buffered saline 0.66× (PBS tablets, Sigma). Ovaries were fixed in PBS 1× 3.7% formaldehyde during 30 min at room temperature and stored at 4°C. General morphology of the ovary was observed under a stereoscopic microscope (Leica MZFL III). Ovaries were labeled in DAPI (5 µg/ml in PBS) and observed under epifluorescence (Leica DMRD).

**Immuno-fluorescence and imaging**

*bab* mutant stocks were balanced over *TM6 Tb*. Female wandering third instar larvae of the Tb+ phenotype were dissected in PBS and whole fat bodies to which ovaries are attached were collected, fixed in PBS containing 2% BSA (Sigma A2058), 3.7% formaldehyde (Sigma), 1% Triton during 30 min at room temperature, washed in PBS 0.3% Triton (PBT), and blocked in PBT, BSA 1% (PBTA). Fixed larval ovaries were incubated in PBTA with the appropriate combination (see Figure 2 legend) of rabbit anti-BAB1 (1 :1000, (Williams *et al.*, 2008)), rat anti-BAB2 (1 :1000, (Couderc *et al.*, 2002)), and mouse anti β-galactosidase (DSHB) overnight at 4°C. Ovaries were incubated during 2 hours at room temperature with the appropriate Alexa 488- and Alexa 568-coupled secondary antibodies (1:500 in PBTA, Molecular Probes). Ovaries were mounted in Citifluor (AF1, Biovalley, FR) and directly observed under an inverted confocal microscope NIKON TE2000-U. Fixation and immuno-fluorescence of ovaries of mutant and control genotypes were performed in the same tube, thus allowing direct comparison of signal levels between genotypes. Control and mutant ovaries were genotyped using *lacZ* reporters. Control ovaries were Canton-S for *bab1P*, and *hh-lacZ* for *bab2E1*. Genotypes were identified after imaging: *bab1P* carries a *lacZ* reporter that is expressed in TF cells, allowing to positively discriminate the *bab1P* homozygotes from Canton-S controls; *bab2E1* mutants were identified by the absence of anti-betaGalactosidase immunostaining, and *hh-lacZ* controls by the presence of anti-betaGalactosidase immunostaining in TF cells (data not shown). The *babAR07* deficiency that covers both *bab1* and *bab2* was used as a control for the specificity of BAB1 and BAB2 signals in a separate experiment (data not shown). Confocal images were analyzed using ImageJ (NIH) and Photoshop CS2 (Adobe) softwares, using identical settings for all samples of the same experimental series.


**Mutants in D. simulans and D. sechellia.**

_Sousa-Neves, Rui, and Youngmin Chu._ Department of Biology Case Western Reserve University

Here we report the isolation of nine new spontaneous mutants in *D. simulans* identified this year, as well as notes on mutants described in Sousa-Neves *et al.* (2009). We also report the genetic
and molecular position of the recessive mutation *zinfandel* (*zn¹*) of *D. sechellia* and *small wings¹* (*swg¹*) of *D. simulans*.

The new mutants of *D. simulans* described in this report are:

1- *Minute 3 Super Los Angeles* (*M(3)SLA¹*)

**ORIGIN:** Isolated in February 2011 from the stock Super Los Angeles.

**PHENOTYPE:** *M(3)SLA¹* is haplo-insufficient, homozygous lethal like most *Minutes* and because of that it has to be selected every generation. Heterozygotes can be easily scored and have excellent viability and fertility.

**LINKAGE:** *M(3)SLA¹* was located on the 3rd chromosome and mapped using *scarlet* (*st*) and *ebony* (*e*) as references on this chromosome. In *D. simulans* *st* and *e* appear 13.7 units apart. *M(3)SLA¹* maps 26.9 units to the left of *st*.

2- *Minute of Rincón* (*M*R)

**ORIGIN:** Isolated in the September 2011 from the Stock Rincón de la Vieja.

**PHENOTYPE:** Haploinsufficient. Strong Minute with excellent viability as heterozygote

**LINKAGE:** not yet determined.

3- *purple maternal* (*pr-m¹*)

**ORIGIN:** Isolated in February 2011 from the stock Tabacón.

**PHENOTYPE:** Recessive. *pr-m¹* flies have eyes with a dark Port wine color, smooth appearance and faint pseudopupils (which appear white or gray in young flies, as opposed to black). Ocelli colored. With age, this color progresses to a thick reddish brown. Males homozygous from *pr-m¹*
are viable and fertile. However, homozygous females lay fertilized eggs that die as embryos with extreme pattern defects. Very rare escapers can be obtained and those appear absolutely normal.

