Mutation Notes

The *Drosophila melanogaster straw* locus is allelic to *laccase2*.

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In the course of our studies on the *apterous* locus (Gohl *et al*., 2008; Gohl and Müller, unpublished), we have isolated a PiggyBac insertion in the nearby *laccase2* gene (CG42345). The new insert *PBac{WH}laccase2*$^{5151\text{-}37A}$ was obtained by mobilization of *PBac{WH}ap*^{f00451} and it was molecularly mapped to near the 3' end of the *laccase2* gene (see Figure 1). The enzyme Laccase2 is part of the catecholamine pathway leading to pigmentation and sclerotization of the adult fly cuticle (Riedel *et al*., 2011). It oxidizes dopamine to dopamine quinone, which, in the presence of the Yellow protein, polymerizes to form black melanin. Laccase2 enzyme also oxidizes N-β-alanyldopamine to a quinone, which mediates cuticle protein cross-linking (sclerotization) (Riedel *et al*., 2011).

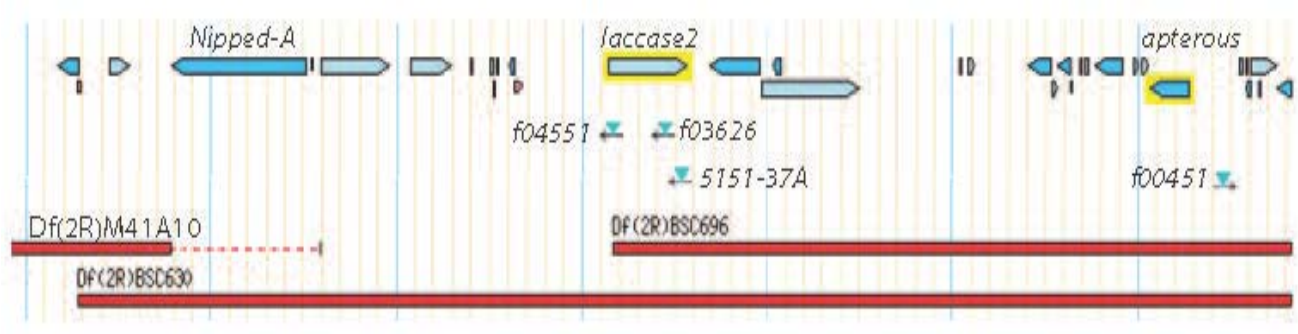

Figure 1. 700-kb genomic interval surrounding the *laccase2* locus at the base of chromosome arm 2R (adapted from FlyBase). In the upper part of the panel, gene spans of all loci in the region are shown. Genes *Nipped-A*, *laccase2*, and *apterous* are highlighted. Below the gene spans, all PiggyBac insertion sites mentioned in the text are indicated. At the bottom of the panel, the complete length of deficiencies *Df(2R)BSC630* and *Df(2R)BSC696* are depicted by a red bar. For the much larger deletion *Df(2R) M41A10,* only the approximate position of its distal break point is shown.

In order to genetically characterize the new allele, complementation crosses among stocks *PBac{WH}laccase2*$^{5151\text{-}37A}$*/SM6*, *PBac{WH}laccase2*^{f04551}*/CyO*, *PBac{WH}laccase2*^{f03626}*/CyO*, *Df(2R)M41A10/ CyO*, *Df(2R)BSC630/CyO*, and *Df(2R)BSC696/CyO* were set up (insertion sites and deficiency breaks are depicted in Figure 1). The results indicated that *laccase2* alleles *PBac{WH}laccase2*^{f04551}, and *PBac{WH}laccase2*^{f03626} are lethal. The new allele *PBac{WH}laccase2*$^{5151\text{-}37A}$ is viable over deficiency as well as over both of the two lethal *PBac{WH}* alleles (see Table 1). Such trans-heterozygous flies show a consistent pigmentation defect reminiscent of *yellow* null alleles: bristles and wings lose their characteristic dark color. However, and in contrast to *yellow*, pigmentation on abdominal tergites remains largely unchanged (data not shown). Similar phenotypes were previously obtained by RNAi-mediated knockdown of the *laccase2* gene (Riedel *et al*., 2011).

