo, J. Balanyà, M. Papaceit, M. Pascual, M. Riutort, R. Romero, and C. Segarra 2016a, Dros. Inf. Serv. 99 (in press); Mestres, F., T. Adell, S. Araujo, J. Balanyà, M. Papaceit, M. Pascual, M. Riutort, R. Romero, and C. Segarra 2016b, *In: EDULEARN16 Proceedings* (IATED ed.). IATED Academia, València (Spain), pp. 6418-6423; Ryder, E., M. Ashburner, R. Bautista-Llacer *et al.* 2007, Genetics 177: 615–629.

CRISPR/Cas9 induced mutations of the white gene of haplo-X and diplo-X *Drosophila melanogaster.*

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Genome editing is the process of changing the DNA structure of a gene by deleting or replacing nucleotides, or by replacing an old gene with a new DNA sequence (Cox *et al*., 2015). The CRISPR/Cas9 bacterial system has revolutionized the genome editing process (Haimovich *et al*., 2015; Govindan and Ramalingam, 2016). This genome editing system consists of a CRISPR guide RNA that locates the gene of interest and a Cas9 endonuclease that cleaves the targeted DNA to form a double-strand break. Mistakes in DNA repair of this breakage often lead to base deletions or insertions. This technique can be used in any organism where the sequence of a gene of interest is known. For example, CRISPR/Cas9 has been used to eliminate the HIV virus in human cells (Kaminski *et al*., 2016), knock out genes involved in aging (Harel *et al*., 2015), correct disease-causing mutations (Wu *et al*., 2013; Yin *et al*., 2014; Nelson *et al*., 2016), inactivate pig retroviruses in human cells (Yang *et al*., 2015), drive genes and populations of *Drosophila* and mosquitoes to extinction (Gantz and Bier, 2015; Hammond *et al*., 2016), and modify the gene that causes mushrooms to brown (Waltz, 2016).

Experimental Plan

In this study two *Drosophila melanogaster* stocks were used to induce mutations in the white gene using the CRISPR/Cas9 system; mutations caused a change of brown or red eyes to white eyes. One stock (GRN-0006) has the CRISPR RNA guide sequence for the white gene, while the second stock (CAS-0001) contains the Cas9 endonuclease. These two stocks, received from the Genetic Strains Research Center, National Institute of Genetics, Japan, were first crossed as follows in Cross Scheme One. In these crosses, y^1 or y^2 = yellow body color, y^+ = grey body color, *f* = forked bristles, cho^2 v^1 = brown eyes, CyO/+ = curly wings, CyO/CyO flies die as early embryos, C(1)DX = two X chromosomes joined at their centromeres, attP40{U6.2-w-ex6-1, y^+} = CRISPR, and attP40{nos-Cas9} = Cas9 (Lindsley and Zimm, 1992; Kondo and Ueda, 2013). Note that the G2 males in this cross contain the full CRISPR/Cas9 system, with the white-gene guide RNA and Cas9 together on the second chromosomes.

Cross Scheme One

G1 GRN-0006 (y^2 cho^2 v^1; attP40{U6.2-w-ex6-1, y^+})/CyO) CRISPR females

×

CAS-0001 (y^2 cho^2 v^1 / Y; attP40{nos-Cas9})/CyO) Cas9 males

↓

G2 GRN-0006 (y^2 cho^2 v^1 / Y; attP40{U6.2-w-ex6-1, y^+})/CAS-0001 (attP40{nos-Cas9}) males (not CyO)

×

C(1)DX, *y f* / Y females

↓

Score G3 males for brown or white eyes (new mutations at the white locus).

As controls, GRN-0006/CyO females were mated to wild-type (Canton-S) males, and CAS-0001/CyO males were mated to wild-type (Canton-S) females. Then G2 males from each cross that did not have curly wings were mated with C(1)DX, *y f* / Y females, and G3 males were scored for red or white eye colors. Neither control cross should exhibit mutations of the white gene, showing that CRISPR and Cas9 must be together in the same fly to induce mutations.

To test the efficiency of the CRISPR/Cas9 system, we also determined if mutations could be induced at both white genes in wild-type females with attached-X chromosomes, using the following Cross Scheme Two.

