Phenotypic analysis of *split ends* (*spen*) overexpression in *Drosophila.*

Muñoz-Descalzo, Silvia, Yaiza Belacortu, and Nuria Paricio*. Department of Genetics, University of Valencia, Doctor Moliner, 50, E-46100, Burjassot, Valencia, Spain; Author for correspondence (e-mail: nuria.paricio@uv.es).

Introduction

The *Drosophila melanogaster split ends* (*spen*) gene encodes a large nuclear protein containing three RNP-type RNA binding motifs and a SPOC domain (Spen Paralogous and Orthologous C-terminal) that may act as a conserved transcriptional co-repressor-interacting domain at the C-terminus (Wiellette *et al.*, 1999; Mace and Tugores, 2004). Genetic analyses have indicated that *spen* interacts with several pathways and may participate in several developmental processes. Thus, mutations in *spen* have been found to act as modifiers of mutations in components of the Raf pathway (Dickson *et al.*, 1996); of the Hox pathway, where Spen acts in parallel with the Hox pathway to repress the development of head-like sclerites in the *Drosophila* trunk (Gellon *et al.*, 1997; Wiellette *et al.*, 1999); and of the EGFR/Ras/MAPK pathway (Rebay *et al.*, 2000; Therrien *et al.*, 2000), where it acts as a positively acting component (Chen and Rebay, 2000). Moreover, mutations in *spen* have been also identified as enhancers of gain-of-function phenotypes caused by overexpression of E2F or Cyclin E in eye cells (Staehling-Hampton *et al.*, 1999; Lane *et al.*, 2000). Other studies have showed that overexpression of Spen may interfere with Notch signalling during the development of adult external sensory organs (Abdelilah-Seyfried *et al.*, 2000), and that *spen* function is required for the maternal expression of the Notch transcription factor encoded by *Suppressor of Hairless* (*Su(H)*) during neuronal cell fate and axon extension in the *Drosophila* embryo (Kuang *et al.*, 2000). Some evidences also suggest that *spen* may participate in the transduction of the Wingless (Wg) signal within a subset of cells in the wing imaginal disc (Lin *et al.*, 2003). In addition, it seems that *spen* is required for the maintenance of positional information during *Drosophila* development, including planar cell polarity (PCP) establishment in the wing and thorax (Mace and Tugores, 2004). Finally, it has been shown that *spen* mutants have defective epidermal integrity (Mace *et al.*, 2005).

Results and Discussion

During a gain-of-function genetic screen performed to look for genes involved in PCP generation, the *EP(2)2583* line, inserted in the *spen* locus (Abdelilah-Seyfried *et al.*, 2000), was isolated. In such screen *sev*-Gal4 and *ap*-Gal4 flies were crossed to the EP collection (Rørth *et al.*, 1998) and the progeny was scored for polarity defects in the eye, thorax and/or wing considering that the overexpression of genes required in planar polarity signalling at the relevant time often results in defects that are similar to the loss-of-function mutant phenotypes; *e.g.* Fz and Dsh (Boutros *et al.*, 1998; Krasnow *et al.*, 1995; Strutt *et al.*, 1997). Using adjacent rescued sequence available in the Flybase, we mapped the *EP(2)2583* insertion in the first intron of the *spen-RA* and *spen-RB* transcripts, and upstream of the first exon of the *spen-RC* transcript (Figure 1A-B).

We found that flies overexpressing *spen* in the eye with the *sev*-Gal4 driver, that directs the expression in cells posterior to the morphogenetic furrow of the eye disc, have externally rough eyes (Figure 2A). Tangential sections of these eyes revealed the presence of typical polarity defects with misrotated and symmetrical ommatidia. In the *Drosophila* eye, PCP is reflected in the mirror-

symmetric arrangement of ommatidial units relative to the dorso-ventral midline (the equator), which adopt opposite chirality depending on their dorsal or ventral positions. Polarity defects are manifested in the loss of mirror-image symmetry, with ommatidia adopting random chirality, remaining symmetrical, or being misrotated (Adler, 2002; Klein and Mlodzik, 2005; Mlodzik, 2002). *spen* overexpression in the eye also causes defects in photoreceptor differentiation, with ommatidia showing an incorrect number of photoreceptor cells (Figure 2B-C).

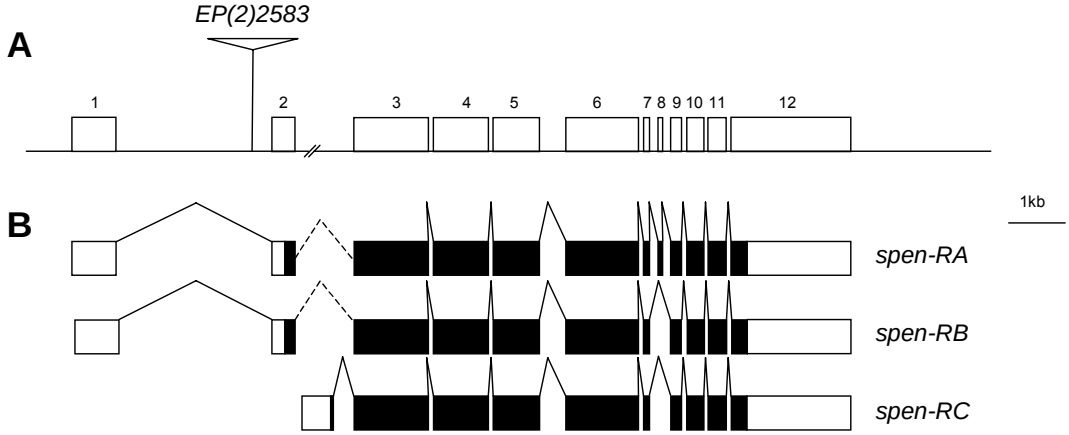

Figure 1. Molecular structure of *spen*. A. Genomic structure of the *spen* locus. The location of the EP-element inserted in the *spen* genomic region is indicated. Numbered boxes indicate *spen* exons. B. Structure of *spen* transcripts: *spen-RA*, *spen-RB* and *spen-RC*. Coding regions are shown in black, non-coding regions, in white. Introns are depicted as open triangles. The second intron of *spen-RA* and *spen-RB* is not in scale (dotted lines).

spen overexpression with the *ap*-Gal4 driver in the thorax and dorsal part of the wing was partially lethal, but some escapers displayed PCP defects on the thorax like misoriented microchaetae (Figure 2D). On the thorax, PCP is reflected by the regular orientation of the bristles along the anterior-posterior axis. Polarity defects in this tissue alter the orientation and number of hairs and bristles (Adler, 2002; Klein and Mlodzik, 2005; Mlodzik, 2002).

In order to determine the consequences of *spen* overexpression in the wing, we used *ptc*-Gal4 and *dpp*-Gal4, which direct the expression in a narrow stripe of cells in the central part of the wing disc, and *en*-Gal to express the gene in the posterior compartment of the wing. Flies that overexpress *spen* with *dpp*-Gal4, *ptc*-Gal4 or *en*-Gal4 also showed typical polarity defects, like disoriented and multiple wing hairs (Figure 2E). In the wing each cell generates one hair at the distal vertex that points distally, and PCP defects are reflected in an incorrect orientation and subcellular localization of the hair, as well as in the number of hairs produced by each cell (Adler, 2002; Klein and Mlodzik, 2005; Mlodzik, 2002). Furthermore, flies overexpressing *spen* with those drivers displayed other defects in the wing non-related to PCP, like problems in vein pattern establishment. They developed ectopic vein tissue, but also loss of veins could be observed. Moreover, we also detected loss of wing tissue, probably due to cell death (Figure 2F-G).

Taken together, our results suggest that *spen* is involved in PCP establishment in the eye, wing and thorax. This hypothesis is supported by previous work done by Mace *et al.* (2004) that showed that *spen* mutant mitotic clones in the wing and thorax display PCP defects, reflected by an abnormal orientation of the wing hairs and spatial distribution of both micro and macrochaetae. Although our results do not show how this gene could be involved in this process, we can hypothesize that it could be through the Ras/MAPK or Notch pathways, which are involved in PCP establishment (Mlodzik,

2002; Gaengel and Mlodzik, 2003), as *spen* interacts with both (Rebay *et al.*, 2000; Therrien *et al.*, 2000; Chen and Rebay, 2000; Abdelilah-Seyfried *et al.*, 2000; Kuang *et al.*, 2000). Another possibility is that *spen* could have a role in the Fz/PCP pathway.

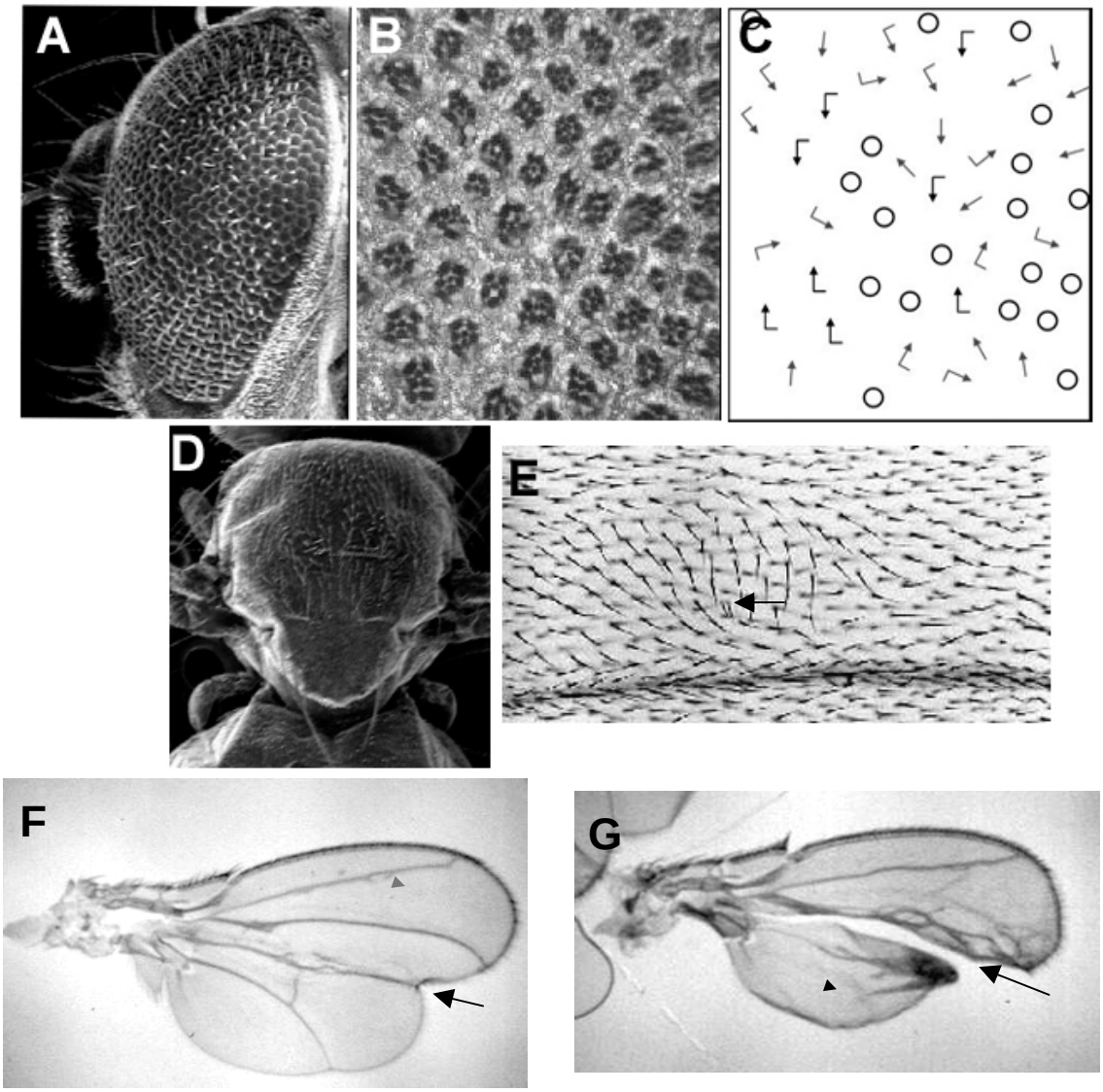

Figure 2. *spen* overexpression phenotypes. A-C. *spen* overexpression in the eye. In all cases anterior is left, dorsal is up. A. SEM image of *sev*-Gal4/*EP(2)2583* eyes. B. Tangential section of *sev*-Gal4/*EP(2)2583* eyes. C. Schematic representation of B. Black arrows with a flag represent correctly oriented ommatidia; grey arrows with a flag, misrotated ommatidia; grey arrows, symmetrical ommatidia; and circles mark unscorable ommatidia due to missing or malformed photoreceptors. D. SEM image of an *ap*-Gal4/*EP(2)2583* thorax. Misorientation of microchaetae can be observed. Anterior is up. E. *en*-Gal4/*EP(2)2583* wing. *spen* overexpression in the wing causes misorientation of hairs and also multiple wing hairs (arrow). F-G. *ptc*-Gal4/*EP(2)2583* wings showing ectopic vein material (grey arrowhead in F) and loss of vein tissue (black arrowhead in G). Loss of wing tissue is also evident (arrows). Anterior is up, proximal is left.

Other phenotypes caused by *spen* overexpression in eyes and wings may be explained considering that *spen* is involved in several signalling pathways. Changes in photoreceptor cell number could be related to *spen* function during cell cycle progression (Lane *et al.*, 2000). Changes in wing vein tissue could be caused by EGFR pathway activation, in which *spen* acts as a positively acting component (Rebay *et al.*, 2000; Therrien *et al.*, 2000; Chen and Rebay, 2000). Finally, cell death induced by *spen* overexpression in the central part of the wing, could be due to JNK pathway activation (Adachi-Yamada *et al.*, 1999).

A mechanistic model has been suggested to explain Spen function (Mace and Tugores, 2004). This model proposes that Spen is necessary for the maintenance of correct cell position during growth, ensuring that structures that are determined during development are correctly positioned in the adult. For this function, Spen interacts with several signalling pathways that are required and/or precisely specify the spatial organization during development. This could explain the variety of phenotypes caused by *spen* overexpression observed in this study.