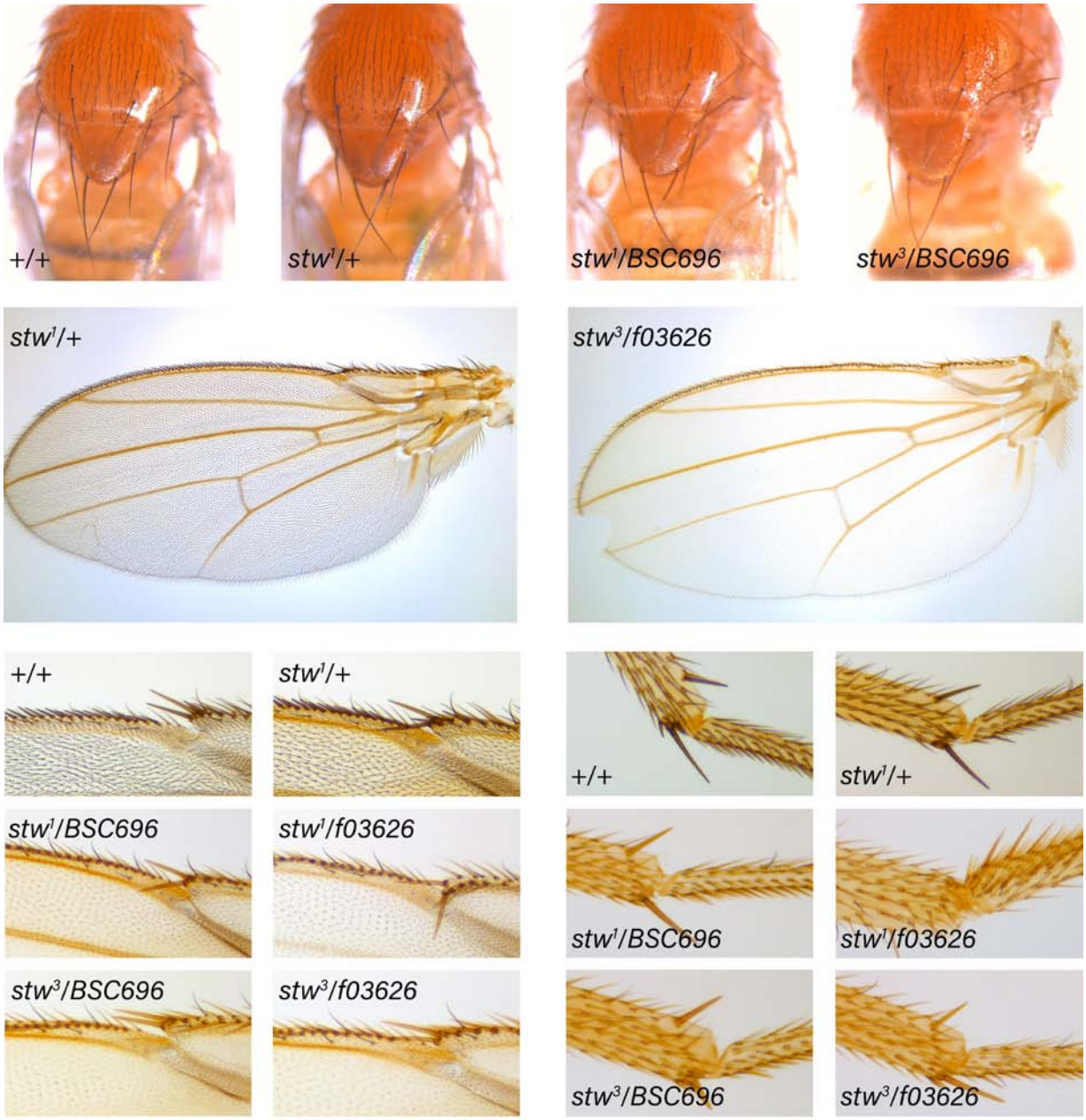

Figure 2. Adult phenotypes of *stw*^{1} and *stw*^{3}. In the top 4 pictures, the change in thoracic bristle pigmentation is shown. Bristle color in OregonR (+/+) and heterozygous *stw*^{1} (*stw*^{1}/+) flies is clearly darker than in *stw*^{1} (*stw*^{1}/*BSC696*) and *stw*^{3} (*stw*^{3}/*BSC696*) hemizygotes. In the middle of the panel, the difference in appearance of the wing blade between heterozygous *stw*^{1}/+ and *stw*^{3}/*f03626* flies is documented. Note the yellowish appearance of the blade in *stw*^{3}/*f03626* as compared to *stw*^{1}/+. Wing blade *stw*^{3}/*f03626* is slightly damaged at the tip. Wings were dissected and embedded in Hoyer's before images were taken. At the bottom left, blow-ups of proximal-anterior wing margins are shown. Note the loss of dark pigment in hemizygous (*stw*^{1}/*BSC696*, *stw*^{3}/*BSC696*) or trans-heterozygous (*stw*^{1}/*f03626*, *stw*^{3}/*f03626*) *stw*^{1} and *stw*^{3} conditions as compared to OregonR (+/+) or *stw*^{1}/+. At the bottom right, blow-ups of adult legs are depicted. Bristles acquire a distinct yellowish color in hemizygous (*stw*^{1}/*BSC696*, *stw*^{3}/*BSC696*) or trans-heterozygous (*stw*^{1}/*f03626*, *stw*^{3}/*f03626*) *stw*^{1} and *stw*^{3} conditions as compared to OregonR (+/+) or *stw*^{1}/+. Pictures of the same morphological structures were taken under identical illumination conditions.

