Research Notes

A clustering of P-element insertions within the first exon of the *modifier of rudimentary* gene.

Lam, Woei, and Stuart I. Tsubota. Department of Biology, Saint Louis University, St. Louis, MO 63103, tsubota@slu.edu

The *modifier of rudimentary* gene, *mod(r)*, was isolated as a regulatory gene of *rudimentary, r*. It encodes a protein, whose function within the cell has not been determined, although initial experiments indicate that it may encode a novel transcription factor that normally activates *r* transcription (Begley *et al.*, 1995). The original *mod(r)* mutation, $mod(r)^{p1}$, was isolated as a suppressor of the mutant wing phenotype of a weak *r* allele (Begley *et al.*, 1995). $mod(r)^{p1}$ is an insertion of an unmarked P element in the first exon upstream of the start of translation.

A.

```
tggcggcaagctagccaaggtggcagcactgaaatggtcacagAacgaaaatgcaacttcgatcggg
gcatataaaaccagtgcttccaatccgaaggcaaagCataaaaGatcagaacatcagtAgccgaaga
ttggctgagtagcacggacagcgggcaagtcctttgaaacgttggtagtttgcaaccgggtttgcca
acttcctttggagttcagtggtgctcaactatcgacacaactatcctcggctttcgcaaaactcagt
aaaccggtaaacttccgttcatacagttcacaattctaaatttaaaatagatctaacgcagacacat
tgacattcgaaaattgggattgaaaactcaagATGccgactacaccacaggatcttgcccttgccca
ctctcttgctcaaagacctccgaccgatagcagtgaggccaaggagcaggaggccggcgaatcggac
aacctgcccaacctgtgcactttgtcgctggacgaactgaaac
```

B.

$mod(r)^{p1}$	cataaaag
$mod(r)^{p2}$	gatcagaa
$mod(r)^{KG07005}$	gatcagaa
CB-0688-3	agccgaag

Figure 1. Insertion of P elements into the first exon of *mod(r)*. A. Sequence of the 5' end of the *mod(r)* gene showing the insertion sites. The nucleotide indicating the start of transcription is capitalized and underlined. The first base of the 8-bp site duplication of each insertion is capitalized. The start of translation (ATG) is capitalized. B. 8-bp site duplications of the four P-element insertions. $mod(r)^{KG07005}$ was isolated by the Gene Disruption Project members, 2001- (NCBI # BH900899) and *CB-0688-3* was isolated by the Cambridge Deletion Project.

We have isolated another *mod(r)* mutation, $mod(r)^{p2}$, in a similar hybrid-dysgenesis screen for suppressors of *rudimentary*. This mutation is caused by the insertion of a 1.2-kb P element in the *mod(r)* gene. DNA sequencing of this mutation reveals that, like $mod(r)^{p1}$, it is an insertion of an unmarked P element into the first exon (Figure 1). The site of insertion of $mod(r)^{p2}$ is 7 bp downstream of the $mod(r)^{p1}$ insertion site. Two other P-element insertions into the first exon of

mod(r) have been isolated, $mod(r)^{KG07005}$ and CB-0688-3 (Figure 1). The P element in $mod(r)^{KG07005}$ is inserted in exactly the same position as that of $mod(r)^{p2}$. The P element in CB-06883 is located 15 bp downstream of that of $mod(r)^{p2}$. In the later two cases, the elements were genetically-engineered, marked P elements and, importantly, were isolated as random insertions, not as *mod(r)* mutations. Thus, the selection for the *mod(r)* mutant phenotype is not responsible for the clustering of the P-element insertions. These data show that there is a hot spot for P-element insertion in the first exon of *mod(r)*.

References: Begley *et al.*, 1995, Molecular and General Genetics 248: 69-78.

A hot spot for P-element insertion in the *enhancer of rudimentary* gene.

Close, Dan, and Stuart I. Tsubota. Department of Biology, Saint Louis University, St. Louis, MO 63103; tsubota@slu.edu

The *enhancer of rudimentary* gene encodes a small 104-amino-acid protein of unknown function. The original mutant allele of *e(r)*, $e(r)^{p1}$, was isolated in a hybrid-dysgenesis screen as an enhancer of the mutant wing phenotype of a weak *rudimentary* mutation (Wojcik *et al.*, 1994). This mutation is caused by the insertion of a 1.1-kb P element in the first exon, upstream of the start of translation.

tttcgattattttgtcacggcgaggcacgcagcgaagcgctc**a**tctctagcttcagcctcctcttca
aaacccatagaactggcaaaataaaaaaaaacgaatcaagcgctgattttaccgaatctctttactg
aatcagcatattatcata**GTCCATAT**agtccggcgttcgaacgggtaagttgaacactaaacgggag
acatgggaattatttaaaattcgagaaaaatcggtgaaaaggaagaaaaacaagtgattgcatacag
ttagttacacacacgcggcggccgttactccttgtacatgtgtgtgcgtgtctgttcgcgcttccga
atgtttgtgttttagtgaagcggcataagcgtgcattatcttgtacaaatgtacgcatttctccatt
gccaacattcgcttcacacacaaattactcgttttccatgctggaagtgccatcttagtctatttcc
cagttgcaaattggaaaatcaaagagcgttctgctggccagcatgtgtggtgcgcgtgtattggtgt
tgcggcgagagcgcgagcgagatggggcgatgcgttgcatgcggcaacagcatatctcttccatctc
tttcgtttttgttcttttcctttttttttgtctttttttttttgctgacagctctctatgcttct
aataaatatttatgcacaatttttgcagagctggcgaagtaaacttcaacttcgagagtctcggaat
ctttcggactttgctatagaaaaaacgcttgttaaaaaaaaggatgtaagtgatgaagatgaaacgt
aatgtaatgaaacgcacactagtaacatacatacatacatatcgcttgagtacatagatcaccgcca
ataacattaatgttccatttttgccttgcagatcacc**ATG**tcgcacaccatcctattggtacagccg
ggtgctcgtccggagacccgcacttactgtgactacgagagcgtcaacgagtgcatggagggcgtgt
gcaagatctacgaggagca

Figure 1. Insert site of three P elements in *e(r)*. The start of transcription is underlined and the start of translation (ATG) is in upper case letters. The 8-bp site duplication of the three insertions is underlined and in upper case letter.

We have isolated two new hybrid-dysgenesis-induced mutations of *e(r)* as enhancers of the mutant wing phenotype of $r^{hd1\text{-}12}$. One, $e(r)^{p2}$, is an insertion of a 1.1-kb P element, whereas the other, $e(r)^{p3}$, is an insertion of a 0.7-kb P element. The position of each insertion was determined by DNA sequencing and found to be identical to that of $e(r)^{p1}$. The position of the three insertions is shown in

Figure 1. Although $e(r)^{p1}$ and $e(r)^{p2}$ are caused by the insertion of similarly sized P elements, the two insertions are in opposite orientation. The P element in $e(r)^{p1}$ is oriented in the same orientation as the *e(r)* gene, while the P element in $e(r)^{p2}$ is in the opposite orientation. The P element in $e(r)^{p3}$ is also in the opposite orientation with respect to *e(r)*. All three mutations result in similar phenotypes. In an r^{+} background, they look wild-type and have normal viability and fertility. In the background of a weak rudimentary allele such as $r^{hd1\text{-}12}$, they show the enhanced wing truncation that was previously reported (Wojcik *et al.*, 1994).

References: Wojcik *et al.*, 1994, Genetics 138: 1163-1170.

Drosophilid fauna of Mukteshwar in Kumaon region of India.

