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 *TM6Tb*. 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 ß-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 $bab1^{P}$, and *hh-lacZ* for $bab2^{E1}$. Genotypes were identified after imaging: $bab1^{P}$ carries a *lacZ* reporter that is expressed in TF cells, allowing to positively discriminate the $bab1^{P}$ homozygotes from Canton-S controls; $bab2^{E1}$ 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 bab^{AR07} 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.

References: Bardot, O., D. Godt, F.A. Laski, and J.L. Couderc 2002, Genesis 34: 66-70; Barmina, O., and A. Kopp 2007, Developmental Biology 311: 277-86; Couderc, J.L., D. Godt, S. Zollman, J. Chen, M. Li, S. Tiong, S.E. Cramton, I. Sahut-Barnola, and F.A. Laski 2002, Development 129: 2419-33; Godt, D., J.L. Couderc, S.E. Cramton, and F.A. Laski 1993, Development 119: 799-812; Godt, D. and F. A. Laski 1995, Development 121: 173-87; Kopp, A., I. Duncan, D. Godt, and S.B. Carroll 2000, Nature 408: 553-9; Lours, C., O. Bardot, D. Godt, F.A. Laski, and J.L. Couderc 2003, Nucleic Acids Res 31: 5389-98; Randsholt, N.B., and P. Santamaria 2008, Evolution & Development 10: 121-33; Sahut-Barnola, I., D. Godt, F.A. Laski, and J.L. Couderc 1995, Dev. Biol. 170: 127-35; Williams, T.M., J.E. Selegue, T. Werner, N. Gompel, A. Kopp, and S.B. Carroll 2008, Cell 134: 610-23.

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

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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* (*zn*[1]) of *D. sechellia* and *small wings*[1] (*swg*[1]) of *D. simulans*.

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

1- *Minute 3 Super Los Angeles* (*M(3)SLA*[1])

ORIGIN: Isolated in February 2011 from the stock Super Los Angeles.
PHENOTYPE: *M(3)SLA*[1] 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)SLA*[1] was located on the 3[rd] 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)SLA*[1] 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: Haploinsuficient. Strong Minute with excellent viability as heterozygote
LINKAGE: not yet determined.