Table 1. Results of complementation crosses.

Genotype	Viability	straw phenotype
Df(2R)M41A10 / Df(2R)BSC630	lethal	
Df(2R)M41A10 / Df(2R)BSC696	viable	no
Df(2R)M41A10 / PBac{WH}laccase2^{f04551}	viable	no
Df(2R)M41A10 / PBac{WH}laccase2^{f03626}	viable	no
Df(2R)BSC630 / Df(2R)BSC696	lethal	
Df(2R)BSC630 / PBac{WH}laccase2^{f04551}	lethal	
Df(2R)BSC630 / PBac{WH}laccase2^{f03626}	lethal	
Df(2R)BSC696 / PBac{WH}laccase2^{f04551}	lethal	
Df(2R)BSC696 / PBac{WH}laccase2^{f03626}	lethal	
PBac{WH}laccase2^{f03626} / PBac{WH}laccase2^{f04551}	lethal	
Df(2R)M41A10 / PBac{WH}laccase2$^{5151-37A}$	viable	no
Df(2R)BSC630 / PBac{WH}laccase2$^{5151-37A}$	viable	yes*
Df(2R)BSC696 / PBac{WH}laccase2$^{5151-37A}$	viable	yes*
PBac{WH}laccase2^{f04551} / PBac{WH}laccase2$^{5151-37A}$	viable	yes
PBac{WH}laccase2^{f03626} / PBac{WH}laccase2$^{5151-37A}$	viable	yes
Df(2R)M41A10 / stw^{1}	viable	no
Df(2R)BSC630 / stw^{1}	viable	yes
Df(2R)BSC696 / stw^{1}	viable	yes
PBac{WH}laccase2^{f04551} / stw^{1}	viable	yes
PBac{WH}laccase2^{f03626} / stw^{1}	viable	yes
Df(2R)M41A10 / stw^{3}	viable	no
Df(2R)BSC630 / stw^{3}	viable	yes
Df(2R)BSC696 / stw^{3}	viable	yes
PBac{WH}laccase2^{f04551} / stw^{3}	viable	yes
PBac{WH}laccase2^{f03626} / stw^{3}	viable	yes

* These flies also show an *apterous* wing phenotype because the *PBac{WH}laccase2$^{5151-37A}$* chromosome has retained insert *PBac{WH}ap^{f00451}* and *Df(2R)BSC630* and *Df(2R)BSC696* also take out *apterous* (see Figure 1).

PBac{WH}laccase2^{f04551} / CyO (B#18785), Df(2R)M41A10/SM1 (B#*741), Df(2r)BSC630 / CyO (B#25705), Df(2R)BSC696 / CYO* (B#*26548), stw^{1}*(B#*412)* and *lt^{1} rl^{1} stw^{3}* (B#1056 were obtained from the Bloomington stock center. *PBac{WH}ap^{f00451} and PBac{WH}laccase1^{f03626} were* purchased from the Exelixis stock collection at Harvard Medical School.

The first *straw* allele (*stw^{1}*) was discovered by Calvin Bridges exactly 100 years ago in 1917 (Morgan *et al*., 1925). According to FlyBase, the *straw* locus has not been annotated yet. But information available on FlyBase suggests that *straw* could be allelic to *laccase2*: (1) *straw* has been mapped to the base of 2R but distal to *Df(2R)M41A10*; (2) the described *straw* phenotype is very similar to what we have observed for our new allele *PBac{WH}laccase2$^{5151-37A}$*. This assumption was borne out by our observations obtained by complementation crosses between *straw* alleles *stw^{1}* and *stw^{3}* and deficiencies and *laccase2* alleles shown in Figure 1 (see Table 1):

– *Df(2R)M41A10* complements both *straw* alleles but neither *Df(2R)BSC630* nor *Df(2R)BSC696* do. Hemizygous *stw^{1} and stw^{3}* flies are well viable and show the typical straw phenotype (see Figure 2).