Joshi, Mukta, B.K. Singh, and R.S. Fartyal. Cytogenetics Laboratory, Department of Zoology, Kumaon University, Nainital-263002, India.

Introduction

It is now more than two decades since the Drosophilid survey of Kumaon region was started with a view to furnish a complete picture of Drosophilid species of this region. So far, more than 73 species are known from this region, out of which 30 species belonging to different genera of the family Drosophilidae were described as new to science and 20 species were recorded for the first time from India (Singh and Bhatt, 1988; Singh and Negi, 1989, 1992, 1995; Singh, Dash, and Fartyal, 2000; Singh and Fartyal, 2002).

Though studies associated with the geographic distribution of Drosophilidae in India have taken rapid strides in last few years, yet only a cursory survey has been undertaken in certain areas, whereas a vast area of the Indian subcontinent still awaits exploration. In view of the above situation, the Drosophilid fauna of Mukteshwar in Kumaon region, which is a completely virgin field, was explored. The Mukteshwar in Kumaon region is a hilly area located at an elevation of about 2250 meters (7,500 feet) from the sea level on the northeast periphery of the state of Uttaranchal. It includes six border districts of the state viz., Nainital, Almora, Pithoragarh, Udham Singh Nagar, Champawat, and Bageshwar. The area is characterized by having dense evergreen vegetation consisting of broad leaved and conifer species, such as *Quercus floribunda, Quercus leucotrichopora, Quercus lanuginosa, Rhododendron arboretum, Litsea umbrosa, Euonymus pendulus, Machilus duthiei, Cedrus deodara, Pinus wallichiana, Pinua roxburghii, Myrica esculenta, Pyrus pashia etc.* Depending on climatic variations, the year is broadly divisible into (1) a dry and warm summer season (March to mid June), (2) a wet and warm rainy season (mid June to mid September), and (3) a dry and cold winter season (mid September to February). In winters frost formation is severe and snowfall occurs.

The rocks exposed around Mukteshwar area are chlorites, phyllonites, garnetiferous, mica schists and very coarse to fine grained augen gneisses, rich in sericite, biotite and chlorite. According to Valdiya (1980) the rocks exposed at Mukteshwar are quartzites and genisses of the Almora Group. They belong to the lover amphibolite facies of regional metamorphism and are emplaced by plutonic bodies of granodiorites and granites.

Table 1. The genus, subgenus, species and collection localities of the Drosophilid flies collected from Mukteshwar in Kumaon region of India.

Genus / Subgenus	Species	Collection locality
Genus- *Amiota* Loew	1. *bandes* Singh and Negi, 1992	Sargakhet
	2. *biprotrusa* Chen and Toda, 1998	Gahna
Subgenus- *Phortica* Schiner	3. *pseudotau* Toda and Peng, 1990 (new record)	Mukteshwar
Genus- *Dettopsomyia* Lamb	4. *nigrovittata* (Malloch, 1924)	Latoli
Genus- *Drosophila* Fallen	5. *analspina* Singh and Negi, 1995	Letibuga
Subgenus- *Drosophila* Sturtevant	6. *bishtii* Singh and Negi, 1995	Sargakhet
	7. *bizonata* Kikkawa and Peng, 1938	Mukteshwar
	8. *immigrans* Sturtevant, 1921	Sheetla
	9. *lacertosa* Okada, 1956	Dhari
	10. *mukteshwarensis* (new species)	Mukteshwar
	11. *nainitalensis* Singh and Bhatt, 1988	Ganguachaur
	12. *notostriata* Okada, 1966	Kashialekh
	13. *painai* Singh and Negi, 1995	Bhatelia
	14. *parazonata* Dwivedi and Gupta, 1980	Sargakhet
	15. *repleta* Wollaston, 1858	Latoli
	16. *sulfurigaster* Duda, 1923	Letibuga
	17. *trizonata* Okada, 1966	Dhari
Subgenus- *Sophophora* Sturtevant	18. *bifasciata* Pomini, 1940	Sheetla
	19. *hubiensis* Sperlich and Watabe, 1997 (new record)	Sargakhet
	20. *jumbulina* Parshad and Paika, 1964	Dhari
	21. *melanogaster* Meigen, 1830	Gahna
	22. *kikkawai* Burla, 1954	Sheetla
	23. *nepalensis* Okada, 1955	Bhatelia
	24. *sarswati* Singh and Dash, 1995	Mukteshwar
	25. *sargakhetensis* (new species)	Sargakhet
	26. *suzukii indicus* Parshad and Paika, 1964	Latoli
	27. *takahashii* Sturtevant, 1927	Bhatelia
Genus- *Gitona* Meigen	28. *distigma* Meigen, 1830	Dhari
Genus- *Hirtodrosophila* Duda	29. *hexaspina* Fartyal and Singh, 2000	Letibuga
	30. *quadrivittata* Okada, 1956	Sheetla
Genus- *Leucophenga* Mik	31. *albiceps* de Meijere, 1914	Ganguachaur
	32. *angulata* Singh, Dash and Fartyal, 2000	Gahna
	33. *angusta* Okada, 1956	Sargakhet
	34. *bellula* (Bergroth, 1894)	Mukteshwar
	35. *clubiata* Singh, Dash and Fartyal, 2000	Dhari
	36. *neolacteusa* Singh and Bhatt, 1988	Latoli
	37. *ornata* Wheeler, 1959	Kashialekh
	38. *subpollinosa* (de Meijere, 1914)	Sheetla
Genus- *Lissocephala* Malloch	39. *parasiatica* Takada and Momma, 1975	Mukteshwar
Genus- *Paraleucophenga* Hendel	40. *neojavanaii* Singh and Negi, 1992	Dhari
Genus- *Scaptomyza* Hardy	41. *elmoi* Takada, 1970	Latoli
	42. *quadruangulata* Singh and Dash, 1993	Letibuga
Genus- *Scaptodrosophila* Duda	43. *actinia* Okada, 1991	Dhari
	44. *coracina* Kikkawa and Peng, 1938	Bhatelia
	45. *hirsuata* Singh and Dash, 1998	Sargakhet
Genus- *Zaprionus* Coquillett	46. *indianus* Gupta, 1970	Mukteshwar

Materials and Methods

The materials for the present study were collected from Mukteshwar area in Kumaon region viz., Sargakhet, Gahna, Bhatelia, Letibuga, Sheetla, Latoli, Ganguachaur, Kashialekh, Dhari, and Dhanachauli. Following two methods were used to collect Drosophilid flies, (1) Trap-bait-method, and (2) By net-sweeping.

The collected flies were etherized, categorized and species were identified under Wild-Leitz Stereozoom Microscope. The males were studied as such but the individual females, which could not be identified, were isolated and allowed to breed in separate vials containing standard laboratory food medium. The progeny obtained from such single gravid females were used for species identification.

Observations

Altogether 46 species representing 11 genera of the family Drosophilidae were collected from July 1999 to July 2002. Out of 46 species, 2 species are described as new to science, 2 species are recorded for the first time from India, and 2 species are recorded for the first time from Kumaon region. Table 1 shows the genus, subgenus, species and collection localities of the Drosophilid flies collected from Mukteshwar in Kumaon region in India.

Drosophila virilis miniature-like m^{42} mutant. Unusual wing vein microstructure.