References: Abdelilah-Seyfried, S., Y.M. Chan, C. Zeng, N.J. Justice, S. Younger-Shepherd, L.E. Sharp, S. Barbel, S.A. Meadows, L.Y. Jan, and Y.N. Jan 2000, Genetics 155: 733-752; Adachi-Yamada, T., K. Fujimura-Kamada, Y. Nishida, and K. Matsumoto 1999, Nature 400: 166-169; Adler, P.N., 2002, Genetics 160: 1535-1547; Boutros, M., N. Paricio, D.I. Strutt, and M. Mlodzik 1998, Cell 94: 109-118; Chen, F., and I. Rebay 2000, Curr. Biol. 10: 943-946; Dickson, B.J., A. van der Straten, M. Dominguez, and E. Hafen 1996, Genetics 142: 163-171; Gaengel, K., and M. Mlodzik 2003, Development 130: 5413-5423; Gellon, G., K.W. Harding, N. McGinnis, M.M. Martin, and W. McGinnis 1997, Development 124: 3321-3331; Klein, T.J., and M. Mlodzik 2005, Annu. Rev. Cell Dev. Biol. 21: 155-176; Krasnow, R.E., L.L. Wong, and P.N. Adler 1995, Development 121: 4095-4102; Kuang, B., S.C. Wu, Y. Shin, L. Luo, and P. Kolodziej 2000, Development 127: 1517-1529; Lane, M.E., M. Elend, D. Heidmann, A. Herr, S. Marzodko, A. Herzig, and C.F. Lehner 2000, Genetics 155: 233-244; Lin, H.V., D.B. Doroquez, S. Cho, F. Chen, I. Rebay, and K.M. Cadigan 2003, Development 130: 3125-3135; Mace, K., and A. Tugores 2004, BMC Dev. Biol. 4: 15; Mace, K.A., J.C. Pearson, and W. McGinnis 2005, Science 308: 381-385; Mlodzik, M., 2002, Trends Genet. 18. 564-571; Rebay, I., F. Chen, F. Hsiao, P.A. Kolodziej, B.H. Kuang, T. Laverty, C. Suh, M. Voas, A. Williams, and G.M. Rubin 2000, Genetics 154: 695-712; Rørth, P., K. Szabo, A. Bailey, T. Laverty, J. Rehm, G.M. Rubin, K. Weigmann, M. Milán, V. Benes, W. Ansorge, and S.M. Cohen 1998, Development 125: 1049-1057; Staehling-Hampton, K., P.J. Ciampa, A. Brook, and N. Dyson 1999, Genetics 153: 275-287; Strutt, D.I., U. Weber, and M. Mlodzik 1997, Nature 387: 292-295; Therrien, M., D.K. Morrison, A.M. Wong, and G.M. Rubin 2000, Genetics 156: 1231-1242; Wiellette, E.L., K.W. Harding, K.A. Mace, M.R. Ronshaugen, F.Y. Wang, and W. McGinnis 1999, Development 126: 5373-5385.

Altitudinal variation of morphometric traits of *Drosophila malerkotliana* of Chamundi hill.

Guru Prasad, B.R., and S.N. Hegde. Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore-570 006, India. Email: malerhegde@yahoo.com.

Morphometric variation observed in natural populations is frequently attributed to natural selection, but the roles of non-genetic modification by the environments have been neglected (Coyne and Beecham, 1987). In *Drosophila* studies on several species have shown the adapative nature of

body size. Latitudinal clines and seasonal changes have also been reported in *D. melanogaster* (David and Bocquet, 1975). Although a few such studies have been made to analyze the morphological variation in natural populations (Parsons, 1983; Hegde *et al.,* 2000), there are no reports on altitudinal variation of morphometeric traits. Therefore, the present investigation was undertaken to study the variation in morphometeric traits such as sternopleural bristles, scutellar bristles, and wing length of *D. malerkotliana* in four different altitudes of Chamundi hill located in Mysore (South Karnataka, India).

To study morphometeric traits *D. malerkotliana* flies were collected from 680, 780, 880, 980m altitudes of Chamundi hill situated at 11º 36' N latitude and 76º 55' N longitude. The total height of the hill from the foot is only 400m. This hill is covered by the scrub layers with small patches of evergreen type forest. *D. malerkotliana* is the most common and abundant species in this hill throughout the year (Hegde, 1979), hence this species was used. Flies were captured using net sweeping method. Males obtained from nature were directly used for making measurements. One *D. malerkotliana* female obtained from the F_1 progeny of each naturally inseminated female (Isofemale line) was used for measuring morphological traits. Fifty males and fifty females from each altitude were used for the analysis.

To analyse the morphometric traits, sternopleural bristles, of the left sides of the body and scutellar bristles on the thorax were counted. Then the left wing of each fly was removed from the base, mounted on a transparent glass slide with a drop of water and the length was measured from humeral cross vein to the tip of the wing with an ocular micrometer (1 unit = 100 μm) under microscope (100×). Means and standard errors of all these three characters were calculated, and analysis of variance followed by DMART was applied separately for both sexes.

Table 1. Shows metric characters for males and females in different altitudes of Chamundi hill.

	male			females		
Altitude	Sternopleural	Sctullar	Wing length	Sternopleural	Sctullar	Wing length
680m	6.48 ± 0.149 [a]	4.08 ± 0.038 [a]	1.55 ± 0.110 [a]	6.76 ± 0.105 a	4.44 ± 0.075 [a]	1.76 ± 0.013 [a]
780m	6.90 ± 0.095 [b]	4.14 ± 0.049 [a]	1.59 ± 0.008 [b]	7.28 ± 0.094 [b]	4.30 ± 0.095 [a]	1.84 ± 0.015 [b]
880m	7.00 ± 0.098 [b]	4.18 ± 0.054 [a]	1.62 ± 0.005 [c]	7.20 ± 0.085 [b]	4.50 ± 0.122 [a]	1.84 ± 0.014 [b]
980m	7.06 ± 0.112 [b]	4.16 ± 0.052 [a]	1.63 ± 0.005 [c]	7.22 ± 7.054 [b]	4.43 ± 0.056 [a]	1.88 ± 0.015 [b]
F value	5.096*	0.769	17.081**	6.687*	0.644	13.375**

*P< 0.01; **P< 0.00;
The strains with same alphabet in superscript are not significantly different at 5% level according to DMART

The scrutiny of Table 1 shows that the female *D. malerkotliana* has more sternopleural and scutellar bristles than males. The wing length of females was also more than males. These morphometric characters are the indices of size (Monclus and Prevosti, 1971), hence it can be concluded that as in most *Drosophila, D. malerkotliana* females are larger than males. Table 1 also shows the variation in morphometeric characters of males and females of *D. malerkotliana*. The sternopleural bristles number in males varied from 6.48 in 680m altitude to 7.06 in 980m, while in females it varied from 6.76 in 680m to 7.28 in 780m. The difference in number of sternpleurals between 680m and others were significant, while between 780m, 880m, and 980m, the difference was insignificant. In males mean number of scutellar bristles increased (4.08 to 4.16) with increase in altitude. In females mean number of scutellar bristles varied from 4.44 in 680m to 4.50 in 880m. However, the differences in number of scutellar bristles was insignificant both in males and females.

The mean wing length varied from 1.55 in 680m altitude to 1.63 in 980m in males, while it varied from 1.76 in 680m to 1.88 in 980m in females. The difference in wing length at different

altitudes was statistically significant. The interesting feature of the study is that with the increase in altitude there is a progressive increase in these metric traits in males. In females no such increase is noticed. This indicates that the male size increase with altitude. In females on the other hand, although differences exist in these traits, at different altitudes there is no distinct trend. The absence of clinal variation in the metric traits in females suggests that they are more heterogenous than males. This is because the females are exposed to higher selection pressures than males.

References: Coyne, J.A., and E. Becham 1987, Genetics 177: 737; Hegde, S.N., 1979, Ph.D. Thesis, University of Mysore; Hegde, S.N., M.K. Naseerulla and M.S. Krishna 2000, Indian J. of Exptl. Biol. 38: 707; David, J.R., and C. Bocquet 1975, Nature 257: 590; Parsons, P.A., 1983, Cambridge Univ. Press, Cambridge, 20; Monclus, M., and A. Prevosti 1971, Evolution 25: 214.

The nuclear export factor gene *small bristles (sbr)* is involved in the control of early embryonic mitoses in *Drosophila melanogaster*.

Golubkova, Elena V.[1], Seppo Nokkala[2], and Lyudmila Mamon[1]. [1]Department of Genetics, St. Petersburg State University, Saint-Petersburg, Russia; [2]Laboratory of Genetics, Department of Biology, FIN-20014, University of Turku, Turku, Finland; Address correspondence to: 199034, Russia, Saint-Petersburg, Universitetskaya nab., 7/9, Tel. +7-812-328 15 90; Fax: +7-812-328 05 41; e-mail address: gelena@EG10217.spb.edu; elena_golubkova@mail.ru

Keywords: *Drosophila*, early embryonic lethality, embryonic mitoses, nuclear export factor, *small bristles (sbr)*.

Abstract

The *small bristles* (*sbr*) gene of *Drosophila melanogaster* belongs to the family of *nuclear export factor* (*NXF*) genes that participate in mRNA nuclear export. The sbr^{10} *(l(1)ts403)* thermosensitive allele together with a null allele has a strong sterilizing effect on sbr^{10} / $Df(1)v^{L4}$ or $sbr^{10}/0$ hemizygotic females at a permissive temperature. Early embryonic lethality among offspring distinguishes $sbr^{10}/0$ females from the females that are heterozygous for other lethal alleles of the *sbr* gene. Among disruptions detected during early embryonic stages of sbr^{10}/0 female offspring, the most characteristic effects were asynchrony of the first cleavage divisions, changes in the morphology of nuclei, and the appearance of zones without nuclei or, on the other hand, with large chromosome-clusters. Such disruptions allow supposing that *sbr* gene is involved in the control of early embryonic mitoses.

Introduction

As a rule, genes that control the basic processes in cell metabolism are evolutionarily conserved and have orthologous genes in different organisms, belonging to evolutionarily remote taxons. Comparing such genes and their products in different organisms has become a promising area of research in genomics. The *small bristles (sbr)* gene of *D. melanogaster* is orthologous to the *TAP (hNXF1)* gene of *Homo sapiens* (Yoon *et al*., 1997; Kang and Cullen, 1999; Wilkie *et al.*, 2001), *NXF1* of *Caenorhabditis elegans* (Tan *et al*., 2000), and *Mex67* of *Saccharomyces cerevisiae* (Segref

et al., 1997). All these *NXF (nuclear export factor)* genes are involved in controlling active transport of most, if not all, mRNAs from the nucleus to the cytoplasm.

Currently, more data is accumulating, which suggest that a number of components of nucleocytoplasmic transport are also involved in chromosome segregation (Clarke and Zhang, 2001; reviewed in Hetzer, Gruss, and Mattaj, 2002; Babu *et al*., 2003; reviewed in Mamon, 2005).

Previously, we have demonstrated that the sbr^{10} *(l(1)ts403)*, a thermosensitive allele of the *sbr* gene, displays a semidominant effect on chromosome segregation in female meiosis under heat shock (Mamon *et al*., 1990; Nikitina *et al*., 2003). It is worth noting that a single dose of the sbr^{10} allele causes almost complete sterility of $sbr^{10}/Df(1)v^{L4}$ *Drosophila* females (deletion $Df(1)v^{L4}$ removes the *sbr* gene) at normal temperature (Golubkova *et al*., 2004). Early embryonic death is the main reason for the sterility of $sbr^{10}/Df(1)v^{L4}$ females. Taking into consideration the effect of the sbr^{10} allele on chromosome segregation, we have suggested that the reason for the early embryonic lethality of offspring is genome instability during early embryonic mitoses.

To uncover reasons for the early embryonic death, we have in the present study analyzed early embryonic mitoses of offspring of females with different combinations of the alleles of the *sbr* gene.

Materials and Methods

Drosophila Strains and Hybrids

The following strains and hybrids were used in this study:

(1) Females lacking mutation sbr^{10} *(l(1)ts403)* were obtained from crosses between the *v* (*vermilion*) and *y* (*yellow*) strains (hereafter, denoted +/+).

(2) The strain carrying mutation sbr^{10} *(l(1)ts403)* (Arking, 1975) with markers $raspberry^{2}$ and *vermilion*, ras^{2} sbr^{10} *v* (hereafter, sbr^{10}).

(3) The strain carrying mutation sbr^{10} (Arking, 1975) with markers *yellow* and *crossveinless* (hereafter, sbr^{10}).

(4) The strain carrying a null allele of the *sbr* gene, deletion $Df(1)v^{L4}$ of the X chromosome region 9F4-10A2, which removes genes *vermilion*, *raspberry* and *small bristles* (Zhimulev *et al*., 1982): $Df(1)v^{L4}$, *ras* m^{D} / *FM6l*, y^{31d} sc^{8} *dm* / *Dp(1; Y)* $v^{+}y^{+}$. The chromosome with this deletion is hereafter denoted 0.

(5) The strain carrying a lethal allele *l(1)K4* (synonym sbr^{5}) of the *sbr* gene: *l(1)K4 B* / *In(1)FM6l*, y^{31d} sc^{8} *dm B* / *Dp(1;Y)* $v^{+}y^{+}$ (Zhimulev *et al*., 1982). The chromosome with this lethal is hereafter denoted K4.

(6) The strain $w^{a}B$ ($white^{apricot}$ and *Bar*) served as the paternal line in all experiments on fecundity assessment.

(7) Hybrid females (0/+), genotype $Df(1)$ v^{L4}, ras^{2} m^{D} / *v*; obtained from crosses between strains $Df(1)v^{L4}$, *ras* m^{D} / *FM6L*, y^{31d} sc^{8} *dm B* / *Dp(1; Y)* $v^{+}y^{+}$ and *v*.

(8) Hybrid females (0/sbr^{10}), genotype $Df(1)$ v^{L4}, ras^{2} m^{D}/ras^{2} sbr^{10} *v*; obtained from crosses between strains $Df(1)v^{L4}$, *ras* m^{D} / *FM6l*, y^{31d} sc^{8} *dm B* / *Dp(1; Y)* $v^{+}y^{+}$ and ras^{2} sbr^{10} *v*.

(9) Hybrid females (sbr^{10}/+), genotype ras^{2} sbr^{10} *v* / *v*; obtained from crosses between the corresponding strains.

(10) Hybrid females (K4/sbr^{10}), genotype *l(1)K4 B* / *y* sbr^{10} *cv*; from crosses between strains *l(1)K4 B* / *In(1)FM6l*, y^{31d} sc^{8} *dm B* / *Dp(1;Y)* $v^{+}y^{+}$ and *y cv* sbr^{10}.

(11) Hybrid females (K4/+), genotype *l(1)K4 B* / *y*; obtained from crosses between strains *l(1)K4 B* / *In(1)FM6l*, y^{31d} sc^{8} *dm B* / *Dp(1;Y)* $v^{+}y^{+}$ and *y*.

Genotypes of the strains are described according to Lindsley and Zimm (1992). The flies of all strains and hybrids were reared at +24.0 ± 0.5°C.

Staining

Adult three-day-old female flies were placed together with w^aB males in 0.9-l liter bottles at a ratio of 1:1. Eggs were laid on plates with agar medium smeared with yeast suspension.
The embryos were collected for a 15-min period and incubated at 24°C for a fixed period. Embryos to be stained were first dechorionated in 50% household bleach, washed in 0.01% Triton in PBS and fixed for 20 to 30 minutes in a 1:1 solution of 4% paraformaldehyde in PBS (pH 7.4) and heptane. Devitellinisation was performed by removing the aqueous phase containing fixative and washing the remaining heptane layer with an equal volume of methanol with shaking. The embryos without vitelline membrane descend to the bottom. The devitellinized embryos were washed at least three times with methanol. The embryos can be used immediately or stored at -20°C in methanol for at least one year. We subsequently used two methods for staining.
1) After methanol the embryos were washed 3 × 10 min with PBS. Then the embryos were mounted in Vectashield supplemented with 2 μg/ml DAPI (4,6-Diamindine-2-phenylindole dihydrochloride).
2) Embryos were rehydrated in 70%, 50%, and 30% methanol, and then embryos were briefly washed with dH_2O. The slides were made and stained according to the method of Nokkala and Nokkala (2003).