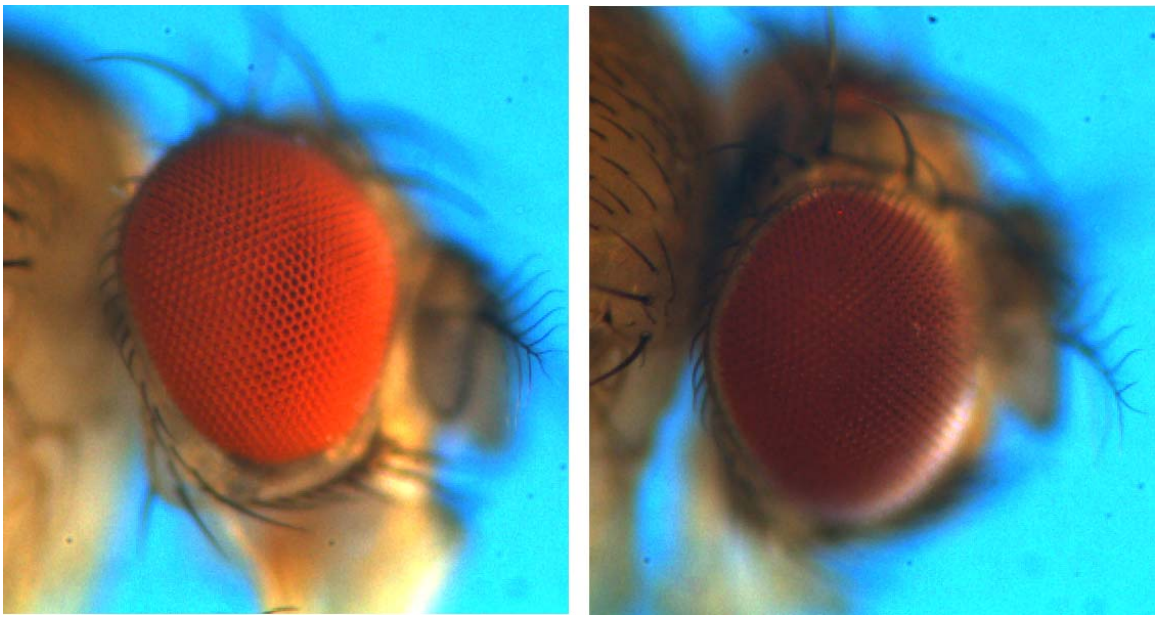

Figure 1. *purple maternal* (*pr-m*[1]) mutant. Wild type eye (left) and mutant *pr-m*[1] (right).

3- *purple maternal* (*pr-m*[1])

ORIGIN: Isolated in February 2011 from the stock Tabacón.
PHENOTYPE: Recessive. *pr-m*[1] 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-m*[1]

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})

ORIGIN: Isolated in June 2011 from the Stock Super Los Angeles.
PHENOTYPE: Strong allele of *singed* with macrochaeta extremely curled. Complements the *D. simulans f* ^{66} and fails to complement *sn*^{X2} 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

Figure 2. *singed*^{3} (*sn*^{3}) mutant. Note the severely distorted thoracic bristles.

5- *scarlet* (*st*^{3})

ORIGIN: Isolated in February 2011 from the Stock Super Los Angeles.
PHENOTYPE: Recessive. Eyes with a bright red color like *vermilion, cinnabar,* and *scarlet.* Ocelli colorless. Allelic to the *D. simulans st*^{1}.
LINKAGE: 3L

6- *curly of Rincón*^{1} (*cyR*^{1})

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

Figure 3. *curly of Rincón*^{1} (*cyR*^{1}) mutant female. Note the curled wings and the lack of humeral bristles (white arrow). For comparison, see the humerals of the individuals in Figure 5.

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} (*rof* ^{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.