Cross Scheme Two

G1 C(1)DX, *y f* / Y females

×

GRN-0006 (y^2 cho^2 v^1; attP40{U6.2-w-ex6-1, y^+})/CyO males

↓

G2 C(1)DX, *y f* / Y; GRN-0006 (y^2 cho^2 v^1; attP40{U6.2-w-ex6-1, y^+})/ + females (not Cy)

×

CAS-0001 (y^2 cho^2 v^1 / Y; attP40{nos-Cas9})/CyO males

↓

G3 C(1)DX, *y f* / Y; GRN-0006 (attP40{U6.2-w-ex6-1, y^+})/CAS-0001 (attP40{nos-Cas9}) females (y+ and not Cy)

`

×

Sibling males

↓

The G4 females were scored for white eyes, caused by mutations in both of the white loci of the C(1)DX, *y f* / Y G3 female gametes and for red eyed females, which will have either no mutations at the white loci, or mutations in just one of the two white loci in G3 female gametes (since white mutations are recessive). As a positive control, G4 white-eyed males should be recovered as expected, because some sibling G3 males from this cross will contain both CRISPR and Cas9.

Anticipated Results

Based on results of preliminary crosses of GRN-0006 and CAS-0001stocks, we expect up to 90 percent of G3 males in Cross Scheme One to have white eyes due to CRISPR/Cas9 induced mutations in the white gene (Kondo and Ueda, 2013). Although we do not know the percentage of the G4 C(1)DX, *y f* / Y females in the Cross Scheme Two that will have white eyes, they should occur at a lower frequency than in males of Cross Scheme One, which only have one copy of the X-linked white gene. We also expect no mutants will be recovered in the two control crosses, as CRISPR and Cas9 together are required to induce mutations in the targeted gene.

Results and Discussion

Controls: In 20 crosses where chromosome two contained CRISPR only (CRISPR/+) or Cas9 only (Cas9/+), no white-eyed mutant males were observed among 639 red-eyed males (Table 1).

Table 1. Number of white-eyed mutant males and red-eye, non-mutant, males containing either CRISPR (CRISPR/+) or Cas9 (Cas9/+), but not both.

Vial number	Red eyed males	White eyed males	Total # of males
1	54	0	54
2	25	0	25
3	27	0	27
4	27	0	27
5	30	0	30
6	22	0	22
7	34	0	34
8	31	0	31
9	33	0	33
10	19	0	19
11	50	0	50
12	35	0	35
13	54	0	54
14	43	0	43
15	21	0	21
16	21	0	21
17	39	0	39
18	20	0	20
19	38	0	38
20	16	0	16
Totals	639	0	639

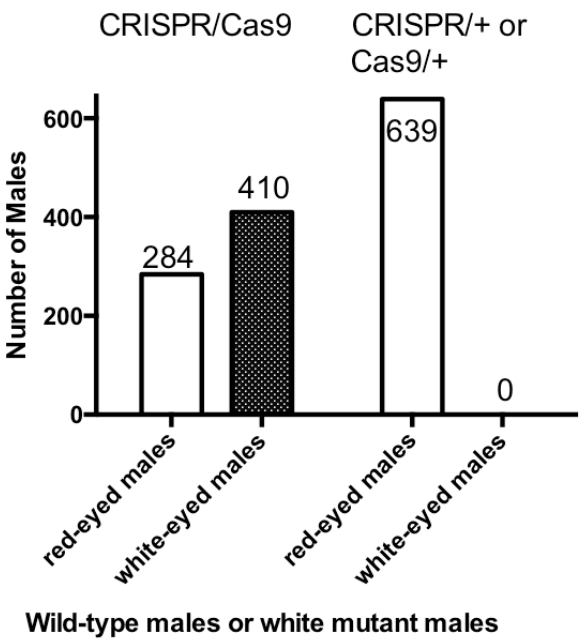

Figure 1. Recovery of white mutations in males

Mutations in males: In 28 crosses of Cross Scheme One, there were 410 white eyed males and 284 red eyed males, for a frequency of 59.1% white-eyed mutant males (410/694 = 59.1%; see Table 2 and Figure 1). White-eyed mutant males were observed in each of the 28 crosses. A total of 24 white-eyed mutant males were mated with C(1)DX, *y f* /Y females, and all bred true as white mutants in subsequent generations. The percent of white-eyed mutants in the CRISPR/Cas9 crosses is significantly different from the lack of mutants recovered in the CRISPR/+ or Cas9/+ crosses ($P < 0.001$ for 410/649 *vs* 0/639).