Results

In order to understand reasons for early embryonic death of offspring of $sbr^{10}/0$ females at a permissive temperature, we have cytologically analyzed the eggs laid by the females of different genotypes, collected no later than within three hours after the eggs have been laid. The main disruptions of the early stages of embryogenesis were asynchrony during early stages of mitosis (Figure 1), the disruption of the division of nuclei (Figure 2), and lack of correspondence between the morphology of embryos and their age, determined as the time from the moment the egg was laid.

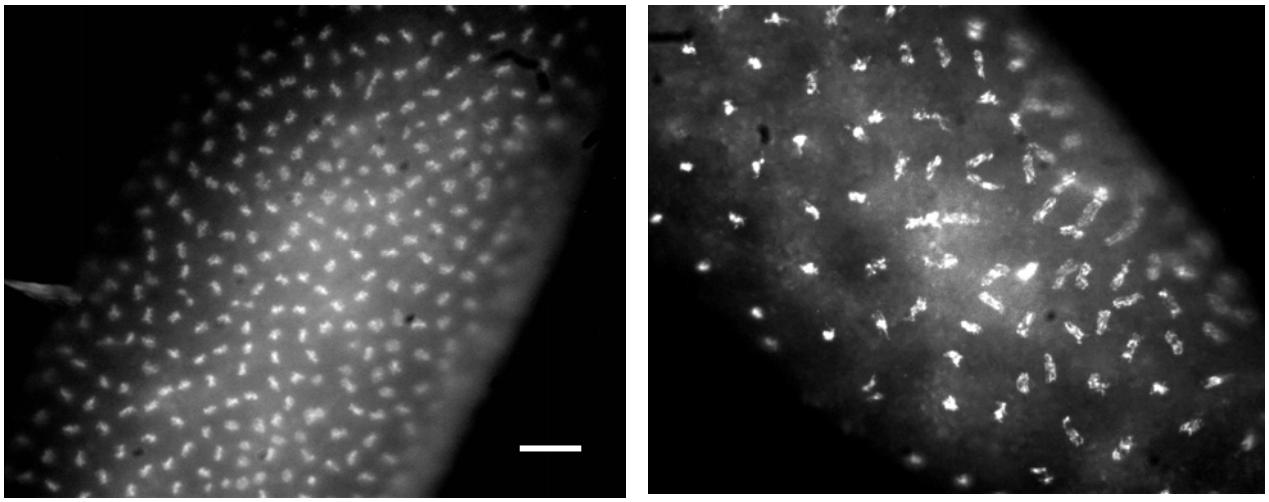

Figure 1. Embryos of *Drosophila melanogaster* in the age ~1 hour after the laying of eggs (DAPI staining). A (left) Embryos in progeny of females + / +, synchronous mitosis - all nucleus at a stage metaphase. B (right) Embryos in progeny of females $0/sbr^{10}$, asynchronous divisions - nucleus at stages telophase, metaphase, anaphase. Bar, 25 μm.

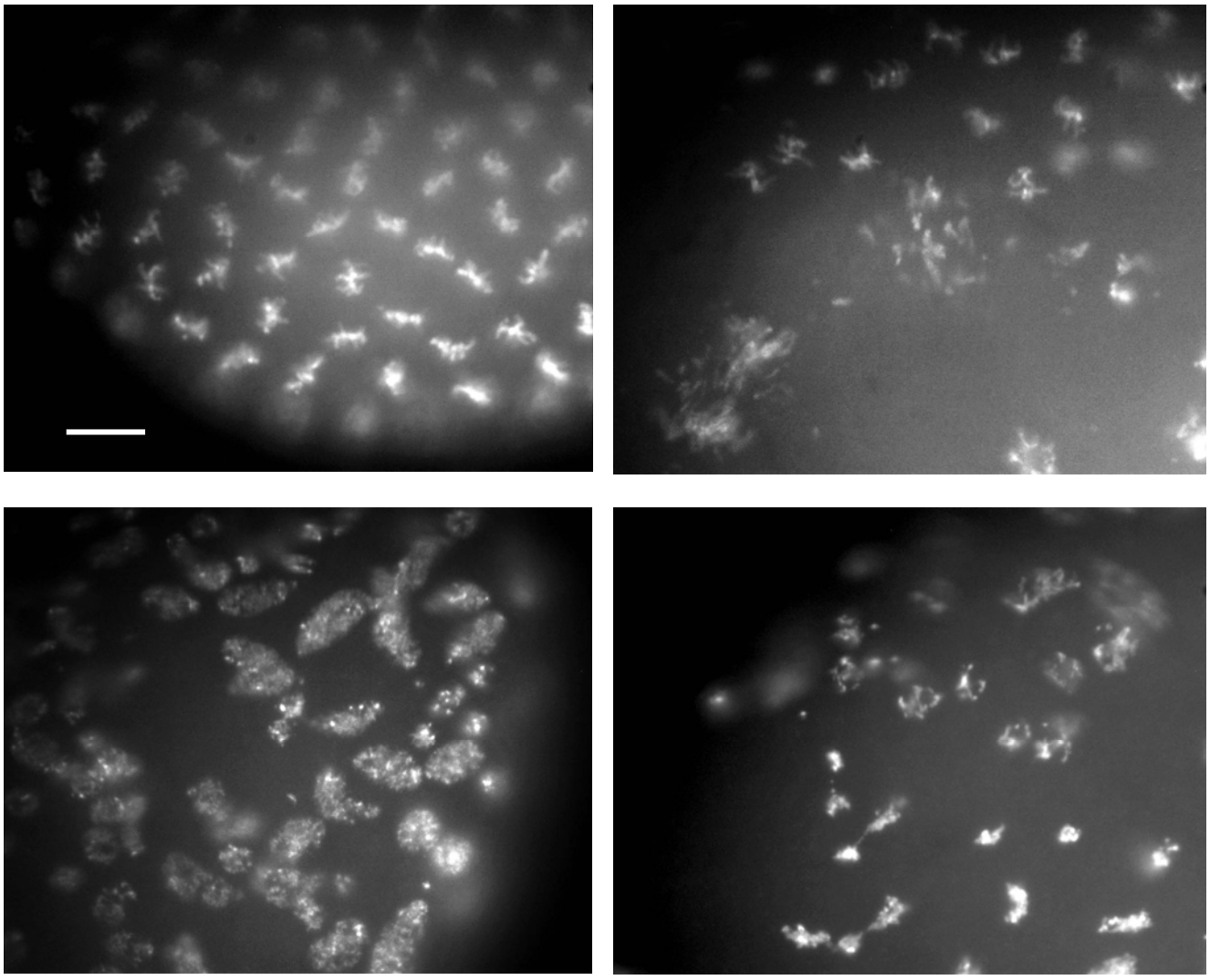

Figure 2. Early embryonic mitosis of *Drosophila melanogaster* (DAPI staining). A (top left) Normal metaphases in embryo in the age ~ 1 h. 30 min., obtained from female + / +. B (top right), C (bottom left) and, D (bottom right) Disturbance of divisions in embryos in the age ~1 h. 30 min., obtained from female $0/sbr^{10}$. We can see an accumulation of numerous chromosomes surrounded by an empty space (B), changes in the size and shape of the nuclei and empty spaces without nuclei (C, D). Bar, 10 μm.

The frequency of embryos with asynchronous division in the offspring of $0/sbr^{10}$ females reaches 8.9% (Table 1), which allowed us to distinguish statistically $0/sbr^{10}$ females from the females of other studied genotypes. The frequency of embryos with asynchronous division during early stages of embryogenesis in offspring of sbr^{10}/sbr^{10}, $sbr^{10}/+$, 0/+ females did not differ from that of +/+ females. We have found some differences in frequencies of embryos with disruptions of synchrony of first embryonic mitosis in the offspring of females carrying the allele *l(1)K4*.

We have also found embryos with evident disruptions of the morphology of the nucleus in the offspring of sbr^{10}/sbr^{10}, $0/sbr^{10}$, K4/+, and $K4/sbr^{10}$ females. The frequency of embryos with these disruptions in the offspring of sbr^{10}/sbr^{10} and $0/sbr^{10}$ females was 12.1 and 14.1%, respectively, and 8.3 and 12.9% in the offspring of K4/+ and $K4/sbr^{10}$ females, respectively (see Table 1). Normally,

synchronously dividing nuclei of early embryos are of the same size and characterized by a characteristic distribution within the embryo, depending on the stage of development. The embryos with disrupted nuclear divisions displayed areas, in the center of which there was an accumulation of numerous chromosomes surrounded by an empty space (Figure 2b). Among the disruptions during the early embryogenesis, there were changes in the size and shape of the nuclei (Figure 2c) and empty spaces without nuclei (Figure 2d), as well as pycnotic nuclei (Figure 3). It is possible that the areas lacking nuclei are formed where a nucleus died leaving an empty space during subsequent synchronized divisions of embryonic nuclei. We assume that the reason for such anomalies is a disruption in the formation of the envelope that surrounds the dividing nuclei. The frequency of embryos with disruptions in nuclear division of the 0/*sbr*^{10} female was not different from that of K4/*sbr*^{10} and *sbr*^{10}/*sbr*^{10} females, but was different from that of other females, including K4/+. Interestingly, the frequency of embryos with these disruptions was 8.3% for the offspring of K4/+ females (see Table 1).

Table 1. Frequency (%) of embryos with various types of disturbances in early embryonic mitoses.

Genotype of parental females	Number of embryos	Frequency of embryos with various types of disturbance (%)			
		Asynchronous of early mitoses	Disturbance of division	Development delay	One pronucleus
+/+	156	0.6	0.6	1.3	0
sbr^{10}/*sbr*^{10}	99	2.0	12.1~	8.1	0
K4/*sbr*^{10}	140	4.3#	12.9~	22.1	0
0/*sbr*^{10}	135	8.9*^	14.1~	24.4	10.4*^
sbr^{10}/+	205	1.0	2.4	3.9	0
K4/+	192	5.7#	8.3#	21.9	0
0/+	124	1.6	1.6	24.2	0

The note: statistically authentic distinctions are marked at P < 0,05:
* - between females *0/sbr*^{10} and females other investigated genotypes.
^ - between females *0/sbr*^{10} and females with genotype *K4/sbr*^{10}.
# - between females carrying the allele *l(1)K4* and females other investigated genotypes.
~ - between females *0/sbr*^{10}, *sbr*^{10}/ *sbr*^{10}, *K4/sbr*^{10} and females other investigated genotypes

The eggs with a single pronucleus with a frequency of 10.4% were found only among the eggs laid by 0/*sbr*^{10} females (see Table 1).

Approximately one fourth of the offspring of 0/+, 0/*sbr*^{10}, K4/*sbr*^{10}, and K4/+ females were characterized by delayed embryogenesis. Since the timing of all the stages of the embryonic development of *Drosophila* have been determined precisely (Campos-Ortega, Hartenstein, 1985), each time interval of embryogenesis determines strict morphological characteristics of the embryo. If the embryogenesis is delayed, we see a discrepancy between age and morphology. In the offspring of the females homozygous with *sbr*^{10}, only 8.1% of the embryos showed delayed development.

The presence of *Df(1)v*^{L4} and *Df(1)v*^{L3} deletions and the *l(1)K4* lethal allele in the genotype of females have a lethal effect in their hemi- and homozygote progeny and undoubtedly increases the frequency of embryonic lethality, since half of the sons, hemizygous for the deletion or lethal allele, died at the embryo stage (Pougatchova *et al*., 2002). Females that are heterozygous for the *Df(1)v*^{L3} deletion have been used as a positive control, because *Df(1)v*^{L3} and *Df(1)v*^{L4} deletions are partly overlapping and have a lethal effect in hemi- and homozygote, but *Df(1)v*^{L3} does not remove the *sbr* gene (Zhimulev *et al*., 1982). The frequency of embryonic lethality of the offspring of 0/+, K4/+, L3/*sbr*^{10}, and L3/+ varied from 24 to 29%, which is within the expected 25% range of offspring

death, in particular, males that are hemizygous for the respective deletion or lethal allele. Most of the embryos, in fact, died during the later stages of embryonic development (Pougatchova *et al*., 2002; Golubkova *et al*., 2004).

Thus, *sbr*^{10}/0 females were different from the other studied genotypes in the frequency of producing embryos with disruptions in synchrony during initial division of the nucleus, as well as in the presence of eggs with a single pronucleus. Such disruptions may be the cause of the high frequency of early embryonic lethals, which distinguishes *sbr*^{10}/0 females from the other genotypes that we have studied.

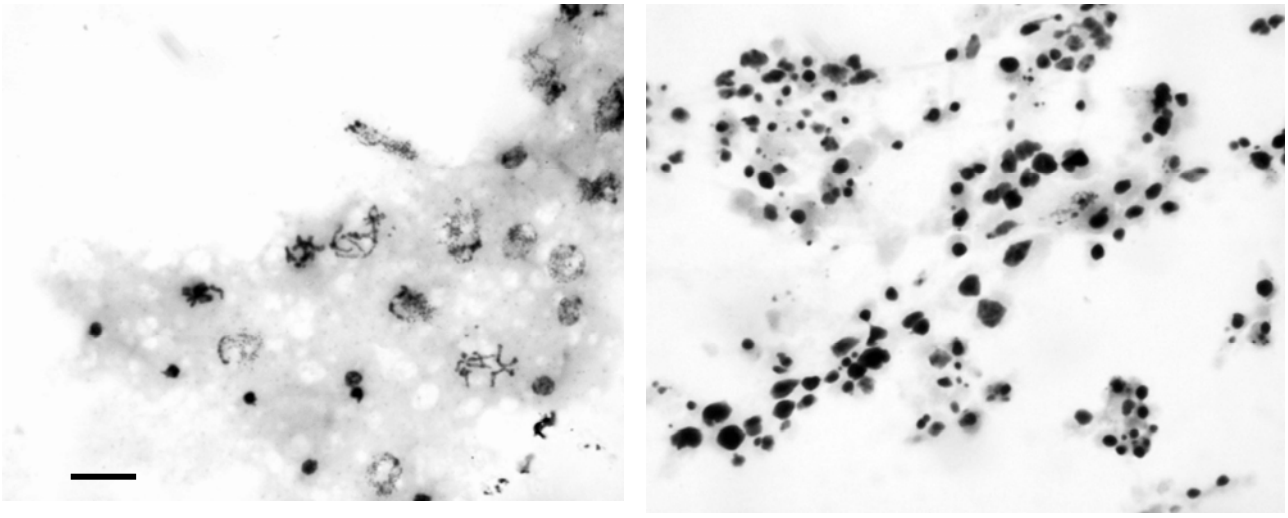

Figure 3. Staining with Giemsa according to the method of Nokkala and Nokkala (2003). A) Normal nuclei in wild type embryo. B) Pycnotic nuclei in embryos obtained from female 0/*sbr*^{10}. Bar, 4 μm.