Gubenko, I.S.[1], R.P. Subbota[1], I.A. Kozeretska[2], and S.N. Gorb[3,4]. [1]Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kyiv 03143, Ukraine; [2]Department of General and Molecular Genetics, Biological Faculty, Taras Shevchenko Kyiv University, Ukraine; [3]Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Chmelnicky str. 15, Kyiv, 01030 Ukraine; [4]Evolutionary Biomaterials Group, Max-Planck-Institut fur Metallforschung, Heisenbergstrasse 3, D-70569 Stuttgart, Germany; S.Gorb@mf.mpg.de

A large number of genes, genetic interactions, and several different signaling pathways regulate strictly specific gene expression pattern including individual and general wing vein development programs in *Drosophila*.

The *miniature*-like *D. virilis* m^{42} mutant associated with multiple defects of wing vein and wing blade (Figure 1) has recently been isolated from a dysgenic cross progeny of the strain 9 females (wild type Batumi population) and the strain 160 males [all autosomes marked with recessive mutations: *broken* (*b*: 2-188.0), *gap L2* (*gp*: 3-118.5), *cardinal* (*cd*: 4-32.5), *peach* (*pe*: 5-203.0), and *glossy* (*gl*: 6-1.0)]. *D. virilis* m^{42} mutation was genetically mapped by recombination with *Beadex* (*Bx*: 1-94.5) and *white* (*w*: 1-105) to a proximal end region of the X chromosome. Hence, more accurate chromosome localization of m^{42} is still not clear. Cytological analysis of salivary gland chromosomes did not reveal obvious chromosome aberrations in heterozygous m^{42}/+ females.

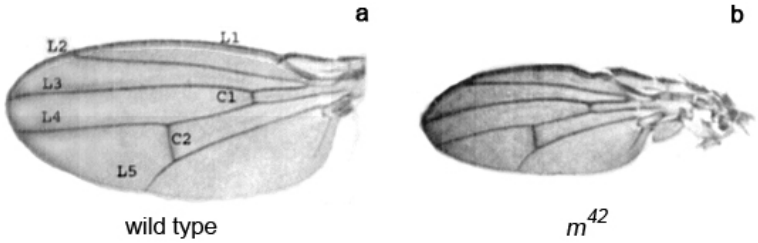

Figure 1. Wings of wild type (**a**) and m^{42} mutant (**b**) flies.

D. virilis m^{42} mutant with its phenotype features is similar to *miniature* alleles of the complex *miniature-dusky* locus in *D. melanogaster* that are known for a long time and influence the morphology of epidermal wing cells (Dobzhanzky, 1929; Slatis and Willerment, 1954; Dorn and Burdick, 1962; Green, 1975; Newby *et al*., 1991; Jackson and

Newby, 1993; Newby and Jackson, 1995). As it has been recently shown (Di Bartalomeis *et al*., 2002; Akten *et al*., 2002; Roch *et al*., 2003), this complex locus of *D. melanogaster* encodes the transmembrane proteins containing an extracellular ZP (zona pellucida) domain, a motif common to the large family of cuticulin-related apical matrix products in vertebrates and invertebrates (Sebatiano *et al*., 1991; Holt *et al*., 2002); it is specifically involved in the interactions between apical membrane, cytoskeleton and the cuticle being formed.

In *D. melanogaster*, the *miniature* mutations are well known to lead to the extreme induction in wing dimension including small wing phenotype due to decreased size/shape of epidermal cells; increased cell density in mutant smaller wings; kept cell boundaries (outlines) in adult *miniature* wings (at the same time, the epidermal wing cells are completely degenerated after emergence from pupal case in adult wild type flies). The regular proximal to distal orientation of wing hairs is sometimes changed in mutant wings, but the hairs on the apical wing surface formed by mutant and wild type flies are almost of the same morphology.

We have obtained similar results and conclusions during a more extensive analysis of m^{42} mutant in *D. virilis*. Moreover, both light and phase contrast microscopy permit one to see the most remarkable, having not yet described, feature of the *D. virilis* m^{42} mutant phenotype – the presence of unusual microstructure elements in the wing veins (Figure 2, b,d); these structures being totally absent in the wild type wings (Figure 2, a,c).

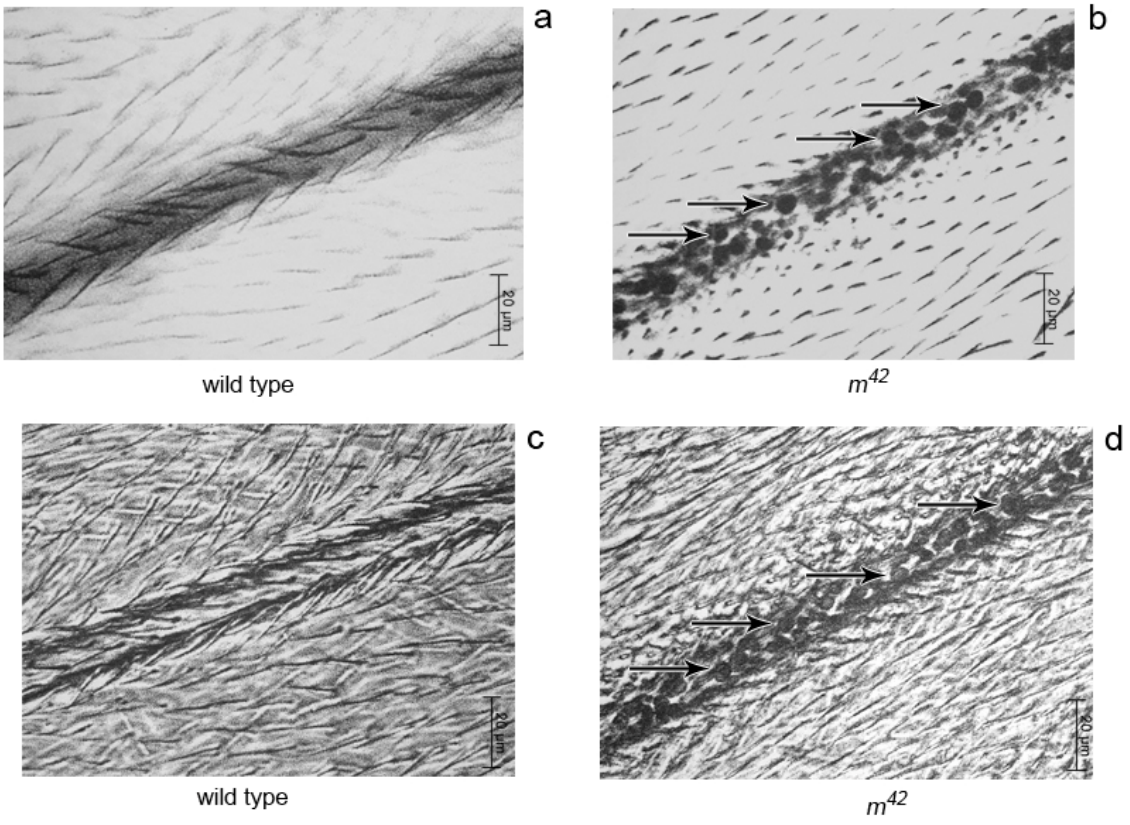

Figure 2. Wing vein microstructure of wild type (**a,c**) and m^{42} mutant (**b,d**) flies. Light (**a,b**) and phase contrast (**c,d**) microscopy. Several neomorphic vein structures (NVS) are shown by arrows.