Mutations in both white genes in C(1)DX, *y f* / Y females: As expected white-eyed mutant males were recovered showing that the CRISPR/Cas9 system was functioning in Cross Scheme Two. Furthermore, in 30 crosses of Cross Scheme Two, 83 C(1)DX, *y f* / Y females had white eyes and 404 had red eyes, for a frequency of 17% females with both white genes mutated by the CRISPR/Cas9 system (see Table 3). Six of the G4 females with mutations in both white genes were crossed and the white mutation bred true into the G5 generation.

Mosaic eyes (spots of red and white pigment) were also observed in G4 females (these females were also *y* and *f*, confirming that they were C(1)DX, *y f* / Y) and in males of cross scheme two (Figure 2). Some of these G4 mosaic flies were also *Cy*, suggesting that they did not carry CRISPR and Cas9 in their somatic cells. It could be that these mosaics were caused by maternal deposition of CRISPR and/or Cas9 into the embryo (Lin and Potter, 2016). Two G4 mosaic flies also gave rise to white-eyed G5 progeny, showing that the white-eyed mutation in mosaics can include germ-line tissues.

Table 2. Number of white-eyed mutant males and red-eye, non-mutant, males from Cross Scheme One CRISPR/Cas9 males.

Vial number	Red eyed males	White eyed males	Total # of males
1	8	20	28
2	11	6	17
3	12	16	28
4	1	21	22
5	10	29	39
6	8	11	19
7	6	27	33
8	8	14	22
9	7	19	26
10	7	4	11
11	12	9	21
12	20	29	49
13	15	31	46
14	30	20	50
15	4	33	37
16	16	19	35
17	18	4	22
18	13	15	28
19	8	17	25
20	24	16	40
21	9	3	12
22	6	9	15
23	7	5	12
24	5	2	7
25	4	16	20
26	4	1	5
27	3	7	10
28	8	7	15
Totals:	284	410	694

Figure 2. Mosaic eyes of a G4 female of cross scheme two.

The results of this study clearly show that the CRISPR/Cas9 genome editing system induces a high percentage of mutations at the targeted white locus in haplo-X males (59.1%) and in diplo-X females (17%). One can compare the frequency of CRISPR/Cas9 induced white mutations in males (59.1%) with the observed spontaneous frequency of white mutations in males (10/668,631 = 0.002%) (Woodruff *et al*., 1983). The female results also mean that both copies of the targeted genes in autosomes could be mutated by this system. The percentage of diplo-X females with mutations in both white genes (17%), however, is signifiantly lower than would be expected if the CRISPR/Cas9 system in these females was the product of the frequency in haplo-X males ($0.591 \times 0.591 = 35\%$; $p < 0.0001$).

A class discussion of the results of this study could include: 1) The CRISPR/Cas9 system can also be used to insert a new DNA sequence into a gene of choice. For example, insertion of a mutant *Sonic hedgehog*

Table 3. Number of white-eye mutant, C(1)DX, *y f* / Y, females and red-eye non-mutant, C(1)DX, *y f* / Y, females from Cross Scheme Two.

Vial Number	Red eyed females	White eyed females	Total # of females
1	16	0	16
2	15	1	16
3	7	0	7
4	12	0	12
5	18	4	22
6	7	0	7
7	4	1	5
8	5	1	6
9	12	2	14
10	5	0	5
11	7	0	7
12	12	1	13
13	5	0	5
14	17	6	23
15	18	0	18
16	8	3	11
17	10	1	11
18	14	4	18
19	18	11	29
20	25	9	34
21	11	18	29
22	14	14	28
23	27	2	29
24	11	0	11
25	16	1	17
26	19	2	21
27	24	0	24
28	20	0	20
29	8	0	8
30	19	2	21
Total	404	83	487

gene from a python into a mouse caused the mice to develop little nubs of legs, suggesting that the ancestors of snakes may have lost their legs by a similar mechanism (Kvon *et al*., 2016). 2) Has the CRISPR/Cas9 system been used in humans? Yes. For example, this system has been used to inactivate HIV in somatic cells and to inactivate a gene in triploid zygotes (Liang *et al*., 2015).