Discussion

D. melanogaster provides unique possibilities for studying chromosome behavior in early embryonic mitoses. The specific features of the early stages of embryogenesis of *D. melanogaster* are that the first 13 embryonic mitoses occur synchronously (Foe and Alberts, 1983) and are controlled by the factors produced during oogenesis (Edgar *et al*., 1994). Expression of zygotic genes almost never occurs in early embryogenesis before cycle 8 (Pritchard and Schubiger, 1996); therefore, there is no transcription and no need for nucleocytoplasmic export of mRNAs. Thus, the function of SBR protein as a transport receptor during early embryogenesis is not needed. Most of the factors that control early embryogenesis of *D. melanogaster* are provided as mRNAs by the mother in oogenesis (reviewed in Bashirullah, Cooperstock, and Lipshitz, 1998). Among the products accumulated during oogenesis is the mRNA of *sbr* gene (Korey *et al*., 2001). Since the concentration of the *sbr* mRNA decreases greatly by the time the zygotic genome activates (Korey *et al*., 2001), it is safe to assume that SBR protein, which is the product of maternal *sbr* mRNA, is necessary during early stages of embryonic development. It is, however, uncertain whether the function of accumulated *sbr* mRNA is limited to providing embryonic nuclei with transport receptors for the beginning of expression of the zygotic genome, or whether this gene has any other functions.

Currently, we are witnessing the appearance of even greater data testifying for the connection between the processes of nucleocytoplasmic transport and chromosome segregation during cell division. There are known factors (Ran GTPase, Rae 1, and some nucleoporins) involved in nucleocytoplasmic transport of macromolecules during interphase which demonstrate a connection

with the mitotic apparatus during cell divisions (reviewed in Hetzer, Gruss, and Mattaj, 2002; Babu *et al*., 2003; reviewed in Mamon, 2005). Genes have been discovered whose mutations show pleiotropic effects, disrupting both nucleocytoplasmic transport of macromolecules and chromosome segregation during cell division (Tange, 2002). Such pleiotropic effect is also characteristic of the *sbr*^{10} mutation of *D. melanogaster*, which disrupts mRNAs transport (reviewed in Tretyakova *et al*., 2001) and increases the frequency of non-disjunction and loss of sex chromosomes in meiosis (Nikitina *et al*., 2003).

Among other things, genome instability has been observed to cause cancer, premature aging of the organism, and male and female sterility (Cahill *et al*., 1999; Martin *et al*., 1985; Hristova *et al*., 2002). Most of these signs are characteristic of the *sbr*^{10} mutation exposed to a heat shock (37°C, 1 h), the primary effect being male and female sterility. At the same time, *sbr*^{10}/0 females are almost sterile at a permissive temperature. Their sterility is primarily determined by increased frequency of the so-called embryonic lethals and early larval deaths of offspring (Golubkova *et al*., 2004). When evaluating the role of the *sbr* gene in female sterility, it is worth noting that a single dose of a normal allele does not have any significant effect on the fertility of +/0 females, whereas the lack of the *sbr*^{10} thermosensitive mutation causes almost complete sterility in *sbr*^{10}/0 females. The *sbr*^{10} (*l(1)ts403*) allele is a C to T mutation at nucleotide position 416, which replaces proline to leucine at residue 139, thus disrupting the RNA-binding domain of SBR protein (Korey *et al*., 2001). The presence of a double dose of mutant protein in homozygous *sbr*^{10} females lowers their fertility only insignificantly, whereas a single dose renders *sbr*^{10}/0 females almost sterile. Importantly, the frequency of embryos with asynchronous divisions of the nucleus is high in the offspring of both *sbr*^{10}/0 females and the females homozygous at *sbr*^{10}. Therefore, the disruption in divisions of the nucleus of early embryos is recessive and controlled by the *sbr*^{10} allele, since it is not observed the difference in the offspring of *sbr*^{10}/+ and +/+ females.

Most of the indications we have studied – embryo death, the fertility of females, the average number of eggs laid by the female during 24 hours (Golubkova *et al*., 2004), the presence of larvae with characteristic disruptions of the Malpighian tubules (data not shown) and the asynchrony of early mitoses – show that the *l(1)K4* (*sbr*^{5}) allele in combination with the *sbr*^{10} allele has a lesser effect, in comparison with the null allele in combination with the *sbr*^{10} allele. This allows us to assume that the *sbr*^{5} allele is not a null-allele and corresponds to a mutant protein. Such indication as the fertility of *sbr*^{5}/*sbr*^{10} females is no different from those of the females homozygous at the *sbr*^{10} allele. At the same time, the *sbr*^{10} allele in homozygote females has a greater effect on the average number of eggs laid over a period of 24 hours, than the *sbr*^{5} (*l(1)K4*) allele together with the *sbr*^{10} allele (data not shown). The *sbr*^{5} allele has a deletion with the size of 494 kb that affects exon 8 and exon 9 and removes 146 amino acids from the SBR^{K4} protein, disrupting the domain responsible for binding with nucleoporins, but does not affect the RNA-binding domain (Markova, E.G., A.V. Markov, O.S. Sotnikova, I.V. Tretyakova, E.V. Golubkova, and L.A. Mamon, unpublished). The *sbr*^{10} allele is a point mutation that damages the RNA-binding domain (Korey *et al*., 2001). It is possible that the interaction of the two different mutant products somehow results in a compensatory effect, for example in regard to the average number of eggs laid by the female during 24 hours. At the same time we have also found some effects of the *sbr*^{5} (*l(1)K4*) allele in compound with the wild type allele of the *sbr* gene as for frequency of embryos with disruptions in division of the nucleus or for frequency of embryos with asynchronous of early mitoses.

The easiest explanation for this is inter-allele complementation, whose mechanism is characteristic of multimeric proteins (Gross, 1962). There is, however, no data testifying that orthologous proteins –TAP, Mex 67p, or SBR – which act as the transport receptors are capable of forming homo-multimers. At the same time, such interaction may be carried out through other proteins, with which transport receptors interact.

Since SBR is a polydomain protein (Korey *et al*., 2001), the disruption of one of its domains may be crucial for carrying out one of its functions, while the disruption of another domain may affect another function. In heteroallele combinations, such interactions may mutually compensate for functional defects of interacting alleles when there are several products specializing in carrying out different functions correspond to the gene. The data that we have obtained confirm this assumption: there are at least three tissue-specific transcripts that correspond to the *sbr* gene (Ivankova, N.A., I.V. Tretyakova, G.T. Lyozin, O.G. Zatsepina, M.B. Evgeniev, and L.A. Mamon, unpublished). In turn, the existence of similar compensatory effects in interacting mutant alleles may confirm the assumption of the multifunctional nature of the *sbr* gene. Nucleocytoplasmic transport may not be the only function of transport receptors, such as TAP protein and SBR protein. Our data show that the *sbr* gene is involved in regulating embryonic mitoses. The presence of a single dose of the sbr^{10} mutation causes sterility of females in consequence of embryonic death. The wild type allele of the *sbr* gene is not haploinsufficient, at the same time the sbr^{10} allele proves haploinsufficiency.

In conclusion, a distinctive feature of female $sbr^{10}/0$, hemizygous for the mutation sbr^{10}, is the frequent occurrence of embryos with disturbances in synchronization of nuclear divisions. Moreover, these females laid eggs only with one pronucleus. It remains to be elucidated whether SBR protein itself directly participates in the regulation of chromosomal segregation or the effect on chromosomal segregation is a consequence of transport functions of the given protein. The last possibility is of great interest, because of the role of SBR protein in biogenesis of maternal mRNA pulse translation which synchronizes nuclear divisions in early embryogenesis of *D. melanogaster*.

Acknowledgments: The study has been supported by grants SciSch-2214.2003.4 and CRDF-Ministry of Education ST-012, awarded by the leading schools and RFBR-06-04-49519.

References: Arking, R., 1975, Genetics 80: 519-537; Babu, J.R., K.B. Jeganathan, D.J. Baker, X. Wu, N. Kang-Decker, and J.M. Van Deursen 2003, J. Cell Biol. 160: 341-353; Bashirullah, A., R.L. Cooperstock, and H.D. Lipshitz 1998, Annu. Rev. Biochem. 67: 335-394; Cahill, D.P., K.W. Kinzker, B. Vogelstein, and C. Lengauer 1999, Trends Cell Biol. 9: M57-M60; Campos-Ortega, J.A., and V. Hartenstein 1985, *The Embryonic Development of* Drosophila melanogaster, Springer-Verlag, Berlin, Heidelberg; Clarke, P.R., and C. Zhang 2001, Trends Cell Biol. 11: 366-371; Edgar, B.A., F. Sprenger, R.J. Duronio, P. Leopold, and P.H. O'Farrell 1994, Genes Dev. 8: 440-453; Foe, V.E., and B.M. Alberts 1983, J. Cell Sci. 61: 31-70; Golubkova, E.V., O.M. Pugachova, E.P. Demina, A.S. Musorina, A.N. Shershabova, and L.A. Mamon 2004, Russian J. of Genetics 40: 369-376; Gross, S.R., 1962, PNAS 6: 922–930; Hetzer, M., O.J. Gruss, and W. Mattaj 2002, Nature Cell Biol. 4: 177-184; Hristova, R., E. Ko, C. Greene, A. Rademaker, J. Chernos, and R. Martin 2002, Biology of Reproduction 66: 1781-1783; Kang, Y., and B.R. Cullen 1999, Genes Dev. 13: 1126-1139; Korey, C.A., G.S. Wilkie, I. Davis, and D. Van Vactor 2001, Genetics 159: 1659-1670; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster, Academic Press, San Diego, p. 1133; Mamon, L.A., E.L. Mazur, I.V. Churkina, and L.V. Barabanova 1990, Russian J. of Genetics 26: 554-556; Mamon, L.A., 2005, Tsitologiya 47: 263-276; Martin, G.M., A.C. Smith, D.J. Ketterer, C.E. Ogburn, and C.M. Disteche 1985, Isr. J. Med. Sci. 3: 296-301; Nikitina, E.A., A.V. Komarova, E.V. Golubkova, I.V. Tretyakova, and L.A. Mamon 2003, Russian J. of Genetics 39: 341-348; Nokkala, S., and C. Nokkala 2003, Methods in Molecular Biology, vol. 247: *Drosophila* Cytogenetics Protocols, (Henderson, D.S., Ed.). Humana Press Inc., Totowa, NJ, pp. 129-135; Pougatchova, O.M., A.N. Shershabova, A.S. Musorina, E.V. Golubkova, E.A. Belyaev, and L.A. Mamon 2002, 43th Annual *Drosophila* Research Conference, San Diego, California, p. 141; Pritchard, D.K., and G. Schubiger 1996, Genes Dev. 10: 1131 –1142; Segref, A., K. Sharma, V. Doye, A. Hellwig, J. Huber, R. Luhrmann, and E. Hurt 1997, EMBO J. 16: 3256-3271; Tan, W., A.S. Zolotukhin, J. Bear, D.J. Patenaude, and B.K. Felber 2000, RNA 6: 1762-1772; Tange, Y., A. Hirata, and O. Niwa 2002, J. Cell Sci. 115: 4375-4385; Tretyakova, I.V., G.T.

Lyozin, E.G. Markova, M.B. Evgen'ev, and L.A. Mamon 2001, Russian J. of Genetics 37: 593-602; Wilkie, G.S., V. Zimyanin, R. Kirby, C. Korey, H. Francis-Lang, D. Van Vactor, and I. Davis 2001, RNA 7: 1781-1792; Yoon, D.W., H. Lee, W. Seol, M. DeMaria, M. Rosenzweig, and J.U. Jung 1997, Immunity 6: 571-582; Zhimulev, I.F., E.S. Belyaeva, G.V. Pokholkova, G.V. Kotchneva, O.V. Fomina, A.V. Bgatov, Ju. Khudyakov, I. Patzevich, V.F. Semeshin, E.M. Baritcheva, M.G. Aizenzon, P. Kramers, and J. Eeken 1982, Dros. Inf. Serv. 58: 210-214.

Sequence analyses of the acid phosphatase gene (*Acph-1*) from *D. virilis* and a comparison with the *Acph-2*. Gene expression may be regulated by repeated sequences.

Kitagawa, Hiroko T. Laboratory of Biochemistry, Department of Chemistry, Faculty of Science, Josai University, Sakado, Saitama, 350-0295 Japan; TEL: 81-492-71-7961; FAX: 81-492-71-7985; e-mail:hkita@josai.ac.jp. The nucleotide sequence data reported in this paper appeared in the DDBJ / EMBL / Gen Bank nucleotide sequence databases with accession number AB271538.

The four allelic forms of ACPH specified by the *Acph* locus of *D. virilis* have been found in the Japanese population (Ohba, 1977). The *Acph-2* comprises more than 98% of the genes in the population. These allozyme proteins, glycoproteins with M.W. of 44000 (Narise and Kitagawa, 2001), electrophoretically migrate in the order of ACPH-1, -2, -3 and -4 from the origin at the same intervals from each other. Furthermore, these enzymes show activity differences in terms of the intensities of the electrophoretic bands; ACPH-2 has higher activity than ACPH-1 and lower than ACPH-4 (Narise, 1976). In order to elucidate the activity differences through the analysis of nucleotide sequences of the genes, I recently investigated the nucleotide sequence of the *Acph-2* gene and its transcripts (Kitagawa, 2003). The *Acph-2* gene is organized into six exons. Three types of the transcripts ascribed to alternative splicing in the last intron are identified by RACE analysis. Here I report the DNA sequences of the low-activity variant (*Acph-1*) gene and a comparison with the *Acph-2* gene.

The genomic library was constructed with the genome from adult flies of the *Acph-1* strain. The *Acph-1* gene was screened by the *Acph-2* cDNA as a probe. The positive clones were subcloned with pBluescript SK^+. The sequence analysis was carried out by using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) with an ABI PRISM 310 Genetic Analyzer.

Figure 1 shows the nucleotide sequences of the *Acph-1* including 931 bp of the 5' region and 1461 bp of the 3' region. The organization and the length in the exon and intron of the *Acph-1* were the same as those of the *Acph-2*. When the sequences of the *Acph-1* were compared with those of the *Acph-2*, four nucleotide changes in the coding region were found at the exon 4 (Figure 1). The change at 746 bp (C-A) out of them results in the amino acid replacement of glutamine (CAG) of the ACPH-2 protein with lysine (AAG) in the ACPH-1. Therefore, it is clear that this change altered the electrophoretic mobility at an interval of one step. However, this replacement does not seem to be the main cause for low activity of the ACPH-1, for it does not occur in the catalytic residues and also in the glycosylation sites. The transcriptional start sites, exon-intron junctions, and poly-A signals in the *Acph-2* mRNA previously reported (Kitagawa, 2003) were all conserved in the *Acph-1*. These results predict that the three transcripts will be produced by alternative splicing in the *Acph-1* as the same as in the *Acph-2*.