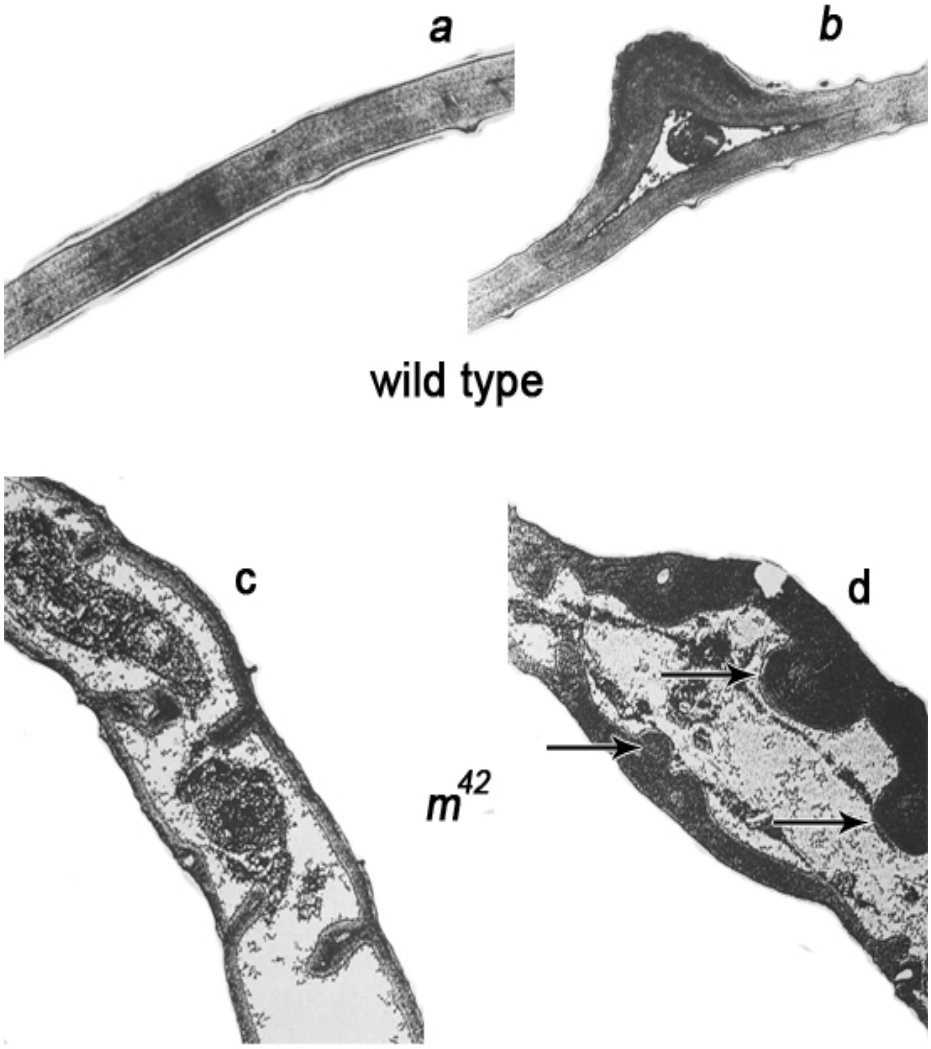

Figure 3. Cytoskeleton reorganization during wing morphogenesis in both wild type (**a,b**) and m^{42} mutant (**c,d**) flies in intervein (**a,c**) and vein (**b,d**) regions. Transmission electron microscopy. Cuticle "bulges" are shown by arrows (**b**).

Characteristic m^{42} mutant numerous **neomorphic vein structures** (NVS) are 2-8 μm wide and usually round (rarely they are oval or irregular). The longitudinal veins, especially the L3 and L5, are very abundant in NVS, and few NVS only are found in C1 and C2 cross veins. Such NVS are visible constantly inside the wing veins of the homozygous females and hemizygous males. Like other m^{42} recessive markers, the NVS presence is inherited as a fully penetrant one. In order to understand the nature of the NVS development we used transmission electron microscopy (TEM) to analyze transverse ultrathin wing blade sections of m^{42} and wild type flies. The formation of cuticulin envelope is a common feature of all arthropod cuticles. In wild type epidermal wing cells the apoptosis undergoes, the dorsal and ventral cuticle surfaces formed independently are closely apposed by their basal sides (Figure 3, a,b). At the same time dorsal and ventral parts of cuticulin envelope are separated in the m^{42} mutant wings (Figure 3, c,d): cuticular material is present inside the wing preventing the apposition of dorsal and ventral layers. These cuticular elements of intervein regions (Figure 3c) appear to be the cellular fragments of degenerated epidermal cells between two cuticle components. In contrast, wild type wings are cleaned from cell debris (Figure 3a). Very thickened dorsal and ventral cuticle layers of m^{42} mutant become more marked in certain sites of wing vein regions (Figure 3d). These characteristic cuticle "bulges" inside the veins are seen (that is perfectly obvious!) in the wing vein (Figure 2, b,d) by light and phase contrast microscopy. Thus, NVS are presented not as some separate cuticle

elements, but as characteristic "bulges" of both dorsal and ventral uninterrupted cuticulin envelope layers in shortened m^{42} mutant wings.

Taking into consideration the wing vein role in the development of the wing skeleton (Biehs *et al*., 1998; de Celis, 1998), m^{42} allele is suggested to be a possible component of the mechanism aiding the epidermal cells to control the properties of the cuticle secreted by them.

References: Akten, B., J. Suh, G. Genova, M.A. Roberts, and F.R. Jackson 2002, Genesis 34: 156-159; Biehs, B., M.A. Sturtevant, and E. Bier 1998, Development 125: 4245-4257; de Celis, J.F., 1998, Inter. J. Dev. Biol. 42: 335-343; Di Bartolomeis, S.M., B. Akten, G. Genova, M.A. Roberts, and F.R. Jackson 2002, Mol. Genet. Genomics 267: 564-576; Dobzhansky, Th. 1929, Arch. Entwicklungsmech. Org. 115: 363-379; Dorn G.L, and A.B. Burdick 1962, Genetics 47: 503-518; Green, M.M., 1975, Mut. Res. 29: 79-84; Holt, R.A., G.M. Subramanian, A. Halpern, G.G. Sutton, R. Charlab, D.R. Nusskern, P. Wincker, and A.G. Clark 2002, Science 295: 129-149; Jackson, F.R., and L.M. Newby 1993, Comp. Biochem. Physiol. A. Comp. Physiol. 104(4): 749-756; Newby, L.M., L. White, S.M. DiBartolomeis, B.J. Walker, H.B. Dowse, J.M. Ringo, N. Khuda, and F.R. Jackson 1991, Genetics 128: 571-582; Newby, L.M., and F.R. Jackson 1995, Dev. Genet. 16(1): 85-93; Roch, F., C.R. Alonso, and M. Akam 2003, J. Cell Sci. 116: 1199-1207; Sebastiano, M., F. Lassandro, and P. Bazzicalupo 1991, Dev. Biol. 146: 519-530; Slatis, H.M., and D.A. Willermet 1954, Genetics 39: 45-58.

A new geographical record for *Drosophila desertorum* and a review of the *D. ritae* cluster in the repleta group of *Drosophila*.

Etges, William J.,[1] and William B. Heed.[2] [1]Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701; [2]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson AZ 85701. wetges@uark.edu; WHeed@email.arizona.edu .

The *D. repleta* group is currently comprised of ca 100 species endemic to North and South America. Several of these species are poorly known, sometimes described from only a single specimen or individuals from a single collection (Wasserman 1992). One such species, *D. desertorum*, was described from a collection made in 1958 from northeast of Pachuca, Hidalgo, Mexico, and a second collection made at San Pedro Mines, Hidalgo, ca 120 km north of Mexico City (Wasserman, 1962; Vilela, 1983). Based on its polytene chromosome similarities, Wasserman (1992) included this species in the *ritae* cluster, comprised of *D. ritae*, *D. mathisi*, and *D. desertorum*, within the *D. mulleri* complex. Another related species, *D. brevicarinata*, was described by Patterson and Wheeler (1942) from a single collection. It was described as morphologically similar to *D. ritae*, including the latter's metaphase chromosomes, but the type specimen was lost and no record of its genitalia is known (Vilela, 1983). Thus its relation to the *D. ritae* cluster cannot be re-evaluated here. The purpose of this report is to describe a new geographical location for *D. desertorum* and to untangle the species and geographical relationships of members of the *ritae* cluster given this unforeseen new location for *D. desertorum*.