References: Cox, D.B.T., R.J. Platt, and F. Zhang 2015, Nature Medicine 21: 121-131; Gantz, V.M., and E. Bier 2015, Science 348: 442-444; Govindan, G., and S. Ramalingam 2016, J. Cell. Physiol. 231: 2380-2392; Haimovich, A.D., P. Muir, and F.J. Isaacs 2015, Genetics 16: 501-516; Hammond, A., *et al.*, 2016, Nature Biotechnology 34: 78-83; Harel, L., *et al.*, 2015, Cell 160: 1013-1026; Kaminski, R., *et al.*, 2016, Sci. Rep. 6: 22555; doi: 10.1038/srep22555; Kondo, S., and R. Ueda 2013, Genetics 195: 715-721; Kvon, *et al*., 2016, Cell 167: 633-642; Lang, *et al*., 2015, Protein and Cell 6: 363-372; Lin, C., and C.J. Potter 2016, G3 6: 3785-3691; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster. Academic Press, New York; Nelson, C.E., *et al*., 2016, Science 351: 403-407; Waltz, E., 2016, Nature 532: 293; Woodruff, R.C., B.E. Slatko, and J.N. Thompson, jr. 1983, *The Genetics and Biology of* Drosophila, Vol. 3c, pp. 37-124 (Ashburner, M., H.L. Carson, and J.N. Thompson, jr., eds.), Academic Press, New York; Wu, Y., *et al.*, 2013, Cell Stem Cell 13: 659-662; Yang, L., *et al.*, 2015, Science 350: 1101-1104; Yin, H., *et al.*, 2014, Nature Biotechnology 32: 551-553.

Guide to Authors

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Green, R.L., 1998, Heredity 121: 430-442.
Waters, R.L., J.T. Smith, and R.R. Brown 1990, J. Genet. 47: 123-134.

Note the initials are before each name except for the senior author.

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Other Reports

57th Annual *Drosophila* Research Conference

The 57th Annual *Drosophila* Research Conference was held on 13-17 July 2016 at the Orlando World Center Marriott in Orlando, Florida. The Conference Organizers were Susan Celniker, Chair, (Lawrence Berkeley National Laboratory), David Bilder (University of California, Berkeley), Nancy Bonini (University of Pennsylvania, HHMI), and Ross Cagan (Mount Sinai School of Medicine). The conference was sponsored by The *Drosophila* Board in association with the Genetics Society of America, 9650 Rockville Pike, Bethesda, MD 20814-3998.

Opening Remarks

Susan Celniker: Welcome
Thom Kaufman: Remembering Bill Gelbart

Opening Session Panel: Discovery of the Homeobox

Michael Levine (Princeton University), William McGinnis (University of California, San Diego), Matthew Scott (Carnegie Institute for Science), Cassandra Extavour, Moderator (Harvard University)

Plenary Lectures (in presentation order)

Adam Martin (MIT). Organizing the contraction that changes tissue shape.

Ingrid Lohmann (Heidelberg University). Hox transcription factors and their cell type-specific role in development.

Annette Schenck (Radboud University Medical Center). Modelling intellectual disability disorders in *Drosophila*: From genes to functional modules and clinical applications.

Doujia Pan (Johns Hopkins School of Medicine). Hippo signaling in growth control and beyond.

Iris Salecker (Francis Crick Institution). Coordination of neuroepithelial specification and neurogenesis modes in the *Drosophila* visual system.

Pierre Leopold (Institut Valrose Biologie). Growth coordination mechanisms during *Drosophila* development.

Pamela Geyer (University of Iowa). Networking at the nuclear periphery: Contributions of *Drosophila* LEM domain proteins.

Artyom Kopp (University of California, Davis). Molecular genetics of sex-specific evolutionary innovations.