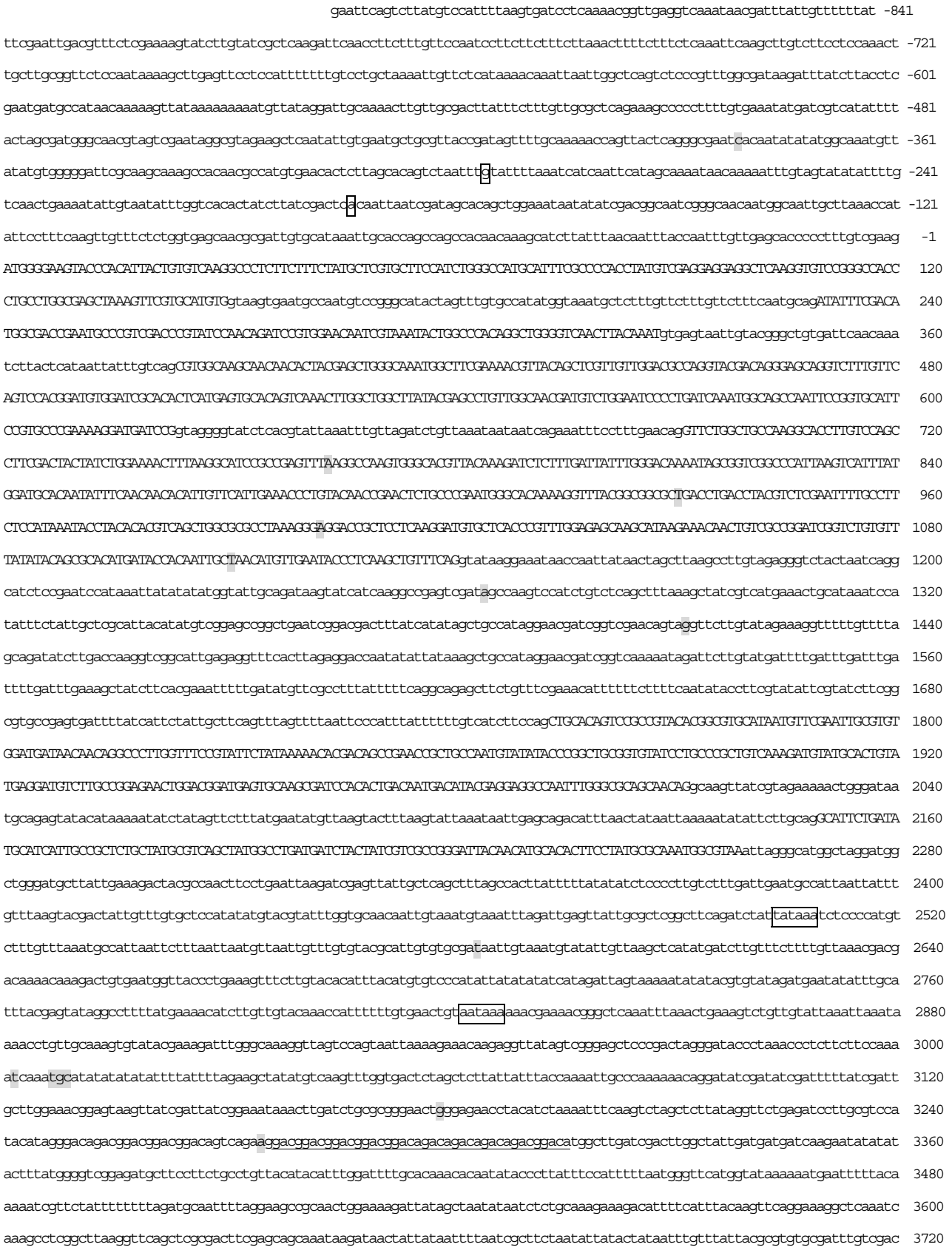
gaattcagtcttatgtccattttaagtgatcctcaaaacggttgaggtcaaataacgatttattgttttttat -841
ttcgaattgacgtttctcgaaaagtatcttgtatcgctcaagattcaaccttctttgttccaatccttcttctttcttaaacttttctttctcaaattcaagcttgtcttcctccaaact -721
tgcttgcggttctccaataaaagcttgagttcctccatttttttgtcctgctaaaattgttctcataaaacaaattaattggctcagtctcccgtttggcgataagatttatcttacctc -601
gaatgatgccataacaaaaagttataaaaaaaaaatgttataggattgcaaaacttgttgcgacttatttctttgttgcgctcagaaagccccctttgtgaaatatgatcgtcatatttt -481
actagcgatgggcaacgtagtcgaataggcgtagaagctcaatattgtgaatgctgcgttaccgatagttttgcaaaaaccagttactcagggcgaatcacaatatatatggcaaatgtt -361
atatgtgggggattcgcaagcaaagccacaacgccatgtgaacactcttagcacagtctaatttgtatttttaaatcatcaattcatagcaaaataacaaaaatttgtagtatatattttg -241
tcaactgaaaatattgtaatatttggtcacactatcttatcgactcacaattaatcgatagcacagctggaaataatatatcgacggcaatcgggcaacaatggcaattgcttaaaccat -121
attcctttcaagttgtttctctggtgagcaacgcgattgtgcataaattgcaccagccagccacaacaaagcatcttatttaacaatttaccaatttgttgagcacccccttttgtcgaag -1
ATGGGGAAGTACCCACATTACTGTGTCAAGGCCCTCTTCTTTCTATGCTCGTGCTTCCATCTGGGCCATGCATTTCGCCCCACCTATGTCGAGGAGGAGGCTCAAGGTGTCCGGGCCACC 120
CTGCCTGGCGAGCTAAAGTTCGTGCATGTGgtaagtgaatgccaatgtccgggcatactagtttgtgccatatggtaaatgctctttgttctttgttctttcaatgcagATATTTCGACA 240
TGGCGACCGAATGCCCGTCGACCCGTATCCAACAGATCCGTGGAACAATCGTAAATACTGGCCCACAGGCTGGGGTCAACTTACAAATgtgagtaattgtacgggctgtgattcaacaaa 360
tcttactcataattatttgtcagCGTGGCAAGCAACAACACTACGAGCTGGGCAAATGGCTTCGAAAACGTTACAGCTCGTTGTTGGACGCCAGGTACGACAGGGAGCAGGTCTTTGTTC 480
AGTCCACGGATGTGGATCGCACACTCATGAGTGCACAGTCAAACTTGGCTGGCTTATACGAGCCTGTTGGCAACGATGTCTGGAATCCCCTGATCAAATGGCAGCCAATTCCGGTGCATT 600
CCGTGCCCGAAAAGGATGATCCGgtaggggtatctcacgtattaaatttgttagatctgttaaataataatcagaaatttcctttgaacagGTTCTGGCTGCCAAGGCACCTTGTCCAGC 720
CTTCGACTACTATCTGGAAAACTTTAAGGCATCCGCCGAGTTTAAGGCCAAGTGGGCACGTTACAAAGATCTCTTTGATTATTTGGGACAAAATAGCGGTCGGCCCATTAAGTCATTTAT 840
GGATGCACAATATTTCAACAACACATTGTTCATTGAAACCCTGTACAACCGAACTCTGCCCGAATGGGCACAAAAGGTTTACGGCGGCGCTGACCTGACCTACGTCTCGAATTTTGCCTT 960
CTCCATAAATACCTACACACGGTCAGCTGGCGCGCCTAAAGGGAGGACCGCTCCTCAAGGATGTGCTCACCCGTTTGGAGAGCAAGCATAAGAAACAACTGTCGCCGGATCGGTCTGTGTT 1080
TATATACAGCGCACATGATACCACAATTGCTAACATGTTGAATACCCTCAAGCTGTTTCAGgtataaggaaataaccaattataactagcttaagccttgtagagggtctactaatcagg 1200
catctccgaatccataaattatatatatggtattgcagataagtatcatcaaggccgagtcgatagccaagtccatctgtctcagctttaaagctatcgtcatgaaactgcataaatcca 1320
tatttctattgctcgcattacatatgtcggagccggctgaatcggacgactttatcatatagctgccataggaacgatcggtcgaacagtaggttcttgtatagaaaggtttttgtttta 1440
gcagatatcttgaccaaggtcggcattgagaggtttcacttagaggaccaatatattataaagctgccataggaacgatcggtcaaaaatagattcttgtatgattttgatttgatttga 1560
ttttgatttgaaagctatcttcacgaaatttttgatatgttcgcctttatttttcaggcagagcttctgtttcgaaacatttttttcttttcaatataccttcgtatattcgtatcttcgg 1680
cgtgccgagtgattttatcattctattgcttcagtttagttttaattcccatttatttttttgtcatcttccagCTGCACAGTCCGCCGTACACGGCGTGCATAATGTTCGAATTGCGTGT 1800
GGATGATAACAACAGGCCCTTGGTTTCCGTATTCTATAAAAACACGACAGCCGAACCGCTGCCAATGTATATACCCGGCTGCGGTGTATCCTGCCCGCTGTCAAAGATGTATGCACTGTA 1920
TGAGGATGTCTTGCCGGAGAACTGGACGGATGAGTGCAAGCGATCCACACTGACAATGACATACGAGGAGGCCAATTTGGGCGCAGCAACAGgcaagttatcgtagaaaaactgggataa 2040
tgcagagtatacataaaaatatctatagttctttatgaatatgttaagtactttaagtattaaataattgagcagacatttaactataattaaaaatatattcttgcagGCATTCTGATA 2160
TGCATCATTGCCGCTCTGCTATGCGTCAGCTATGGCCTGATGATCTACTATCGTCGCCGGGATTACAACATGCACACTTCCTATGCGCAAATGGCGTAAattagggcatggctaggatgg 2280
ctgggatgcttattgaaagactacgccaacttcctgaattaagatcgagttattgctcagctttagccacttatttttatatatctccccttgtctttgattgaatgccattaattattt 2400
gtttaagtacgactattgtttgtgctccatatatgtacgtatttggtgcaacaattgtaaatgtaaatttagattgagttattgcgctcggcttcagatctattataaatctccccatgt 2520
ctttgtttaaatgccattaattctttaattaatgttaattgtttgtgtacgcattgtgtgcgataattgtaaatgtatattgttaagctcatatgatcttgtttcttttgttaaacgacg 2640
acaaaacaaagactgtgaatggttaccctgaaagtttcttgtacacatttacatgtgtcccatattatatatatcatagattagtaaaaatatatacgtgtatagatgaatatatttgca 2760
tttacgagtataggccttttatgaaaacatcttgttgtacaaaccatttttgtgaactgtaataaaaaacgaaaacgggctcaaatttaaactgaaagtctgttgtattaaattaaata 2880
aaacctgttgcaaagtgtatacgaaagatttgggcaaaggttagtccagtaattaaaagaaacaagaggttatagtcgggagctcccgactagggataccctaaaccctcttcttccaaa 3000
atcaaatgcatatatatatatttatttagaagctatatgtcaagtttggtgactctagctcttattatttaccaaaattgcccaaaaaacaggatatcgatatcgatttttatcgatt 3120
gcttggaaacggagtaagttatcgattatcggaaataaacttgatctgcgcgggaactgggagaacctacatctaaaatttcaagtctagctcttataggttctgagatccttgcgtcca 3240
tacatagggacagacggacggacggacagtcagaaggacggacggacggacggacagacagacagacagacggacatggcttgatcgacttggctattgatgatgatcaagaatatatat 3360
actttatggggtcggagatgcttccttctgcctgttacatacatttggattttgcacaaacacaatatacccttatttccatttttaatgggttcatggtataaaaaatgaatttttaca 3480
aaaatcgttctatttttttagatgcaattttaggaagcgcaactggaaaagattatagctaatataatctctgcaaagaaagacattttcatttacaagttcaggaaaggctcaaatc 3600
aaagcctcggcttaaggttcagctcgcgacttcgagcagcaaataagataactattataattttaatcgcttctaatattatactataatttgtttattacgcgtgtgcgatttgtcgac 3720

Figure 1 (facing page). The nucleotide sequences of the *Acph-1* gene of *D. virilis*. The coding and noncoding sequences are shown in uppercase and lowercase letters, respectively. The nucleotides that vary from those of the *Acph-2* are shown by shadow. The transcriptional start sites and poly-A signals deduced from those of the *Acph-2* are boxed. Repeated sequences in the 3' region are underlined.

The non-coding sequences in the *Acph-1* differed from those of the *Acph-2* by 7 single-base changes and another one change involving more nucleotides. In the 3' region, gacg and gaca repeated sequences were present at the 449 bp downstream of the second poly-A signal (Figure 1, underlined). Whereas 13 repetitions of the gacg motif were identified by the additional sequence analyses of this region in the *Acph-2* gene (data not shown). These facts suggest that the activity difference between the ACPH-1 and the ACPH-2 is not due to the structural differences of the enzyme proteins, but rather to the transcriptional activity difference of these alleles. Recently, several studies have reported that tandemly repeated sequence motifs may have a function in transcription; the repeated sequences increased the basal transcription up to 9 fold *in vitro* (Meloni *et al*.,1998) and the alteration of stretches of the repeat affected the transcriptional activity (Albanese *et al*., 2001). If it is the case with the *Acph* locus, the difference of the motifs and stretches of the tetranucleotide repeats between the *Acph-1* and the *Acph-2* may change the transcriptional activity. It is necessary to examine the effect of variation of the tetranucleotide repeats on the quantitative difference of the three types of transcripts in the *Acph* genes.

References: Albanese, V., N.F. Biguet, H. Kiefer, E. Bayard, J. Mallet, and R. Meloni 2001, Hum. Mol. Genet. 10: 1785-1792; Kitagawa, H.T., 2003, DNA seq. 14: 135-139; Meloni, R., V. Albanese, P. Ravassard, F. Treilhou, and J. Mallet 1998, Hum. Mol. Genet. 7: 423-428; Narise, S., 1976, Jap. J. Genet. 51: 428; Narise, S., and H.T. Kitagawa 2001, Dros. Inf. Serv. 84: 24-27; Ohba, S., 1977, Population Genetics. UP Biology Series, Tokyo University Press, Tokyo, pp 99-104.

Drosophilid fauna so far described and recorded from Kumaon region, Uttaranchal, India.

Upadhyay, Kamla[1], and B.K. Singh[2]. Cytogenetics Laboratory, Department of Zoology, Kumaon University, Nainital-263002, Uttaranchal, India; [1]kamla_zoo@yahoo.com, [2]bksingh_dsb@yahoo.com

Introduction

The study of systematics of Drosophilidae in Kumaon region was started in 1984 by Singh and Bhatt. Since then a number of Drosophilid species have been described and recorded from this region (Singh and Negi,1989, 1992, 1995; Singh and Dash, 1993, 1998; Singh, Dash, and Fartyal, 2000, 2004; Singh and Fartyal, 2002; Fartyal, Singh, and Toda, 2005; Joshi, Fartyal, and Singh, 2005). The Kumaon region includes six border districts of the state Uttaranchal, *viz*., Nainital, Almora, Pithoragarh, Bageshwar, Champawat, and Udham Singh Nagar. This area is characterized by having dense evergreen coniferous forest with medium to very steep slopes and extremely moist condition due to heavy rainfall. The present paper deals with all the new species and new records of Drosophilidae so far known from Kumaon region in Uttaranchal.