On March 14-15 2004, eight *D. desertorum* males were collected over banana baits in the Chisos Basin group camping area at 5100 ft elevation in Big Bend National Park, Texas. This site is ca 1100 km northwest from the Hidalgo site from where the species was first described (op. cit. above). The most common species were *D. longicornis* (207 adults) and *D. pseudoobscura* (134

adults), along with a few *D. hydei*. Species identifications were made by dissecting the aedeagus from several males upon return to the laboratory and comparing morphologies to the diagrams in Vilela (1983). The invagination of the tip of the aedeagus and the two pairs of "spurs" or posterior projections are distinctive enough to correctly identify live male *D. desertorum* (males under CO_2 anesthesia will sometimes evert their aedeagus allowing species identification). Overall differences from *D. longicornis* include longer wings, longer legs, lemon yellow testes, and an overall brownish background body color. None of these latter characters are, however, as diagnostic as the morphology of male genitalia.

Prior to this time, only the other two members of the ritae cluster were thought to exist north of Mexico. *D. ritae* was first recorded in Texas (including the Chisos Mountains), New Mexico, and into southern Arizona (Patterson and Wheeler, 1942). Subsequently, *D. ritae* was found in Chihuahua, Coahuila, Durango, Nuevo Leon, and throughout southern Mexico in Guanajuato, San Luis Potosi (Etges, unpubl. data), Mexico City, Jalisco, Michoacan, Puebla, and Oaxaca (Patterson and Mainland, 1944). It is also chromosomally quite polymorphic for gene arrangements (Wasserman, 1992). Wasserman (1962) also described a new, closely related species from Mexico City, *D. tira*. However, Vilela (in Wasserman, 1992) found that the aedeagus of *D. tira* was indistinguishable from that of *D. ritae*, and thus *D. tira* was synonomized with *D. ritae*, and the name "*tira*" became invalid. Apparently, the reason Wasserman (1962, 1967) decided that *D. tira* was a new species was due to the particular strain of "*D. ritae*" (A 6.4 from Patagonia, Arizona) he used in laboratory isolation tests with "*D. tira*". Crosses of the new "*D. tira"* with this strain of "*D. ritae*" produced no offspring and differed in gene arrangements. This strain (A 6.4) of "*D. ritae*" was then recognized as a cryptic undescribed species, "from Arizona" (Wasserman, 1982) that was subsequently named *D. mathisi* (Vilela, 1983). This species has been subsequently found in much of southern Arizona and New Mexico and is cytologically monomorphic. A form with a *mathisi*-like aedeagus has also been reported from Merida, Venezuela, but little more is known about these latter flies (Wasserman, 1992).

Both allozyme and DNA-based analyses suggest a weaker affiliation between Mexican *D. desertorum* and other members of the *D. ritae* cluster. For several enzyme loci, *D. desertorum* shares identical allozymes with *D. longicornis* cluster species (*D. pachuca, D. propachuca*, and *D. longicornis*) as opposed to *D. ritae* (Richardson and Smouse, 1976). Members of the *D. ritae* and *D. longicornis* clusters were grouped together, i.e. neither group was monophyletic, based on a phylogenetic analysis including mitochondrial and nuclear DNA sequences combined with inversion data (Durando *et al*., 2000). Possible explanations for these conflicts include possible convergence for allozyme function due to shared patterns of host cactus use (Richardson and Smouse, 1976; Richardson *et al*., 1977) and/or incomplete lineage sorting at the DNA sequence level (O'Grady *et al*., 2002; Oliveira *et al*., 2003). Therefore, the existence of the *ritae* and *longicornis* clusters may be uncertain, but further scrutiny of the evolutionary relationships of these species is required.

The discovery of *D. desertorum* in the Chisos Basin of Big Bend National Park represents a significant range extension for this species assuming that collections made there in the 1940s would have included this species if it had been present. There are few recent records of *Drosophila* collections made in this region of Texas or the Sierra Madre Occidental in Mexico. Perhaps the range of *D. desertorum* is much larger than previously thought simply because it has been encountered so rarely in nature. All three of the *D. ritae* "cluster" species are now known from in and around southern Arizona, New Mexico, and southwest Texas, although only *D. ritae* and *D. mathisi* are known to be sympatric there. *Drosophila ritae* is the most widespread species, occurring from these southwestern states into southern Mexico. Given the protection afforded by the national park, we hope *D. desertorum* will persist there and further collections can be made so we can

determine the degree of genetic differentiation and reproductive isolation from the Mexican population of *D. desertorum*, as well as from *D. ritae* and *D. mathisi*.

Acknowledgments: We thank Marvin Wasserman for helping us to understand the history of the *D. ritae* cluster, Marshall R. Wheeler for valuable background information and editorial advice, the National Park Service for issuing a collecting permit, and the Department of Biological Sciences at the University of Arkansas, Fayetteville, for providing transportation.

References: Durando, C.M., R.H. Baker, W.J. Etges, W.B. Heed, M. Wasserman, and R. DeSalle 2000, Mol. Phyl. and Evol. 16: 296-307; O'Grady, P.M., C.M. Durando, W.B. Heed, M. Wasserman, W.J. Etges, and R. DeSalle 2002, Hereditas 136: 240-246; Oliveira, D.C.S.G., E.H. Wintermute, W.J. Etges, W.B. Heed, P.M. O'Grady, and R. DeSalle 2003, Hereditas 139: 223–227; Patterson, J.T., and G.B. Mainland 1944, Univ. Tex. Publ. 4445: 9-101; Patterson, J.T., and M.R. Wheeler 1942, Univ. Tex. Publ. 4213: 67-109; Richardson, R.H., and P.E. Smouse 1976, Biochem. Genet. 14: 447-465; Richardson, R.H., P.E. Smouse, and M.E. Richardson 1977, Genetics 85: 141-154; Vilela, C.R., 1983. Rev. Bras. Entomol. 27:1-114; Wasserman, M., 1962, Univ. Tex. Publ. 6205: 85-117; Wasserman, M., 1967, Dros. Inf. Serv. 42: 67-68; Wasserman, M., 1982, Evolution of the *repleta* group. In: *The Genetics and Biology of* Drosophila. (Ashburner, M., H.L. Carson and J.N. Thompson, jr., eds.), pp. 61-139. Academic Press, New York; Wasserman, M., 1992, Cytological evolution of the *Drosophila repleta* species group. In: Drosophila *Inversion Polymorphism*. (Krimbas, C.B., and J.R. Powell, eds.), pp. 455-552, CRC Press, Boca Raton.

First record of *Zaprionus indianus* (Diptera: Drosophilidae) in the state of Espírito Santo, Brazil.