Workshops

Automated Tracking for Quantitative Phenotyping
Organizers: Andre Brown (Imperial College London), Gordon Berman (Emory University), and Megan Carey (Champalimaud Centre for the Unknown)

CRISPR-based Genome Engineering
Organizer: Mike Boxem (Utrecht University, Netherlands)

Using CyVerse Cyberinfrastructure to Enable Data Intensive Research, Collaboration, and Education
Organizers: Joslynn Lee and Jason Williams (Cold Spring Harbor Laboratory)

Model Organisms to Face Environmental Problems
Organizers: Cristina Miceli (University of Camerino, Italy), Michael Lynch (Indiana University), and Wei Miao (China Academy of Sciences)

Integrating Research and Teaching: Professional Development for Current and Future Faculty Members
Organizers: Rebeccah Kurzhals (Southeast Missouri State University), Joyce Fernandes (Miami University), Pamela Hanson (Birmingham-Southern College), Paula Checchi (Marist College), Gretchen Edwalds-Gilbert (Claremont McKenna, Pitzer, and Scripps Colleges), Eric Stoffregen (Lewis-Clark State College), and Christina Swanson (Arcadia University)

Informatics Resources to Aid the Genetic Dissection of Neural Circuitry
Organizers: David Osumi-Sutherland (European Bioinformatics Institute [EMBL-EBI]), Owen Randlett (Harvard University), and Paul Sternberg (Caltech University)

Everything You Wanted to Know About Sex
Organizers: Artyom Kopp (University of California, Davis), Michelle Arbeitman (Florida State University), and Mark Van Doren (Johns Hopkins University)

modMetabolome: Model Organism Metabolomics Consortium Workshop
Organizers: Laura Reed (University of Alabama), and Arthur Edison (University of Georgia)

Feeding Behavior, Nutrition, and Metabolism: Emerging Model Organisms
Organizers: Tania Reis (University of Colorado), William Ja (The Scripps Research Institute), Supriya Srinivasan (The Scripps Research Institute), and Amnon Schlegel (University of Utah)

Functional Genomics for Conserved Gene Function Discovery
Organizers: Stephanie Mohr (Harvard Medical School), Brenda Andrews (University of Toronto), Susan Dutcher (Washington University in St. Louis), Norbert Perrimon (Harvard Medical School), and Yi Zhou (Harvard University)

Cell Competition in Flies and Mice
Organizers: Erika Bach (New York University Langone Medical Center), Nicholas Baker (Albert Einstein College of Medicine), and Laura Johnston (Columbia University Medical Center)

Developmental Mechanics
Organizers: Rodrigo Fernandez-Gonzalez (University of Toronto), Guy Tanentzapt (University of British Columbia), and Ronen Zaidel-Bar (National University of Singapore)

Model Systems in Drug Discovery
Organizer: Daniela Zarnescu (University of Arizona)

CRISPR/Cas9 – Techniques and Applications in Fish, Flies, and Mice
Organizers: Lauryl Mj Nutter (The Centre for Phenogenomics), John Seavitt (Baylor College of Medicine), and Edward Ryder (Wellcome Trust Sanger Institute)

Utilizing NCBI Databases for Model Organism Research
Organizer: Terence Murphy (NIH)

Systems Genetics in Complex Populations
Organizers: Martin Ferris (University of North Carolina, Chapel Hill), Fernando Pardo-Manuel de Villena (University of North Carolina, Chapel Hill), and Logan Everett (North Carolina State University)

An Introduction to Using Galaxy Genetic Data Analysis
Organizer: Dave Clements (Johns Hopkins University)

The InterMOD Consortium: A Common Interface to Model Organism Data
Organizers: Rachel Lyne and Julie Sullivan (University of Cambridge)

Spotlight on Undergraduate Research Using Genetics Research Models
Organizers: Eric Stoffregen (Lewis-Clark State College), Elyse Bolterstein (Northeastern Illinois University), Emily Wiley (Claremont McKenna College), Michelle A. Mondoux (College of the Holy Cross), and Mary Miller (Rhodes College)

The Ecdysone Workshop
Organizers: Nick Sokol (Indiana University) and Rebecca Spokony (Baruch College)

Genetic and Genomic Models of Polyploidy
Organizers: Don Fox (Duke University), David Pellman (Harvard University), Andrew Duncan (University of Pittsburgh), and Eduardo Orias (University of California, Santa Barbara)