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

Genus/Subgenus	Species	Collection locality
Genus- *Amiota* Loew	1. *bandes* Singh & Negi, 1992	Okhalkanda, Sargakhet,Chaubattia garden, Bageshwar, Amsysri,Nainital.
Subgenus- *Phortica* Schiner	2. *biprotrusa* Chen & Toda, 1998	Nainital,Gahna.
	3. *pseudotau* Toda & Peng, 1990	Mukteshwar.
Genus- *Dettopsomyia* Lamb	4. *nigrovittata* Malloch, 1924	Latoli, Lariyakanda, Kailakhan, Champawat.
Genus- *Dichaetophora* Duda (New record)	5. *acutissima* Okada, 1956 (New record)	Kausani.
Genus- *Drosophila* Fallén		
Subgenus- *Dorsilopha* Sturtevant	6. *busckii* Coquillett, 1901	Nainital, Almora, Dunagiri, Dwarahat, Ranikhet, Chaukhutia, Kausani, Pinath, Amsyari, Pithoragarh.
Subgenus- *Drosophila* Sturtevant	7. *actinia* Okada,1991	Nainital, Dhari, Pithoragarh, Amsyari.
	8. *analspina* Singh & Negi, 1995	Nainital,Letibuga, Kausani, Chaubattia garden.
	9. *bageshwarensis* Singh, Dash & Fartyal, 2004	Pithoragarh, Lohaghat, Ranikhet, Amsyari, Bageshwar.
	10. *bishti* Singh & Negi,1995	Nainital, Okhalkanda, Sargkhet, Ranikhet.
	11. *bizonata* Kikkawa & Peng, 1938	Nainital, Mukteshwar, Kausani.
	12. *dwarahatensis* (New species)	Dwarahat.
	13. *elongata* Singh, Dash & Fartyal, 2004	Nainital, Dhari.
	14. *hexaspina* Singh, Dash & Fartyal, 2004	Nainital, Dhari.
	15. *hydei* Sturtevant, 1921 (New record)	Kausani, Dwarahat, Nainital.
	16. *immigrans* Sturtevant,1921	Nainital,Sheetla, Ranikhet, Dunagiri, Dwarahat, Chaukhutia, Almora, Pithoragarh, Gangolihat, Kausani, Pinath, Amsyari.
	17. *khansuensis* Singh, Dash & Fartyal, 2004	Nainital, Tanakpur, Chaukhutia, Kausani.
	18. *lacertosa* Okada, 1956	Nainital,Dhari ,Ranikhet, Dunagiri, Dwarahat, Chaukhutia, Almora, Pithoragarh, Gangolihat, Kausani, Pinath, Amsyari, Rudarpur.
	19. *mukteshwarensis* Joshi, Fartyal & Singh,2005	Mukteshwar.
	20. *nainitalensis* Singh & Bhatt, 1988	Ganguachaur, Nainital, Nagpani Chaubattia.
	21. *nasuta* Lamb, 1914	Nainital, Haldwani, Rudrapur.
	22. *notostriata* Okada, 1966	Kashialekh, Nainital.
	23. *paharpaniensis* Singh, Dash & Fartyal, 2004	Nainital ,Okhalkanda, Ranikhet.
	24. *painai* Singh & Negi, 1995	Bhatelia, Nainital, Bhowali, Almora, Dunagiri, Kausani.

	25. *paramarginata* Singh, Dash & Fartyal, 2004	Nainital, Okhalkanda, Champawat (Mayawati Ashram).
	26. *parazonata* Dwivedi & Gupta, 1980	Nainital.
	27. *paunai* Singh & Negi, 1989	Sargakhet,Nainital, Kausani, Pithoragarh, Almora, Ranikhet.
	28. *repleta* Wollaston, 1858	Latoli,Nainital,Lalkuan, Dunagiri, Dwarahat, Ranikhet, Kausani.
	29. *repletoides* Hsu, 1943 (New record)	Dwarahat.
	30. *serrata* Singh, Dash & Fartyal, 2004	Chaubattia garden, Ranikhet.
	31. *sulfurigaster* Duda,1923	Letibuga, Nainital, Pithoragarh, Bageshwar.
	32. *surangensis* Singh, Dash & Fartyal, 2004	Nainital.
	33. *tetradentata* Singh & Gupta, 1980 (New record, Uttaranchal)	Dunagiri, Kausani.
Subgenus- *Sophophora* Sturtevant	34. *trizonata* Okada, 1966	Dhari, Nainital, Bhowali, Dhari, Khansu.
	35. *bifasciata* Pomini, 1940	Sheetla, Nainital,Haldwani, Dunagiri, Kausani,Lohaghat, Tanakpur, Pithoragarh.
	36. *hubiensis* Sperlich & Watabe, 1997	Sargakhet, Nainital, Dunagiri, Kausani.
	37. *jumbulina* Parshad & Paika, 1964	Dhari, Nainital, Dwarahat, Chaubattia Garden, Chaukhutia,Gangolihat.
	38. *kikkawai* Burla, 1954	Sheetla, Nainital,Ranikhet, Dunagiri, Dwarahat, Chaukhutia, Bageshwar, Kausani, Gangolihat.
	39. *malerkotliana malerkotliana* Parshad & Paika, 1964	Pithoragarh (Ghat), Champawat (Mayawati Ashram), Haldwani, Dunagiri, Kausani, Nainital.
	40. *melanogaster* Meigen, 1930	Gahna, Nainital, Sat Tal, Dwarahat, Chaukhutia, Almora, Kausani, Pithoragarh,Rudrapur, Binsar Mahadev (Almora).
	41. *neobaimai* Singh & Dash, 1998	Nainital, Dunagiri, Kausani, Kanalichchina(Pithoragarh).
	42. *neokhaoyana* Singh & Dash, 1998	Nainital, Gangolihat, Dhari.
	43. *nepalensis* Okada, 1955	Nainital, Almora, Ranikhet, Dunagiri, Dwarahat,Chaukhutia, Pithoragarh, Kausani, Pinath, Amsyari.
	44. *punjabiensis* Parshad & Paika, 1964	Nainital, Chaubattia garden.
	45. *saraswati* Singh & Dash, 1995	Kausani,Mukteshwar, Pithoragarh, Bageshwar, Gangolihat.
	46. *sargakhetensis* Joshi, Fartyal & Singh, 2005	Sargakhet.
	47. *suzukii indicus* Parshad & Paika, 1964	Latoli, Nainital, Almora, Ranikhet, Dwarahat,Chaukhutia, Amsyari, Kausani, Gangolihat.
	48. *takahashii* Sturtevant, 1927	Bhatelia, Nainital, Ratighat, Almora,Ranikhet, Dwarahat, Dunagiri, Gangolihat ,Amsyari, Kausani.
Genus- *Gitona* Meigen	49. *distigma* Meigen, 1830	Dhari, Nainital, Lariyakanda.
Genus- *Hirtodrosophila* Duda	50. *hexaspina* Fartyal & Singh, 2000	Letibuga, Nainital.
	51. *quadrivittata* Okada, 1956	Sheetla, Nainital.

Genus- *Leucophenga* Mik	52. *albiceps* de Meijere, 1914	Ganguachaur, Nainital, Lariyakanda, Chaubattia garden, Dunagiri, Kausani, Gangolihat.
	53. *angulata* Singh, Dash & Fartyal, 2000	Gahna, Nainital, Dunagiri, Kausani, Pinath. Sargakhet.
	54. *angusta* Okada, 1956	Nainital, Pithoragarh.
	55. *argentata* de Meijere, 1914 (New record)	Dunagiri, Dwarahat, Gangolihat.
	56. *bellula* (Bergroth, 1894)	Mukteshwar, Nainital, Ratighat, Ranikhet, Dunagiri, Kausani, Pinath, Amsyari.
	57. *champawatensis* Fartyal, Singh and Toda, 2005	Nainital, Kausani, Champawat.
	58. *chaubattiaensis* Fartyal, Singh and Toda, 2005	Chaubattia garden, Ratighat, Nainital.
	59. *clubiata* Singh, Dash & Fartyal, 2000	Dhari, Nainital, Bhowali, Dunagiri, Kausani.
	60. *kumaonensis* Fartyal, Singh and Toda, 2005	Nainital.
	61. *nainae* Fartyal, Singh & Toda, 2005	Nainital, Chaubattia garden.
	62. *neolacteusa* Singh & Bhatt, 1988	Latoli, Nainital, Chaubattia garden.
	63. *neointerrupta* Fartyal, Singh & Toda, 2005	Nainital,Pilkholi, Dwarahat, Kausani, Pinath.
	64. *okhalkandensis* Singh, Dash &Fartyal, 2000	Nainital, Ranikhet, Chaubattia garden,Dunagiri, Kausani.
	65. *ornata* Wheeler, 1959	Kashialekh, Nainital, Chaubattia garden, Dunagiri, Kausani.
	66. *quadripunctata* de Meijere, 1908	Nainital.
	67. *subpollinosa* de Meijere, 1914	Sheetla, Nainital, Bhowali,Chaubattia garden.
	68. *trispina* (New species)	Kausani.
Genus- *Lissocephala* Malloch	79. *parasiatica* Takada & Momma, 1975	Mukteshwar, Nainital, Almora, Ranikhet, Pithoragarh.
Genus- *Lordiphosa* Malloch, (New record, Uttaranchal)	70. *tripartita* Okada 1966 (New record)	Dunagiri (Almora).
Genus- *Microdrosophila* Malloch, P (New record, Uttaranchal)		
Subgenus- *Microdrosophila*	71. *bamanpuriensis* (New species)	Dwarahat (Almora).
Genus- Paraleucophenga Hendel	72. *neojavanaii* Singh & Negi, 1992	Dhari, Nainital, Ratighat.
	73. *todai* Fartyal & Singh	Nainital.
Genus- *Scaptomyza* Hardy	74. *elmoi* Takada, 1970	Latoli, Nainital, Pithoragarh.
	75. *himalayana* Takada, 1970	Nainital, Almora, Ranikhet, Dwarahat, Dunagiri,Chaukhutia, Pithoragarh, Gangolihat, Kausani, Pinath, Amsyari.
	76. *quadruangulata* Singh & Dash, 1993	Letibuga,Bhimtal, Sat Tal, Ramnagar, Nainital, Ranikhet, Dwarahat, Dunagiri, Chaukhutia, Pithoragarh, Gangolihat, Kausani.
Genus- *Scaptodrosophila* Duda	77. *chandraprabhiana* Gupta & Ray-Chaudhuri	Nainital, Bageshwar,Kausani, Champawat, Lohaghat.
	78. *coracina* Kikkawa & Peng, 1938	Nainital, Bhatelia, Champawat, Almora, Dunagiri, Kausani.

	79. *hirsuata* Singh & Dash, 1998	Sargakhet, Haldwani, Nainital, Bageshwar, Amsyari, Rudrapur, Tanakpur.
	80. *subtilis* Kikkawa & Peng, 1938 (New record, Uttaranchal)	Kausani.
Genus- *Stegana* Meigen	81. *nainitalensis* Singh & Fartyal, 2000	Nainital.
Genus- *Zaprionus* Coquillett	82. *indianus* Gupta, 1970	Mukteshwar, Nainital, Almora, Ranikhet, Dwarahat, Dunagiri, Chaukhutia, Pithoragarh, Gangolihat, Kausani, Pinath, Amsyari.

Materials and Methods

This study is the result of Drosophilid collection made in different geographical localities of Kumaon region since July 2003 to July 2005 (warm temperate zone, Nainital, Kausani, Pinath, Ranikhet and Dunagiri; subtropical zone, Dwarahat, Chaukhutia and Amsyari; and transitional zone, Pithoragarh and Gangolihat). The collections were largely made by net sweeping over wild vegetation and by trap bait method. The flies were then preserved in 70% alcohol and examined for their head, thorax, and abdomen under the microscope.

Observations

Total of 82 species (Table-1) belonging to 15 genera of the family Drosophilidae were collected, out of which 3 species were described as new to science, 5 species were recorded for the first time from India and 2 species were recorded for the first time from Uttaranchal. Genus *Lordiphosa* Malloch and genus *Microdrosophila* Malloch were recorded for the first time from Uttaranchal, while genus *Dichaetophora* Duda was recorded for the first time from India.

Acknowledgments: The authors express their gratitude to Dr. M.J. Toda, of Low Temperature Science, Hokkaido University, Sapporo, Japan for confirming the identification of specimens. One of us, Kamla Upadhyay, is thankful to Kumaon University for granting SILVER JUBILEE FELLOWSHIP during the course of this study.

References: Singh, B.K., and N.S. Negi 1989, Proc. Zool. Soc. Cal. 40: 19-26; Singh, B.K., and N.S. Negi 1992, Senckenbergiana Biol. 72: 321-327; Singh, B.K., and N.S. Negi 1995, Entomol. Z. 105 (21): 421-440; Singh, B.K., and S. Dash 1993, Proc. Zool. Soc. Cal. 46: 131-140; Singh, B.K., and S. Dash 1998, Proc. Zool. Soc. Cal. 51: 45-56; Singh, B.K., S. Dash, and R.S. Fartyal 2000, Senckenbergiana Biol. 80 (1/2): 149-154; Singh, B.K., S. Dash, and R.S. Fartyal 2004, Senckenbergiana Biol. 83 (2): 163-176; Singh, B.K., and R.S. Fartyal 2002, Proc. Zool. Col. 50 (1): 11-18; Fartyal, R.S., B.K. Singh, and M.J. Toda 2005, Entomol. Sci. 8: 405-417; Joshi, M., R.S. Fartyal, and B.K. Singh 2005, Entomon. 30 (1): 75-84.

Call for Papers

Submissions to Drosophila Information Service are welcome at any time. The annual issue now contains articles submitted during the calendar year of issue. Typically, we would like to have submissions by 15 December to insure their inclusion in the regular annual issue. but articles can be accepted for this volume until 31 December. Details are given in the Guide to Authors.

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

Marshall R. Wheeler

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

Some issues were given titles and subtitles, but the Publication Number (*e.g.*, UTP 4213) is the best reference. This is the complete list of all the publications:

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

This announcement is reprinted from 2002, *Dros. Inf. Serv*. 85: 106-108.

Ethanol attraction and survival in adult *Drosophila pseudoobscura.*

Ortiz-Barrientos, Daniel*, Eric T. Watson*, and Mohamed A.F. Noor. Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803 USA; *These authors contributed equally; Corresponding author: E-mail: danielo@interchange.ubc.ca.