Culik, Mark P. Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural – INCAPER, Rua Afonso Sarlo 160, CEP 29052-010, Vitória, Espírito Santo, Brasil; E-mail: markculik3@yahoo.com

Zaprionus indianus Gupta, 1970 (Diptera: Drosophilidae), probably of African origin, is a potentially important pest of fruits that has recently been found in South America, where it was first recorded in São Paulo, Brazil, in 1999 (Vilela, 1999). Subsequently it has been recorded in the Brazilian states of Goiás, Minas Gerais, Rio Grande do Sul, Santa Catarina, and Tocantins (Castro and Valente, 2001; De Toni *et al*., 2001; Marchiori, 2003; Marchiori and Silva, 2003; Mata *et al*., 2004), as well as other parts of South America (Goni *et al*., 2001). This note reports the presence of *Z. indianus* in the state of Espírito Santo, Brazil, where it was collected in September 2004, associated with ripe papaya which was used as a bait to attract flies present in the area of the collection site.

Because of the importance of *Z. indianus* as a potential pest (Vilela *et al*., 2001), flies attracted to ripe papaya in a home garden located in Manguinhos, municipality of Serra, Espírito Santo, were collected during August and September 2004, to determine if *Z. indianus* was present in this region. Initial efforts to collect *Z. indianus* in traps such as those described by Marchiori and Silva (2003) were not successful although specimens that appeared to be this species were observed near ripe, rotting papaya fruit. In September 2004, ripe papaya fruit that had been cut open was placed on a bench (height ~0.5m) next to a mature papaya plant. After ~1 hour, flies attracted to the fruit were captured by quickly placing a plastic bag over the fruit and associated flies. In this way, 5 specimens of *Z. indianus* were collected using ripe papaya fruit as a bait in Manguinhos (20º11'34"S

40º12'34"W, altitude ~2 m), Serra, Espírito Santo, Brazil, on 12 September 2004. One live specimen was cultured with banana and produced ~30 progeny over a period of several weeks. Voucher specimens of the flies collected are deposited in the collection of INCAPER. The fact that the species was captured with little effort at a relatively small, isolated town in Espírito Santo suggests that the fly is common and is likely to be more widely distributed in the state.

Acknowledgment: I thank J.A. Ventura, INCAPER, for reviewing this note, J.S. Döge and V.L.S. Valente, UFRGS, for confirming the identification of the specimens of *Zaprionus indianus* collected, and C.H. Marchiori, ULBRA, and C.R. Vilela, USP, for additional information regarding the species.

References: Castro, F.L., and V.L.S. Valente 2001, Dros. Inf. Serv. 84: 15-17; De Toni, D.C., P.R.P. Hofmann, and V.L.S. Valente 2001, Biotemas 14(1): 71-85; Goni, B., P. Fresia, M. Calvino, M.J. Ferreiro, V.L.S. Valente, and L. B. da Silva 2001, Dros. Inf. Serv. 84: 61-65; Marchiori, C.H., 2003, Biotemas 16(1): 129-133; Marchiori, C.H., and C.G. Silva 2003, Braz. J. Biol. 63: 361-362; Mata, R.M., M.F. Kanegae, and R. Tidon 2004, In: XXV Congresso Brasileiro de Zoologia: Resumos (R. Constantino, ed.). p 106. Soc. Brasil. Zool., Brasília, available at <http://www.unb.br/ib/zoo/CBZ/resumos/Insecta.pdf>, consulted 22 Oct 2004; Vilela, C.R., E.P. Teixeira, and C.P. Stein 2001, In: Histórico e impacto das pragas introduzidas no Brasil (Vilela, E.F., R.A. Zucchi, and F. Cantor, eds.). pp 48-52. Holos, Ribeirao Preto; Vilela, C.R., 1999, Dros. Inf. Serv. 82:3 7-39.

A comparative study of mating behavior and fertility in two wild types and two mutants of *Drosophila nasuta nasuta.*

Chayakumari and S.R. Ramesh. Drosophila Stock Center, Department of Studies in Zoology, University of Mysore, Mysore-570 006, India.

Mating behavior of *Drosophila* consists of specific actions, which are accompanied by orientation movements. These actions referred to as courtship displays are made up of several signals, which are performed sequentially. Mating occurs only if the female perceives these signals and reciprocates by exhibiting acceptance signals. In this way, mating behavior forms an important component, which enables a species to maintain its genetic integrity. Several investigations in *Drosophila* have clearly shown that sexual behavior is under genetic control (Spieth, 1983). Different components of sexual behavior have been analyzed in certain species of *Drosophila* (*c.f.*, Sisodia and Singh, 1996). Further, the impacts of various mutations on selective mating and mating propensity have been analyzed in different species of *Drosophila* (*c.f.*, Chatterjee and Singh, 1987). Though investigations on some fitness parameters in wild type and mutant strains of *D. n. nasuta* and *D. n. albomicans* have been made (Ashadevi and Ramesh, 2000; Ashadevi, 2001), different components of mating behavior have not so far been analyzed. In view of this, present investigations were undertaken to understand the degree of divergence in three components of prezygotic reproductive isolation, namely the courtship latency, courtship duration, copulation duration and postmating fitness (fertility) among wild type and mutant strains of *D. n. nasuta*.

For the present investigations we have employed two wild type strains namely Coorg (India; Stock no.201.001), Sy-I (Seychelles Island, Stock No. 201.006) and two mutant strains *viz*., *brown* and *Curly*. These stocks were obtained from Drosophila Stock Center, University of Mysore, Mysore, India. Uniformity was maintained with regard to temperature, space, amount of food,

moisture and the larval population density in raising the populations that are used in the present analysis. Fifty synchronized eggs placed in fresh half-pint-milk bottles containing wheat cream agar medium were raised at 22 ± 1°C. From these cultures, virgin females and unmated males were isolated within 3 hr of their eclosion from the pupal case. After aging for 7 days, these flies were used for the mating experiments. A pair of flies (male and female of the same strain) was aspirated into the mating chamber to avoid etherization before the experiment. Mating components such as courtship latency (the time required to initiate courtship after the male and female are placed together), courtship duration (the time elapsed between the beginning of the first courtship bout and copulation, irrespective of breaks in courtship) and copulation duration (from initiation to the termination of copulation) were documented by direct observation. All pair matings were conducted during morning hours (7-11 am) when the ambient temperature ranged between 22-24°C. To determine fertility each mated female was placed in a separate culture vial for a period of 3 days, which was then transferred to fresh food vials every third day. After giving three successive changes, the total number of flies that emerged from each vial was counted and the mean number of offspring produced per female was calculated. Thirty such replicates were set up for each cross and the data were subjected to ANOVA followed by DMRT to determine the level of significance. Further, correlation co-efficient was calculated to understand the relationship between various parameters analyzed.

Fertility is one of the post mating fitness components. Parsons (1973) has suggested that the individual completing mating and copulation rapidly would most readily leave genes in subsequent generation and thus there is some selective advantage in completing mating and copulation rapidly. The duration of copulation is determined by the male in various species of *Drosophila* and is an expression of rate of sperm transfer (MacBean and Parsons, 1967). Sisodia and Singh (1996) have shown that males copulating for longer durations produce more progeny, which is likely due to higher sperm transfer and a species-specific character. Thus there is direct influence of the variation in prezygotic reproductive isolating parameters of mating behavior on post mating fitness (fertility).

Table 1. Components of mating behavior and fitness in four strains of *D. nasuta nasuta*.

Strain	Courtship latency (min)	Courtship duration (min)	Copulation duration (min)	Fertility (No. of progeny/female)
Coorg	11.13 ± 2.25(a)	21.53 ± 3.43(a)	16.83 ± 0.37(b)	136.87 ± 4.52(a)
Sy-I	8.2 ± 2.15(a)	31.53 ± 4.63(a)	18.83 ± 0.34(a)	119.77 ± 4.99(b)
bw/bw	6.17 ± 1.63(a)	26.17 ± 5.14(a)	18.23 ± 0.28(a)	176.8 ± 5.81(c)
Cy/Cy	23.4 ± 4.72(b)	46.83 ± 6.12(b)	18.36 ± 0.44(a)	98.56 ± 6.07(d)
F-value	6.648	6.809	5.307	45.18

Note: Number of pairs tested: 30; df = (3.116).
The strains with the same letter in the parenthesis are not significantly different at 5% level according to DMRT.

