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Novel mutants in *D. simulans.*

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

Results and Discussion

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

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

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

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

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

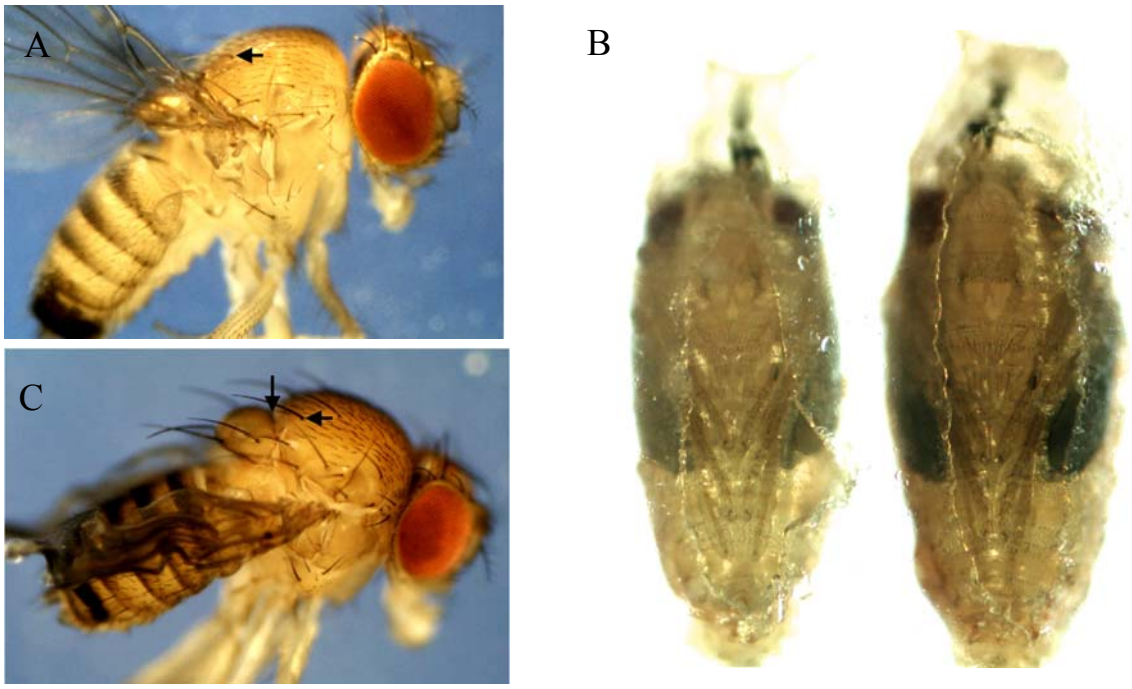

Figure 2. *blond*, *cy* and *tronc* mutants. A) *blond*[1] female. Compare the yellow hairs (arrows) and body color to the female shown in (C). B) *bd*[1] pupae. a *blond*[1] male (left) and a wild type male (right). Note the paler color of the wings of *bd*[1]. C) Double mutant *cy*[S]; *tronc*[1]**.**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