– Importantly, the *straw* alleles are also not complemented by *PBac{WH}laccase2^{f04551}* and *PBac{WH}laccase2^{f03626}* (see Figure 2). In trans-heterozygous flies, pigmentation is lost in wings and bristles.

In conclusion, these complementation tests demonstrate that *straw* is allelic to *laccase2*. Therefore, we propose that according to established *Drosophila* nomenclature practices, the gene name of CG42345 should be changed to *straw*, as it was first called in the Morgan lab in 1917. Our observation "Bridges" the historical gap in understanding the molecular nature of the *straw* mutants discovered 100 years ago.

References: Gohl, D., M. Mueller, V. Pirrotta, M. Affolter, and P. Schedl 2008, Genetics 178: 127-143; Morgan, T.H., C.B. Bridges, and A.H. Sturtevant 1925, The genetics of *Drosophila melanogaster*. Biblphia Genet. 2: 262pp; Riedel, F., D. Vorkel, and S. Eaton 2011, Development 138: 149-158.

History of the FM7 balancer chromosome.

Merriam, John[1]. Revised by Scott Hawley and Danny Miller, 1-22-2014. Figure courtesy of Angie Miller. [1]Molecular Cell and Developmental Biology, University of California, Los Angeles 90095.

Like all modern balancer chromosomes in *D. melanogaster*, *FM7* was constructed from a series of progenitor balancer chromosomes. Inversion heterozygotes, but not homozygotes, suppress crossing over at the inverted portion of the chromosome. Sturtevant (1913) discovered the first example of an inversion, which he named *In(3R)C*, for crossover suppressor in the right arm of chromosome 3. This inversion reverses much of the distal third of the chromosome (from section 92D1 to 100F2) so that the chromosome sections in the right arm distal to the break at 92D1 are in the order centromere–100F2–92D1–telomere, and it suppresses crossing over for this region (from the marker *Dl* (*Delta*) to the telomere). Muller (1918) used *In(3R)C* to make the first permanent heterozygous stock, or "balanced stock", with the marker *Bd*, an allele of the *Serrate* (*Ser*) gene, that has both a dominant wing notching and recessive homozygous lethality. In the language of its time, this stock was "pure breeding"—all progeny had the same phenotype and genotype of their parents—because *Bd/Bd* and *In(3R)C/In(3R)C* homozygotes did not survive, leaving only *Bd/In(3R)C* heterozygotes each generation. In order for this stock to remain heterozygous each generation there must be suppression of crossing over, as it keeps the wild-type Bd^{+} allele from *In(3R)C* from being placed onto the *Bd* chromosome, which would allow for recovery of *Bd+/Bd* non-*In(3R)C* progeny.

The importance of this example was instantly recognized, leading to the identification of dominant crossover suppressor lines for all the linkage groups, and it was applied to maintaining mutant alleles with poor viability and/or multiply-marked chromosomes. Because *Drosophila* stocks cannot be maintained through frozen lines, essentially all of the thousands of mutant alleles in different genes now available must be maintained in balanced stocks without selection. The balancer chromosomes responsible have improved to contain multiple inversions for more complete crossover suppression, as well as a dominant marker for identification and recessive lethal or sterile mutants to prevent the stock from becoming homozygous for its balancer and losing the mutant allele.

Along with balancing mutant alleles, the inverted chromosomes also became essential in screens for new mutants. Muller (1928) recovered a balancer on the *X* (or 1^{st}) chromosome, *In(1)Cl*, also carrying the visible markers *sc v f* (all recessive) and *B* (dominant), as well as a lethal allele in an unknown gene. The middle two-thirds of this chromosome was inverted from 4A5 to 17A6. Maintained as the "ClB" stock, it formed the basis for Muller's assay to determine the fraction of sperm that carried a new *X*-linked lethal mutation after exposure to X-rays, work for which he received the Nobel Prize in 1946. Balancers were also used to identify lines with segregating recessive lethal mutations following mutagenesis, which could be identified as stocks which only gave heterozygous mutagenized-chromosome/balancer progeny. One highlight example is a 1980 paper by Nusslein-Volhard and Wieschaus describing their identification of the embryonic patterning genes through lethal alleles, for which they received the Nobel Prize in 1992.

Both the stock-maintenance and selective-screening uses of balancers depend on their effectiveness in suppressing crossing over. The goal of balancer construction has, therefore, been to add multiple inversions in order to cover as much of the chromosome as possible. *In(1)Cl* suppressed crossing over for most *X* regions except most proximally, but was less useful because of its own recessive lethality. *In(1)dl-49*, the second *X*