Abstract

Resource use in phytophagous insects can influence survivorship as well as the ability to find mates. Therefore, the ability to find a resource and the potential to survive in its presence can have important effects on micro-evolutionary processes in insect populations. Here, we study tolerance of and attraction to varying concentrations of ethanol in *Drosophila pseudoobscura* adult flies. We document substantial heritable variation for ethanol attraction and tolerance among outbred strains and observe a borderline significant correlation between attraction and tolerance. We also note significantly greater survivorship of females over males when exposed to low concentrations of ethanol.

Introduction

Phytophagous insects choose potential hosts based on a variety of ecological cues (*e.g.*, chemical attractants, plant defenses, food availability, or flowering time). These cues may affect host detection, the probability of finding mates, and possibly the performance of eggs and larvae laid during such visits (Thompson, 1994). A good example of this dynamic comes from sympatric host

races of the fruit fly *Rhagholetis pomonella*, where detection of hosts is mediated by chemical volatiles produced by apple or hawthorn trees. Detection of the appropriate host is associated with higher fitness, as offspring from individuals that lay eggs in the alternative host may experience diapause conditions disfavored during winter (Feder *et al.*, 1988; Feder *et al.*, 1994; Feder *et al.*, 1997; Filchak *et al.*, 2000; Feder *et al.*, 2005). In addition, individuals that visit the alternative host could potentially mate with the other host race and produce maladapted hybrids that cannot easily detect either host (Linn *et al.*, 2004). This system illustrates how cues from a host can affect the ability to choose food resources, mating arena, and possibly an advantageous environment for the development of their offspring. Other dynamics are also likely (Jaenike, 1990; Berlocher and Feder, 2002), so studying attraction to, and performance in the presence of, chemicals produced by plants or other food resources is an important step to understanding coevolutionary dynamics between phytophagous insects and their hosts. Here, we investigate the behavioral and physiological responses of adult *Drosophila pseudoobscura* to varying concentrations of ethanol, an important chemical produced by food resources visited by fruit flies.

Many food resources consumed by *Drosophila* ferment and produce ethanol in concentrations up to 11% (Gibson *et al.*, 1981). Because ethanol is both a resource and a toxin (Parsons and Spence, 1981; Fry *et al.*, 2004), *Drosophila* species may exhibit variation in their attraction and tolerance to ethanol. Comparative studies of the *Drosophila* genus have shown that certain species like *D. lebanonensis* can tolerate concentrations of ethanol as high as 25% before half of the study sample dies, while *D. metzei* can tolerate no more than 1% (Chakir *et al.*, 1993). These results and others show that *Drosophila* species feeding at wineries are more tolerant to ethanol than those species feeding on fermenting fruits or mushrooms (Gibson and Wilks, 1988; Mercot *et al.*, 1994). Similarly, attraction studies have shown that *Drosophila* species from the tropics are less attracted to ethanol than species from temperate zones (Parsons, 1980), and there is variation within and among species in oviposition preference for substrates containing ethanol (McKenzie and Parsons, 1972; Parsons and King, 1977; Eisses and Santos, 1997). These physiological and behavioral responses can dramatically affect the ecology of *Drosophila* species and in turn modulate their adaptation to new hosts and mating arena.

Theory predicts that an organism's ability to detect a resource should positively correlate with the effect of that resource on viability (Thompson *et al.*, 1990; Via, 1990; Futuyma, 1991). This correlation could result from higher quality of offspring developing in hosts preferred by parents, or from a covariance between ability to detect a resource and the time spent on the resource while eating or waiting for mates; this could also be understood in terms of selection to prefer a host with the optimal amount of a resource. Both scenarios can create significant variation in attraction to and tolerance of resources that vary among hosts either spatially or temporally. For example, variable sunflower condition due to herbivory affects preference and performance of feeding grasshoppers, since grasshoppers tend to prefer and perform better in sunflowers with less damage (Lewis, 1983). Likewise, fermenting fruits whose ethanol concentrations vary with time can affect both attraction and tolerance to ethanol if insects spend long periods of time feeding on the resource and waiting for mates. These responses could vary among populations and among sexes, but this issue has not been studied in detail within most *Drosophila* species.

Here, we study tolerance and attraction to varying concentrations of ethanol in insects using the North American fruit fly *Drosophila pseudoobscura* as a model system. Although the evolutionary genetics of the system is vastly developed, we still have a poor knowledge of its ecology (Powell, 1997). Nonetheless, this species has a broad geographic range and is found in a diversity of habitats across the western half of the continent, making it an ideal system for studying intraspecific variation in these fitness-related traits. We evaluate attraction and performance in outcrossed lines derived from multiple distinct populations. We have included lines derived from populations in

California, where wineries are common, and from more inland areas. We test the hypothesis that tolerance and attraction to ethanol are positively correlated and explore the potential differential behavior of the sexes across ethanol concentration.

Materials and Methods

Drosophila *stocks*

Inbred lines were established from a set of isofemale lines collected by Noor and Ortiz-Barrientos (Noor, 1995; Noor *et al.*, 2000; Ortiz-Barrientos, unpublished) from various locations in the western United States. Locations relevant to this study included: Flagstaff, Arizona; Squaw Peak, Utah; Mather, California; Mount St. Helena, California; and Mesa Verde, Colorado. We used 10 lines of *D. pseudoobscura* (Flagstaff14, Flagstaff6, Mather16, Mather97, MSH7, MSH12, Squaw Peak18, Squaw Peak4, Mesa Verde17, and Mesa Verde22) to generate outbred strains for our assays, and F_1 progeny of crosses between strains from a single population were used directly in all assays here.

We maintained flies on a standard dextrose-yeast-agar medium in an incubator kept at 18ºC on a 12:12 light-dark cycle. Virgins of each line were collected within 8 hours of eclosion and stored for 7 days prior to each experiment.

Ethanol attraction

Y-maze bait competitor traps were constructed from clear plastic tubing, Y-connectors, and microcentrifuge tubes such that only entry into the trap area (tube) was permitted (maze design elaborated from Woodard *et al.*, 1989). Care was given in the construction of the traps so that all path lengths were equal. In each of the vial traps, yeast-based fly culture medium was placed in the distal end to provide food for trapped flies and to correct for food attraction alone. Two treatments were used to bait the traps: one water-based, and one ethanol-based, both in an agarose vehicle. 1g of agarose was heated and dissolved in 100ml of water+ethanol to a final concentration of 0% (none), 9% (low), or 20% (high) ethanol. 1ml of the resultant agarose gel was used in each trap. With each Y-maze set up with water and an ethanol trap, virgin flies were placed by themselves in the base of the maze and scored 24 hours later. Flies tested in this manner were anesthetized with CO_2 a total of two times: once for separation of sexes upon collection on day 1 and once very briefly for placement into the entrance of the maze on day 7. After 24 hours, only flies trapped in the vials or less than 1 cm from the entrance of the vials were scored – an average of 90% of the flies were thus scored. We present in the Results the proportion of such flies that were observed in or beside the ethanol-containing trap, relative to the random movement expectation of 50%.

Adult ethanol tolerance

To assay for the effects of ethanol on adult survival, flies were maintained in vials containing ethanol-supplemented dextrose-yeast-agar medium. Into each vial, we placed 15 flies of the same sex and line and recorded the number dead after 24 hours. Each line was tested at 0%, 9%, and 20% ethanol supplemented medium simultaneously. Flies tested in this manner were similarly anesthetized with CO_2 a total of two times, and for as little time as possible. We present in the Results the percentage of flies that survived.

Results

We investigated how *D. pseudoobscura* adult flies are attracted to and tolerate ethanol at high and low concentrations. Briefly, we found that some strains of flies are attracted to ethanol, exposure

to ethanol affects their survival, and the flies exhibited hereditary differences in their specific responses to ethanol. Results are presented in Table 1.

Table 1. Levels of tolerance (% surviving flies) and attraction (% released flies attracted to ethanol-containing trap) to varying concentrations of ethanol in *D. pseudoobscura* populations from California [C] or more inland [I] regions including Arizona, Colorado and Utah. Parentheses contain sample sizes (n) for tolerance and attraction experiments at each concentration, respectively (n is equal for both 9% and 20% tolerance treatments, but different between the two attraction treatments).

	Population	Tolerance		*Attraction*	
		9%	*20%*	*9%*	*20%*
I	Flagstaff 6x14 (n=165; 31, 255)	78	4	63	42
I	Mesa Verde 17x22 (n=155; 74, 238)	64	7	59	51
I	Squaw peak 18x4 (n=150; 43, 201)	50	5	49	52
C	MSH 7x12 (n=125; 51, 244)	75	4	67	73
C	Mather 97x16 (n=270; 54, 294)	37	0	54	63

Ethanol attraction in adult D. pseudoobscura

Only the MSH line exhibited significantly greater attraction to the ethanol trap over the water control at the low concentration (9%: $\chi^2 = 5.7$, df = 1, $p = 0.017$). At the higher ethanol concentration (20%), the Flagstaff strain exhibited a significant aversion to the ethanol trap ($\chi^2 = 5.9$, df = 1, $p = 0.015$), while the two strains from California, Mather and MSH, exhibited significant attractions to the ethanol trap (Mather: $\chi^2 = 18.5$, df = 1, $p < 0.0001$; MSH: $\chi^2 = 53.1$, df = 1, $p < 0.0001$). Chi-square analyses of variation among strains for ethanol attraction were nonsignificant at low concentration ($p > 0.3$) but highly significant at high concentration ($\chi^2 = 57.6$, df = 4, $p < 0.0001$, see Table 1). Hence, this species bears variation for attraction to ethanol. However, we did not observe any sex-specific attraction to ethanol (Wilcoxon signed rank test, $p = 0.5$).

Ethanol tolerance in adult D. pseudoobscura

In general, adult flies derived from the 7 different strains studied survived better on low (9%) concentrations of ethanol than in high concentrations (20%) (Mann-Whitney U-test, $p = 0.009$, see Table 1). Chi-square analyses of variation among strains for ethanol tolerance were significant at low ($\chi^2 = 26.6$, df = 4, $p < 0.0001$) and high ($\chi^2 = 16.4$, df = 4, $p = 0.0025$) concentrations (see Table 1), suggesting that this species also bears variation for tolerance of ethanol. Ethanol attraction and tolerance did not significantly positively correlate when flies were exposed to high concentrations of ethanol (20%, $r = 0.33$, $p = 0.6$), likely resulting from the low variance in survival, but there was a strong and borderline statistically significant correlation when considering flies that were exposed to low concentrations of ethanol ($r = 0.86$, $p = 0.063$, see Figure 1). We did observe significantly greater tolerance of females relative to males in low concentrations of ethanol (Wilcoxon signed rank, $p = 0.043$): in every strain, a greater fraction of females relative to males survived, and the average difference was about two-fold.

Discussion

Phytophagous insects depend on plant associations for their survival. Plant-insect associations require cues that allow the insects to detect food resources, mating arenas, and survive on the host while eating or mating (Hassell and Southwood, 1978; Futuyma and Peterson, 1985; Via,

1990; Futuyma, 1991). In this study, we have shown that *D. pseudoobscura* strains exhibit variation in their ability to detect and survive in ethanol, a common chemical in *Drosophila* food resources (Gibson *et al.*, 1981). This variation relates to gender and the levels of ethanol present in the traps, and possibly to geography. We also observed a borderline significant correlation between attraction to 9% (low) ethanol and adult survivorship on 9% ethanol (Figure 1), as predicted by adaptive theories on resource use (Futuyma and Peterson, 1985; Thompson *et al.*, 1990; Via, 1990).

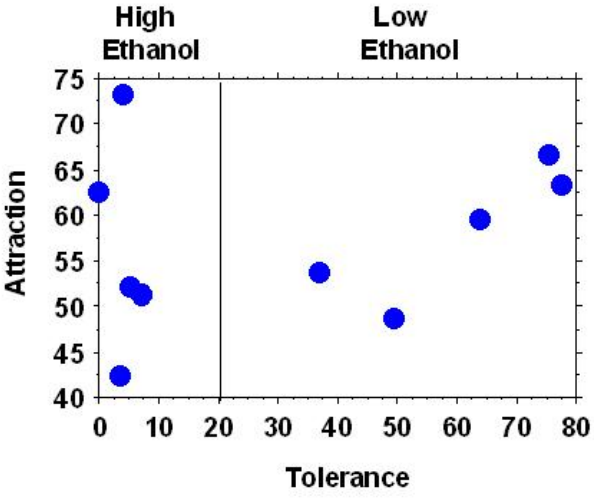

Figure 1. Relationships between survival on and attraction to ethanol in *D. pseudoobscura* strains according to different concentrations of ethanol.

The variation in attraction to high concentrations of ethanol among strains of *D. pseudoobscura* was striking, wherein some strains exhibited statistically significant attraction while others exhibited significant repulsion. Interestingly, we did observe a geographic correlation of attraction vs. repulsion: flies derived from California populations were strongly attracted to high concentrations of ethanol, while those from inland populations either exhibited no significant preference or a slight repulsion. This possible interaction between geographic origin and ethanol concentration could result from differences in vegetation: ethanol concentrations at *D. pseudoobscura* food sources may differ between the two areas, possibly due to the presence of a large number of wineries in California, where *D. pseudoobscura* has been collected in large numbers (Mercot *et al.*, 1994). However, if this finding is general, geographic differences between California flies and those from inland regions exist despite very high levels of gene flow among populations (Prakash *et al.*, 1969; Schaeffer and Miller, 1992; Noor *et al.*, 2000). Other behavioral differences among flies derived from California and inland regions are known in this species, and evidence suggests that natural selection is responsible for maintaining such variation (Noor, 1995).

Males and females can respond differently to environmental cues (*e.g.*, Selander, 1966). In our study, we found that females tolerate higher concentrations of ethanol than males. These differences do not appear to result from changes in life-time span between males and females, since the proportion of surviving males or females in negative control experiments (traps with water) is similar (data not shown). However, it is likely that females tolerate longer periods of exposure to ethanol because they tend to be larger than males and, therefore, would not dry as fast as males. Tolerance of varying concentrations of ethanol appears to have an important genetic component, however, so it is possible that other factors besides body size contribute to this difference between the sexes.

In this study, we assayed only survivorship on varying concentrations of ethanol only in adult flies. Larval survivorship may be more greatly affected by ethanol than adult survivorship, as the larvae are completely immersed in the rotting fruits and would have greater exposures. Future work in this system should explore the extent of correlation between adult and larval tolerance of ethanol to obtain a more complete understanding of this important resource and toxin.

Acknowledgments: We thank J. Feder, N. Kandul, and K. Teeter for helpful comments on this manuscript, and A. Chang technical assistance. This work was supported by a National Science Foundation REU supplement for undergraduate students to ETW, Sigma Xi grant to DOB, and National Science Foundation grants 0211007, 0314552, 0509780, and EPS-0346411, and Louisiana Board of Regents Governor's Biotechnology Initiative grant 005 to MAFN.