LINKAGE: 3

4- singed$^3$ (sn$^3$)

PHENOTYPE: Strong allele of singed with macrochaeta extremely curled. Complements the D. simulans f$^{66}$ and fails to complement sn$^{X_2}$ from D. melanogaster carried in the balancer Binscy. sn$^1$ and sn$^2$ were isolated by Sturtevant in the 1920’s and are presumably lost. Thus, sn$^3$ may be the only existing allele of sn in D. simulans.

LINKAGE: X

5- scarlet (st$^3$)

ORIGIN: Isolated in February 2011 from the Stock Super Los Angeles.

LINKAGE: 3L

6- curly of Rincón$^1$ (cyR$^1$)

PHENOTYPE: Recessive. Wings curled. In addition to curly wings, cyR$^1$ often exhibit a loss of humeral hairs. At this point it is not clear whether the lack of humerals is separable from the wing phenotype.

LINKAGE: Not yet determined.

7-Ultrabithorax-like$^1$ (Ubx-l$^1$)

ORIGIN: Isolated in November 2011 from the Stock sn$^3$; st$^3$.
PHENOTYPE: Recessive. Halteres flat with irregular lobes and partial transformations to wings. Flies are flightless and often exhibit divergent wings.

LINKAGE: Not yet determined.

8-rough-forked$^1$ (raf$^1$)

ORIGIN: Isolated in November 2011 from the stock Rincón the la Vieja.
PHENOTYPE: Eyes rough with ommatidia distorted. In addition to the eye phenotype the posterior dorsocentrals appear gnarled. The gnarling is usually less extreme than $sn^3$. Other bristles may appear distorted. Frequently $rof^1$ flies exhibit divergent wings.

LINKAGE: Autosomal, not yet determined.

Figure 4. *rough-forked*\(^1\) (*rof^1*) mutant. (Left) the eye of a *rof^1* male. (Right) the thorax of the same individual. Compare the disorganized ommatidia of the mutant above with the wild type of Figure 1. The crystalline structure of the wild type ommatidia resembling a mesh is replaced by an irregular tissue. Note the abnormal thoracic bristles (white arrows).

Figure 5. *sternopleurals reduced* (*sr*) mutant. (Left wild type and right *sr*). Observe the extreme reduction of the sternopleural bristles (black arrows). The white arrow points to humeral bristles, which appear wild type in *sr* mutants.
9-sternopleurals reduced (sr)

ORIGIN: Isolated from the Super Los Angeles stock in December 2011.

PHENOTYPE: Sternopleural bristles reduced. Post-scuteal bristles also reduced to a great extent. Body color is not as shiny and with the waxed appearance as the wild type.

LINKAGE: not yet determined.

Updates of mutations previously described in D.I.S. (Sousa-Neves et al., 2009):

Genetic and physical position of the D. simulans small wings (swg)

Previously we reported that swg is an X-linked recessive mutation. Recombination mapping in D. simulans showed that swg is 5.4 units to the right of v. This result suggested that swg might correspond to the D. melanogaster dusky (dy) or minitature (m). However, swg complements m. We tested whether the D. melanogaster deletion Df(1)BSC876 that deletes dy disrupts swg and found that this deletion fails to complement swg.

Genetic and physical position of the D. sechellia zinfandel (zn1)

zn1 is an X-linked recessive mutation that affects eye color. We mapped it by recombination in hybrids D. simulans/D. sechellia at position 23.0. Since the position and phenotype of zn1 is very similar to the D. melanogaster gene carmine (cm) located at the physical position 6.9Mb, we tested whether zn1 corresponds to cm in heterozygous hybrids D. sechellia/D. melanogaster zn1/Df(1)BSC867. We find that Df(1)BSC867 uncovers the recessive zn1. Thus, it seems likely that zn1 corresponds to carmine, which is located between the D. melanogaster molecular coordinates (X: 6875892..6935548).