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

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

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

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

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

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

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

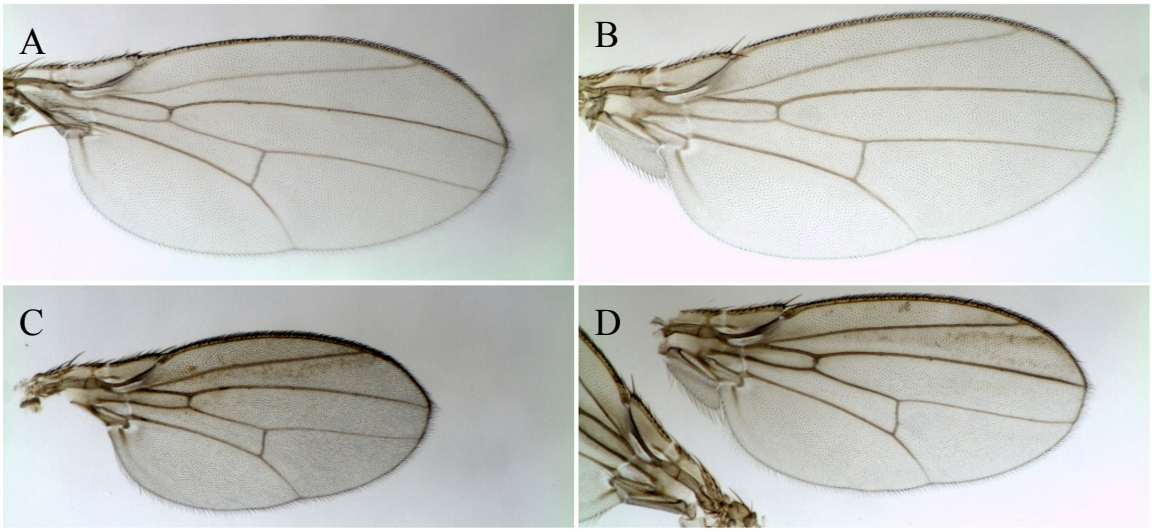

Figure 3. *swg* wings compared to the wild type. A-D same magnification. E and F, same magnification. A, wild type wing size of a *bd*^{1} male. B, wild type wing size of a *bd*^{1} female. C, wing size of a *swg*^{1} male. D, wing size of a double mutant, *swg*^{1} *bd*^{1}. Note the reduction in size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

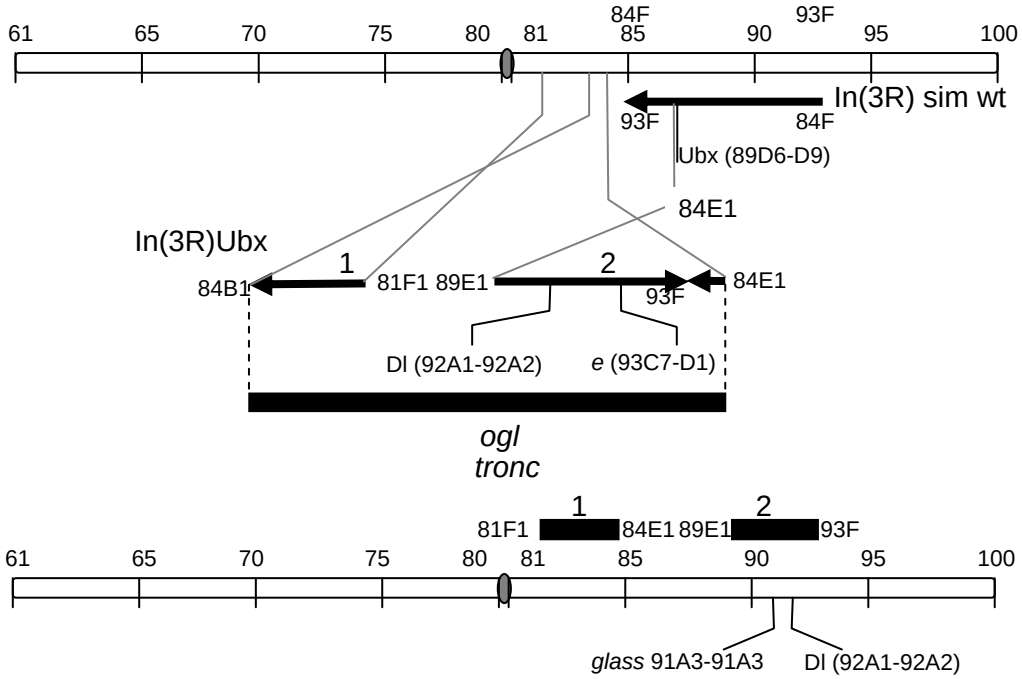

Figure 4. Estimated position of *ogl* and *tronc* based on the breakpoints of In(3R)Ubx**.** Chromosome 3 is represented at the top and bottom of this figure with some divisions of *D. melanogaster*. The wild type inversion In(3R) is shown right below the top chromosome (right to left arrow). Below this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

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

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

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

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

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

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

Recessive mutants, no selection required

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