Microbiota
Organizers: Brooke McCartney (Carnegie Mellon University) and Will Ludington (University of California, Berkeley)

The North American *Drosophila* Board

The Board's duties include: overseeing community resource centers and addressing other research and resource issues that affect the entire *Drosophila* research community. The Board also administers the finances for the annual North America *Drosophila* Research Conference and its associated awards, and it chooses the organizers and the site of the annual meeting. The Board consists of nine regional representatives and four international representatives, who serve 3-year terms. The three elected officers are President, President-Elect, and Treasurer. Years noted in the following list are the last Drosophila Conference in which a Board Member will serve as an officer or regional representative. In addition, the Board has *ex officio* members who represent *Drosophila* community resources or centers. For more information about the Board and the summaries of the annual Board meetings, see: Fly Board under the News menu at the FlyBase web site: flybase.bio.indiana.edu.

Drosophila Board Membership 2015 - 2016

President (2019): David Bilder (University of California, Berkeley)

President-Elect (2020): Laura Johnston (Columbia University, New York, NY)

Past-President (2014): Ken Irvine (Waksman Institute, Rutgers University, Piscataway, NJ)
Past-President (2013): Amy Bejsovec (Duke University, Durham, NC)
Past-President (2012): Michael O'Connor (University of Minnesota, Minneapolis)

Treasurer (2016): Deborah Andrew (Johns Hopkins School of Medicine, Baltimore, MD)

Regional Representatives:

New England: Giovanni Bosco (2017; University of Arizona, Tucson)
Heartland: Michael Galko (2018; University of Texas MD Anderson Cancer Center, Houston)
Midwest: Bing Zhang (2018; University of Missouri, Columbia)
Mid-Atlantic: Jessica Triesman (2016; New York University School of Medicine)
Southeast: Andrea Page-McCaw (2018; Vanderbilt University Medical Center, Nashville, TN)
California: Angelika Stathopoulos (2016; California Institute of Technology, Pasadena)
Great Lakes: Scott Barolo (2018; University of Michigan, Ann Arbor)
Mountain: Sarah Certel (2017; University of Montana, Missoula)
Canada: Esther Verheyen (2018; Simon Fraser University, Burnaby, Canada)

International Representatives:

Asia: Shigeo Hiyashi (Riken, Japan)
Australia/Oceania: Gary Hime (University of Melbourne, Australia)
Europe: Daniel St. Johnston (University of Cambridge, UK)
Latin America: Mariana Melani (Fundacion Instituto Leloir-CONICET, Buenos Aires, Argentina)

Primarily Undergraduate Institution Representative: Alexis Nagengast (Widener University, Chester, PA)

***Ex Officio* – Representing *Drosophila* Resources:**

Susan Celniker (BDGP; Lawrence Berkeley National Laboratory, Berkeley)
Thom Kaufman (Bloomington Stock Center & FlyBase; Indiana University)
Kathy Matthews (Bloomington Stock Center & FlyBase; Indiana University)

Kevin Cook (Bloomington Stock Center & Nomenclature Committee; Indiana University)
Teri Markow (UCSD Drosophila Species Stock Center; University of California, San Diego)
Maxi Richmond (UCSD Drosophila Species Stock Center; University of California, San Diego)
Jim Thompson (Drosophila Information Service; University of Oklahoma)
Liz Perkins (Harvard TRiP; Harvard University)
Hugo Bellen (Bloomington Stock Center Advisory Committee & P Element Project; Baylor College of Medicine)
Allan Spradling (P-Element Project; HHMI/Carnegie Institute)
Stephanie Mohr (Harvard DRSC; Harvard University)
Scott Hawley (Nomenclature Committee; Stowers Institute for Medical Research)
Lisa Meadows (VDRC; Vienna, Austria)
Masanobu Itoh (DGRC, Kyoto; Kyoto, Japan)
Toshiyuki Takano-Shimizu (DGRC, Kyoto; Kyoto, Japan)
Chuck Langley (At-large; University of California, Davis)
Brian Oliver (FlyBase Advisory Board; NIH)

Genetics Society of America:

Adam Fagan, Executive Director (afagen@genetics-gsa.org)
Suzy Brown, Senior Director (sbrown@genetics-gsa.org)