References: Berlocher, S.H., and J.L. Feder 2002, Annu. Rev. Entomol. 47: 773-815; Chakir, M., O. Peridy, P. Capy, E. Pla, and J.R. David 1993, Proc. Natl. Acad. Sci. USA 90: 3621-3625; Eisses, K.T., and M. Santos 1997, Dros. Inf. Serv. 80: 29-30; Feder, J.L., C.A. Chilcote, and G.L. Bush 1988, Nature 336: 61-64; Feder, J.L., S.B. Opp, B. Wlazlo, K. Reynolds, W. Go *et al.*, 1994, Proc. Natl. Acad. Sci. USA 91: 7990-7994; Feder, J.L., U. Stolz, K.M. Lewis, W. Perry, J.B. Roethele *et al.*, 1997, Evolution 51: 1862-1876; Feder, J.L., X. Xie, J. Rull, S. Velez, A. Forbes *et al.*, 2005, Proc. Natl. Acad. Sci. USA 102: 6573-6580; Filchak, K.E., R.B. Roethele, and J.L. Feder 2000, Nature 407: 739-742; Fry, J.D., C.M. Bahnck, M. Maryann, N. Phadnis, and W.C. Slattery 2004, Integ. Comp. Biol. 44: 275-283; Futuyma, D.J., 1991, In: *Plant-Animal Interactions: Evolutionary Ecology in Tropical and Temperate Regions* (Price, P.W., T.M. Lewinsohn, G.W. Fernandes, and W.W. Benson, eds.), pp. 431-454. John Wiley & Sons, Inc.; Futuyma, D.J., and S.C. Peterson 1985, Annu. Rev. Entomol. 30: 217-238; Gibson, J.B., T.W. May, and A.V. Wilks 1981, Oecologia 51: 191-198; Gibson, J.B., and A.V. Wilks 1988, Heredity 60: 403-414; Hassell, M.P., and T.R.E. Southwood 1978, Annu. Rev. Ecol. Syst. 9: 75-98; Jaenike, J., 1990, Ann. Rev. Ecol. Syst. 21: 243-273; Lewis, A.C., 1983, Ecology 65: 836-842; Linn, C.E., H.R. Dambroski, J.L. Feder, S.H. Berlocher, S. Nojima *et al.*, 2004, Proc. Natl. Acad. Sci. USA 101: 17753-17758; McKenzie, J.A., and P.A. Parsons 1972, Oecologia 10: 373-388; Mercot, H., D. Defaye, P. Capy, E. Pla, and J.R. David 1994, Evolution 48: 746-757; Noor, M.A., 1995, Nature 375: 674-675; Noor, M.A.F., M.D. Schug, and C.F. Aquadro 2000, Genet. Res., Camb. 75: 25-35; Parsons, P.A., 1980, Behav. Genet. 10: 183-190; Parsons, P.A., and S.B. King 1977, Experientia 33: 898-899; Parsons, P.A., and G.E. Spence 1981, Aust. J. Zool. 29: 671-678; Powell, J.R., 1997, *Progress and Prospects in Evolutionary Biology: The* Drosophila *Model.* Oxford University Press, New York; Prakash, S., R.C. Lewontin, and J.L. Hubby 1969, Genetics 61: 841-858; Schaeffer, S.W., and E.L. Miller 1992, Genetics 132: 471-480; Selander, R.K. 1966, Condor 68: 113-151; Thompson, J.N., 1994, *The Coevolutionary Process*. University of Chicago Press, Chicago; Thompson, J.N., W. Wehling, and R. Podolsky 1990, Nature 344: 148-150; Via, S., 1990, Annu. Rev. Entomol. 35: 421-446; Woodard, C., T. Huang, H. Sun, S.L. Helfand, and J. Carlson 1989, Genetics 123: 315-326.

Nucleotide sequence variability of *Adh* gene of *Drosophila melanogaster* in the populations of Eurasia.

Vaulin, O.V., and I.K. Zakharov. Institute of Cytology and Genetics of Siberian Branch of the Russian Academy of Sciences, Novosibirsk, 630090, Russia; e-mail: Oleg.v.vaulin@mail.ru

The variability of alcohol dehydrogenase (*Adh*) was studied in a number of model organisms due to its significant genetic variability and convenient use of its products for isoenzymes analysis as well as to its importance in the life functions of organisms (Brogna *et al*., 2001). Two major electrophoretic variants of this enzyme – slow (S-form) and fast (F-form) – were found present in *Drosophila melanogaster*. Clinal variability of allele frequencies of this gene throughout the

different parts of *Drosophila melanogaster* natural habitat was shown. In the natural populations of India, Japan, Western Europe, and North America, the proportion of F-form of this enzyme was shown to grow northwards (Shamina and Parkash, 1993). Meanwhile, the vast North Eurasian areas of *Drosophila melanogaster* habitat remain unstudied. The heterozygous Adh^F/Adh^S flies were shown to have better fitness in the laboratory conditions (Bijlsma-Meels and Bijilsma, 1993). The nucleotide variability of the gene sequence also affects the fitness by the modification of the amount of enzyme synthesized (Laurie *et al.*, 1991; Dunn and Laurie, 1995).

Table 1. Genotypes of *Adh* fragment locus in the natural populations of *Drosophila melanogaster.*

Population (year, region)	Line	Fly genotype
Uman 1984, Cherkassk region, Ukraine	U84101	F/F
	U84399	F/F
	U84526	F/F
Uman 1993, Cherkassk region, Ukraine	U93011	F/F
	U93014	F/F
	U93033	F/F
	U93080	F/F
Nikopol 1997, Dnepropetrovsk region, Ukraine	N97003	F/F
	N97011	F/S_1
	N97021	F/F
	N97025	F/S_2
Biisk 1993, Altai region, Russia	B93235	F/F
	B93343	S_2/S_2
	B93350	F/F
	B93351	F/F
	B93397	F/S_3
	B93364	F/F
Belokurikha 2000, Altai region, Russia	B14	F/F
	B15	F/F
	B16	F/F
	B20	F/F
	B37	F/F
Gorno-Altaisk 1993, Gornoaltaisk republic, Russia	GA93010	S_2/S_2
	GA93066	F/F
	GA93080	F/F
	GA93124	F/F
	GA93146	S_2/S_2
Sochi 2004, Krasnodar region, Russia	S406	F/F
	S407	F/F
	S415	F/F
	S421	F/F
	S426	F/F
	S431	F/F
Izhevsk 2002, Udmurtia, Russia	I295	F/F
	I329	F/F
	I336	F/F
	I341	F/F
	I343	F/F
Nalchik 2006, Kabardino-Balkaria, Russia	N1-2	F/F
	N1-3	F/F
	N1-4	F/F
	N2-2	F/F
	N3-4	F/F
Bishkek 2004, Kirgizia	Bi1	S_2/S_3
	Bi3	F/S_1
	Bi31	S_1/S_1
	Bi62	F/F

In our work, we studied the nucleotide sequences of *Adh* gene fragment obtained from *Drosophila melanogaster* populations of Ukraine, Russia, and Kirgizia (see Table 1). We analyzed a single imago fly from isofemale line, each of which represents the progeny of a female fertilized in the wild and maintained henceforth in the collection of the Laboratory of Genetics of Populations, Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences. The direct and reverse primers for the amplification of *Adh* gene fragment are 5'-ATTG CCGTCAACTACACTGG-3' and 5'-GGTTCGCGAACCCTATGAAC-3', respectively. The resulting amplicon contains intron 3 and exon 4 of *Adh* gene.

The nucleotides starting from position 49 downstream to the forward primer annealing site were used for the analysis. Different sequence variants of S-form of *Adh* enzyme discovered in this work were designated as S_1, S_2 … S_n. A single fast-form *Adh* variant was designated as F.

The obtained sequences were compared to those deposited in DDBJ DNA Sequence Database (http://www.ddbj.nig.ac.jp); the numbers of sequences are as follows: M22210, M17827, M17828, M17830, M17831, M17832, M17833, M17834, M17835, M17836, M17837 M19547, M36580, X04454, X60791, X60792, X60793, and U20765.

The nucleotide sequence heterozygosity of the studied fragment was found conserved in the lines maintained under the laboratory environment over a significant period of time. In particular, heterozygous flies were found in the lines from Bijsk (1993), Bishkek (2004), and Nikopol (1997). Predominance of the F-form was shown for the samples from Ukraine, Caucasus, and the Urals (with the exception for the two lines from Nikopol, which were polymorphic for this locus, while the S-form was found in the populations of Altai and Bishkek; Table 1).

```
Variable sites          1111112222223333334444
                        1523467780012690337867 8
Lines/samples           4989742450068753202158 00
M18733                  CCCGGACGATCTGTCTCCCGGCCA
F                       ........................
N97011  (F/S1)          Y..........SYMYY.MR.M..
N97025  (F/S2)          ......SR....SYMYY.MR...M
GA93010 (S2/S2)         ......GA....CCACT.AA...C
B93397  (F/S3)          ...........S.M.........
Bi31    (S1/S1)         T..........CCACT.AA.A..
M36580                  TT.........C.A.........
M17832                  ...........C.A.T..A....
M17837                  .....G.................
X04454                  ...................A...
M22210                  ................T.A....
M17830                  ...........CCACT.AA.A..
M17827                  T.ACA...TAACCCACT.A..A..
M17831                  ...........CCACT.A.....
X60793                  ......GA....CCACT.AA....
U20765                  ...........C.A.........
```

Figure 1. Variable sites in the annotated and newly studied *Adh* gene sequences of *Drosophila melanogaster*. Positions aligned to the starting position of the studied variants. In bold is the site of the substitutions that determine the electrophoretic mobility of the enzyme (S and F forms). Polymorphic positions in heterozygotes are designated by a 15-letter code. Only unique non-repeated variants, both annotated and newly studied are included.

The *Adh* sequences found in the homozygous flies were found similar to those deposited in the DNA sequence database (Figure 1). F sequences were found in all collections and were identical among themselves as well as to the sequences deposited under the following numbers in the DNA database: M17833, M17834, M17835, M17836, X60791, and X60792. M17833, M17834, M17835, and M17836 variants were shown to derive from the different parts of the habitat (North America, Western Europe, and Africa). The F-variant of *Adh* sequence found in our study is, probably, the prevailing variant in the natural populations of *Drosophila melanogaster*.

The S-variants obtained in our study demonstrate more diversity: three different *Adh* S variants were found, one of those (S_3) corresponding to the sequence variant deposited in the database. S_1 variant was found in *Drosophila melanogaster* populations of Ukraine and Kirgizia, S_2 in the populations of Ukraine, Altai, and Kirgizia, and S_3 in Altai and Kirgizia.

S_1 variant has a number of nucleotide substitutions described for the different annotated *Adh* S variants and, possibly, is the result of crossing-over. Particularly, the recombination between the annotated sequences M17830 and M17827 obtained from the flies from Western Europe (France) and North America, respectively, in the region 4-58 bp would result in the generation of a product identical to S_1 (Figure 1). S1 and X60793 sequences differ from each other in a single substitution, which was not found in any other annotated sequence. S_2 sequence is a derivative variant of X60793.

S_3 variant found in our study only as in heterozygotes is identical to the U20765 fragment, the latter being described in the database as a null allele. The isolated S_3 variant has not been identified as null alleles as yet, as they carry neither stop codons nor microdeletions in the coding part of the studied *Adh* fragment. However, their presence in the remaining unstudied part of the gene cannot be excluded. In case that S_3 represents a null allele, it is the widespread lethal mutation in the Siberian populations of *Drosophila melanogaster*.

Acknowledgments: This work was supported by the Russian Foundation for Basic Research, project 05-04-48838, and the Program of the Russian Academy of Sciences "Biodiversity and Dynamics of Gene Pools."

References: Bijlsma-Meels, F., and R. Bijilsma 1988, Genetics 120 (3): 743-753; Brogna, S., P.V. Benos, G. Gasperi *et al*., 2001, Mol. Biol. Evol. 18 (3): 322-329; Dunn, R.C., and C.C. Laurie 1995, Genetics 140 (2): 667-677; Laurie, C.C., J.T. Birdgham, and M. Choudhary 1991, Genetics 129 (2): 489-499; Shamina, Parkash R., 1993, Dros. Inf. Serv. 72: 97-98.

Flanking regions of *P*-elements inserted in the 3rd chromosome of *Drosophila mauritiana*.

Araripe, Luciana O., Nathan Eckstrand, Daniel Hartl, and Yun Tao. Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, 02138.

The *P* [*lac*-w^+] insertion lines described by True *et al*. (1996) were generated in order to produce an easy-score system for introgressing *D. mauritiana* into its sibling species, such as *D. simulans.* The insertion sites of each *P*-element were determined by *in situ* hybridization to their nearest lettered sub-division of polytene chromosomes (True *et al*., 1996a). In total, 114 lines had *P* [w^+] inserts localized in 94 different positions. These lines have been useful for estimating recombination rates within *D. mauritiana* (True *et al*., 1996a) and for mapping genes causing inter-specific divergence (True *et al*., 1996b; Coyne and Charlesworth, 1997; Laurie *et al*., 1997; Tao *et al*., 2001; Tao *et al*., 2003a-c). Several introgression studies using these lines are now in progress.

Here we report the precise insertion sites for the *P*-elements by inverse PCR. We follow the Universal Fast Walking (UFW) protocol of Myrick and Gelbart (2002) by word, using the same set of primers for each side of the *P* [*lac*-w^+] construct (Bier *et al*., 1989). The only modification is an added round of PCR reaction at the end, in order to increase the amount of PCR product for sequencing.

We obtained 167 sequences for 92 *P*-elements. A total of 77 lines had both flanking sequences obtained, while 8 lines have only the 5'-flanking sequences and 7 lines have only the 3'-flanking sequences. Deletions within the *P*-construct, flanking repetitive sequences or many other complications may have contributed to the failure of some PCR reactions. The sequences obtained were blasted to the *D. melanogaster* genome (NCBI, release 2.2.13). The precise insertion sites are provided by the following information: scaffolds, cytological location and the beginning and end positions for the flanking sequences (Table 1). Remarkably, the original cytological positions reported by True *et al*. (1996a) – column 1 of Table 1 – are in good accord with our data. (All the flanking sequences can be downloaded from Hartl lab website).

References: Bier, E., H. Vaessin, S. Shepherd *et al.*, 1989, Genes Dev. 3: 1273-1287; Coyne, J.A., and B. Charlesworth 1997, Genetics 145: 1015-1030; Laurie, C.C., J.R. True, J. Liu, and J.M. Mercer 1997, Genetics 145: 339-348; Myrick, K.V., and W.M. Gelbart 2002, Gene 284: 125-131; Tao, Y., D.L. Hartl, and C.C. Laurie 2001, PNAS 98(23): 13183-13188; Tao, Y., S. Chen, D.L. Hartl, and C.C. Laurie 2003a, Genetics 164: 1383-1397; Tao, Y., Z-B. Zeng, J. Li, D.L. Hartl, and C.C. Laurie 2003b, Genetics 164: 1399-1418; Tao, Y., and D. Hartl 2003c, Genetics 57(11): 2580-2598; True, J.R., J.M. Mercer, and C.C. Laurie 1996, Genetics 142: 507-523; True, J.R., B.S. Weir, and C.C. Laurie 1996, Genetics 142: 819-837.