Perusal of Table 1 reveals that in different strains analyzed, the mean duration of courtship latency varies from 6.17 min (*bw/bw*) to 23.4 min (*Cy/Cy*), mean courtship duration from 21.53 min (Coorg) to 46.83 min (*Cy/Cy*) and mean copulation duration from 16.83 min (Coorg) to 18.83 min (Sy-I) while fertility (mean number of progeny produced per female) varied from 98.56 (*Cy/Cy*) to 176.8 (*bw/bw*). Analysis of variance of the data revealed that significant variations exist with respect to different parameters. By the present study, it is evident that *Curly* has relatively long courtship

latency and courtship duration, while it has similar copulation duration when compared with other strains except Coorg strain. In spite of this, it is least fertile. These differences are statistically significant. Further, there is no correlation between courtship latency and courtship duration with fertility although Curly strain varies with respect to courtship latency and courtship duration. The wing vibration, scissoring, flicking or the waving actions of wings of males provide the information to the female (Hegde and Krishnamurthy, 1976). Wing is an important morphological structure, which provides necessary stimulus. Curly nature of the wing is believed to have drastic effect on the courtship act resulting in long courtship latency, courtship duration and least post mating fitness. Working with four members of *D. nasuta* subgroup, Spieth (1952) has remarked that the duration of copulation is relatively long when compared to many other species of *Drosophila.* The present study revealed that the mean copulation duration is long in all the analyzed strains except in Coorg strain, which has significantly shorter duration when compared with other strains. Further, there is no significant correlation between the components of mating behavior and fertility. Fulker (1966) has shown that *D. melanogaster* males, which mate more quickly, also copulate successfully and leave more progeny. Batabyal (1972) has reported reduced fitness in mutants stocks of *D. melanogaster*. Similarly, reduction in mating success (Singh *et al*., 1985) and activity of males (Chatterjee and Singh, 1987) has been reported in mutants of *D. ananassae*. Ashadevi and Ramesh (2000) have shown that brown of *D. n. nasuta* has higher viability and they have suggested that this mutant may dominate over the wild type populations under competitive conditions. Similar results of superiority in some fitness components in other mutants such as *sepia* and *crossveinless* of *D. n. nasuta* were also documented. The present investigation has revealed that (in spite of mutation) the brown eye mutant has high mating speed and fertility as compared with the other three strains. Thus, two more components of fitness *viz*., mating speed and fertility can be added to the list of superiorities of brown eye mutant of *D. n. nasuta*.

Acknowledgments: The authors thank the Chairman, Department of Studies in Zoology, for the facilities and University of Mysore for the award of fellowship (to Chayakumari).

References: Ashadevi, J.S., and S.R. Ramesh 2000, Genetica 109: 235-243; Batabyal, A.K., and N.S. Sidhu 1972, Dros. Inf. Serv. 48: 48-49; Fulker, D.W., 1966, Science 153: 203-205; Hay, D.A., 1976, Behav. Genet. 6: 161-170; Hegde, S.N., and N.B. Krishnamurthy 1976, Indian J. Exptl. Biol. 1: 52-55; Parsons, P.A., 1973, *Behavioral and Ecological Genetics. A Study in Drosophila* (Clarendon Press, Oxford); Mac Bean, I.T., and P.A. Parsons 1967, Genetics 56: 233-239; Singh, B.N., S. Chatterjee, and S. Roy 1985, Indian J. Exptl. Biol. 23: 661-662; Sisodia, S., and B.N. Singh 1996, Zool. Sci. 35: 25-29; Spieth, H.T., 1952, Bull. Amer. Natural Hist. 99: 395-474; Spieth, M.T., and J.M. Ringo 1983, *In*: *The Genetics and Biology of* Drosophila, Vol. 3c, (Ashburner, M., H.L. Carson, and J.N. Thompson, jr., eds.), pp. 223-284. Academic Press, New York.

Standing Orders

Several years ago, formal standing orders were discontinued due to the need to obtain prepayment for issues. "Standing Orders" are now handled through a mailing list of active subscribers. All individuals on the active subscriber list will receive notices for forthcoming regular and special issues and a Standing Order Invoice to facilitate prepayment. An email announcement is also sent annually to those on the FlyBase mailing list. If you would like to be added to the Drosophila Information Service mailing list, please write to the editor, Jim Thompson, Department of Zoology, University of Oklahoma, Norman, OK 73019.

Wing asymmetry and lifespan in recombinant inbred lines (RI) of *Drosophila melanogaster*.

Chismar, J[1]., N. Minois[2], A.A. Khazaeli[1], and J.W. Curtsinger[1]. [1]University of Minnesota, Department of Ecology Evolution and Behavior, St Paul, MN55108, USA; jwcurt@tc.umn.edu; [2]Max Planck Institute for Demographic Research, Konrad-Zuse Strasse 1, D-18057 Rostock, Germany; minois@demogr.mpg.de

Introduction

Morphological traits are often under study, especially in relation with fitness components, such as lifespan. The use of those morphological traits is chosen because such variables appear to be easy to measure, and they can be modulated by environmental and genetic factors. Furthermore, morphology is thought to be associated to the general quality of an organism: a bigger organism may be healthier, fitter and live longer than a smaller one, which could have encountered stressful conditions during its growth. However, studies did not report a clear relationship between morphology measured as body size or weight and fitness components. For instance, Zwaan *et al.* (1992) observed only 3 out of 24 significant correlations between wing length and lifespan in fruit flies raised at different temperatures. Nunney (1996) measured longevity and dry weight in selected *D. melanogaster* for fast larval development and observed that selection decreased dry weight but did not alter lifespan.

Asymmetry of bilateral traits can also be measured and correlated with lifespan. It is supposed that more asymmetrical individuals will have reduced lifespan, because it has been observed many times that asymmetry was bigger and lifespan reduced under stressful conditions (*e.g.*, Parsons, 1992). A more symmetrical individual is hypothesized to be of higher genetic quality and thus to have a better program to cope with aging and to live longer. For instance, Møller (1997) reported many studies where asymmetry was negatively correlated with lifespan. However, it does not seem completely general. For instance, Packer and Pusey (1993) found a negative association between vibrissae spots asymmetry and lifespan in male lion *Panthera leo* but a positive one in females. Van Dongen *et al.* (1999) did not observe a significant correlation between asymmetry and lifespan in the winter moth *Operaphtera brumata*. Here we report a study of wing asymmetry in recombinant inbred lines of *D. melanogaster* differing for their lifespan.

Material and Methods

D. melanogaster populations have been selected for age at reproduction and selected lines exhibit greater lifespan (Luckinbill and Clare, 1985). Selected and control lines were then inbred. Sixty recombinant inbred lines with different life spans were obtained by crossing one selected (IL6) and control (IS9) inbred line. Here we report the measurement of wing asymmetry for 40 recombinant inbred lines and the two parental lines.

Both wings of 10 individual male flies for each line were mounted on microscope slides. Pictures of those wings were captured in a computer using a fluorescence E800 Nikon microscope. The pictures were then analyzed using the software Scion Image (Version Beta 3b; NIH, USA). For each wing, three lengths were measured as depicted on Figure 1. The asymmetry for each wing was calculated as the absolute value of the ratio of the left wing over the right wing subtracted to 1 (ratio if the wings were perfectly symmetrical): $\left| 1 - (\text{Left}/\text{Right}) \right|$.