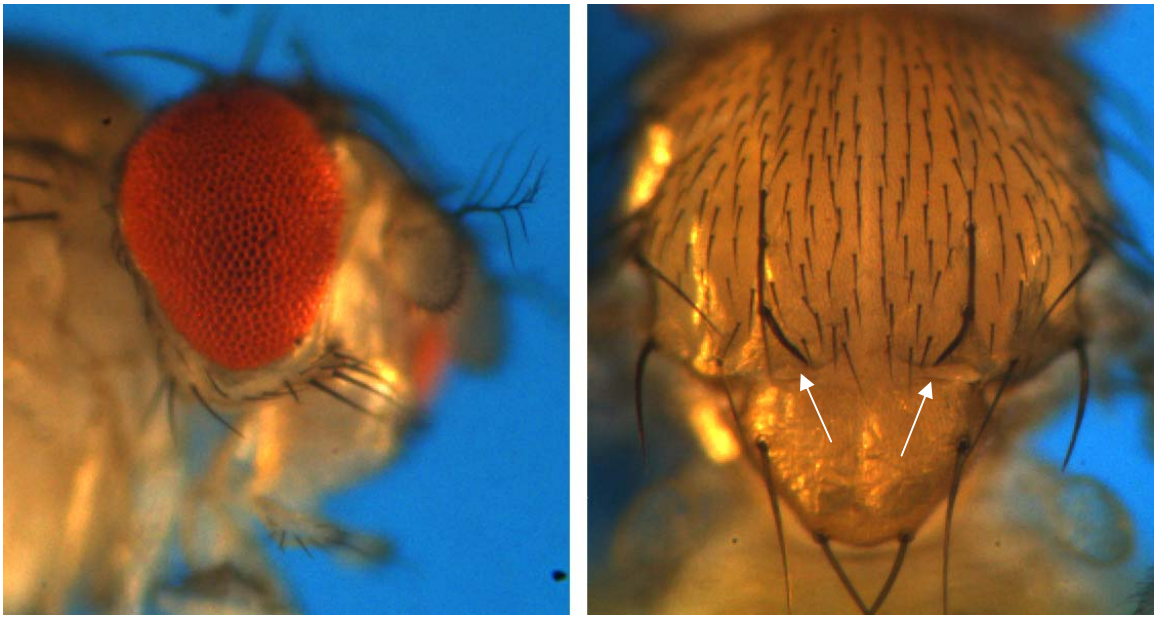

Figure 4. *rough-forked*^{1} (*rof* ^{1}) mutant. (Left) the eye of a *rof* ^{1} male. (Right) the thorax of the same individual. Compare the disorganized ommatidia of the mutant above with the wild type of Figure 1. The crystalline structure of the wild type ommatidia resembling a mesh is replaced by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

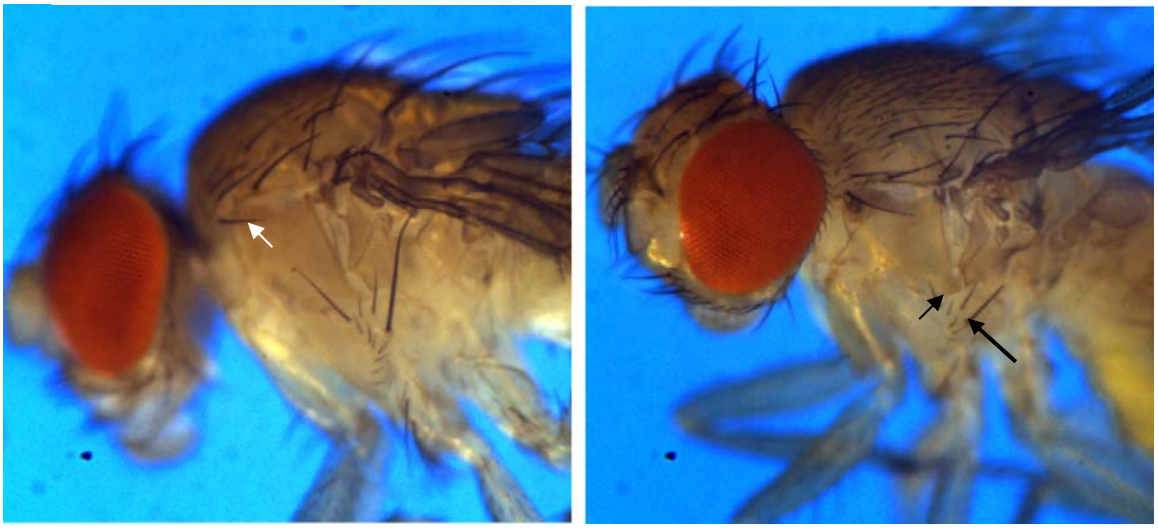

Figure 5. *sternopleurals reduced* (*sr*) mutant. (Left wild type and right *sr*). Observe the extreme reduction of the sternopleural bristles (black arrows). The white arrow points to humeral bristles, which appear wild type in *sr* mutants.

9-*sternopleurals reduced* (*sr*)

ORIGIN: Isolated from the Super Los Angeles stock in December 2011.
PHENOTYPE: Sternopleural bristles reduced. Post-scutellar bristles also reduced to a great extent. Body color is not as shinny 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* (zn^1)

zn^1 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 zn^1 is very similar to the *D. melanogaster* gene *carmine* (*cm*) located at the physical position 6.9Mb, we tested whether zn^1 corresponds to *cm* in heterozygous hybrids *D. sechellia*/*D. melanogaster* zn^1/Df(1)BSC867. We find that Df(1)BSC867 uncovers the recessive zn^1. Thus, it seems likely that zn^1 corresponds to *carmine*, which is located between the *D. melanogaster* molecular coordinates (X: 6875892..6935548).

References: Sousa-Neves, R., J. Schinaman, and J. Cater 2009, Dros. Inf. Serv. 92: 143-147.