The lengths and the 3 measurements of asymmetry were subjected to an one-way ANOVA to study the effect of the genotype (difference between the lines). Individual correlations between the 3 lengths were calculated for each line. Finally, we also calculated the between lines correlations between the 3 measurements of asymmetry and lifespan.

Results

The ANOVAs run on the raw data of the size measurements showed that wing size, both on the length and the width, were very different between lines ($p \leq 0.0001$ in all cases; data not shown).

The analyses showed that asymmetry on measurements 1 and 2 were not different between lines (Figure 2). On the contrary, the asymmetry of measurement 3 was very highly different between lines ($F(41, 333) = 1.87$; $p = 0.002$).

The correlations between measurements 1, 2 and 3 for each lines were all positive and a vast majority of them were significant at the 0.1% level.

Finally, none of the correlations between asymmetry and lifespan were significant (measurement 1: $r = -0.14$; measurement 2: $r = -0.10$; and measurement 3: $r = -0.03$).

Discussion

Wing size does not seem to be a conserved feature in our lines, since all the measurements were very highly different between lines. However, although size itself differed between lines, the difference of size between the two wings of a individual, the asymmetry, was less subject to change. As a matter of fact, only one out of three measurements of asymmetry was different between the lines, showing that a tight regulation of developmental process occurs in those lines. Furthermore, the correlations between the length and the width of the wings were all positive; the longer is the wing, the wider is it; and almost all significant, expressing a highly conserved wing morphology in the lines. Note that the only different asymmetry measurement between lines was the asymmetry on the width of the wing, suggesting that the length of the wing is more tightly regulated than the width.

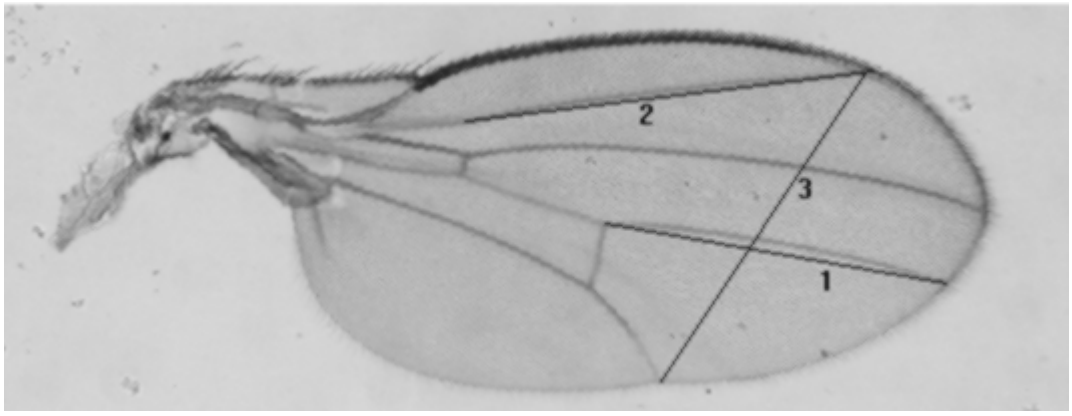

Figure 1. Measurements done on each wing.

Finally, we did not observe any relation between wing asymmetry and the lifespan of the flies. Although all negative, none of the three correlations were significant. It was expected that the correlations between asymmetry of measurements 1 and 2 (Figure 1) and lifespan would not be significant, since we observed no significant difference of the asymmetry of those two measurements

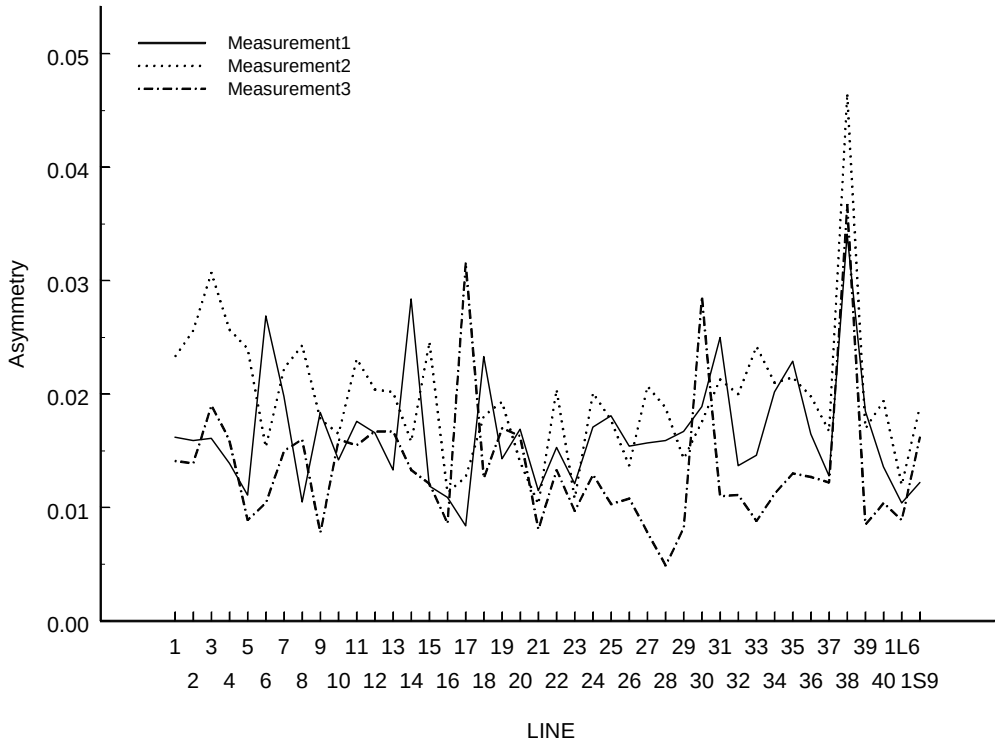

Figure 2. Mean asymmetry of the three measurements for each line.

between lines. On the contrary, the asymmetry on measurement 3 was very different between lines, but the difference did not follow the difference in lifespan.

At variance with the present data, many studies reported a negative relation between asymmetry and lifespan (see references in Møller, 1997). However, as stated by this author, the review of the literature can be biased because usually authors do not submit and publishers do not publish negative results. As a matter of fact, some other studies reported either positive (Suchentruk, 1993), sex-specific (Packer and Pusey, 1993) or no relation (Arcese, 1994; Van Dongen *et al.*, 1999) between asymmetry and longevity. It can be concluded that no clear relationship can be drawn between asymmetry and longevity. The present paper, gathering data of a lot more genotypes than usually studied, supports this lack of association between the two traits.

Acknowledgments: This work was supported by NIH grants AG08761 and AG11722 to J.W. Curtsinger.

References: Arcese, P., 1994, Anim. Behav. 48: 1485-1488; Luckinbill, L.S., and M.J. Clare 1985, Heredity 55: 9-18; Møller, A.P., 1997, Am. Natur. 149: 916-932; Nunney, L., 1996, Evolution 50: 1193-1204; Packer, C, and A.E. Pusey 1993, Nature 362: 595; Parsons, P.A., 1992 Heredity 68: 361-364; Suchentrunk, F., 1993, Acta Theriol. 38: 59-69; Van Dongen, S., E. Sprengers, C. Löfstedt, and E. Matthysen 1999, Behav. Ecol. 10: 659-665; Zwaan, B.J., R. Bijlsma, and R.F Hoekstra 1992, Heredity 68: 123-130.