We also sought to investigate import of rhodopsin into the rhabdomere. In pilot work, we showed that there was no fluorescence 2 hr after heat shock while the fluorescence was nearly fully established at 5 hr. Dissecting this time span, we saw dim rhabdomere fluorescence at 3.5 hr (Figure, bottom right). For a control, we show a fly 26 hr after heat shock (Figure, bottom left); as stated above, both were kept in the dark after heat shock. The striking aspect of the 3.5 hr vista is the haze of fluorescent bodies seen in the cytoplasm of the retinula cells. We presume that we are visualizing membranous vehicles (and, perhaps Golgi apparatus) involved in the import of rhodopsin into the rhabdomere.

All four of our figures, and hundreds of other images we have obtained, show large fluorescent bodies that appear to be in pigment cells between ommatidia. We have always assumed, though we have not proven, that these are the giant unpigmented pigment granules of white eyes (Stark and Sapp, 1988). We thought we should not gloss over this point because, again, with techniques more sophisticated than our 1980's ultrastructural work, there has been a vastly renewed interest in eye color pigment granules.

We hope that our observations are of use to the many research groups using modern techniques and the accessibility of rhodopsin and the compound eye in *Drosophila* to study the broader issue of protein traffic.

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References: Belliveau, B., Thesis, University of Notre Dame, 2008; Chinchore, Y., *et al.*, 2009, PloS Genet. 5(2): e1000377; Selimovic, A., *et al*., 2010, Dros. Inf. Serv. 93: 1-2; Stark, W.S. *et al.*, 1988, J. Neurocytol. 17: 499-509; Stark, W.S., and R. Sapp 1988, Can. J. Zool. 66: 1301-1308; Stark, W.S., and C.F. Thomas 2004, Molec. Vision 10: 943-955 (on line at http://www.molvis.org/molvis/v10/a113); Zinkl, G., *et al.*, 1990, Vis. Neurosci. 5: 429-439.

Polytene chromosome analysis in eye color mutants of *Drosophila willistoni* and their hybrids. The H inversion.

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Introduction

During the course of the linkage analysis of (new) spontaneous mutations in *Drosophila willistoni* from populations of Uruguay, Brazil, and Argentina (Soler and Goñi, 2012, Dros. Inf. Serv., this issue), data on the genetic interaction of some eye color mutants were evaluated, among other genetic data to construct the linkage groups. Within the referent eye color mutations, *brown (bw)*, reported to be linked to chromosome 2 by Spassky and Dobzhansky (1950), produces white eye color in young flies to uniformly blotch brownish eye color in old flies with *cardinal (cd*) (Figure 1 in Soler and Goñi, 2012). The presence of this new eye color in F_2 progeny was interpreted as the occurrence of the eye color genetic interaction between the tested mutations and concluded that the

cd locus was linked to chromosome 3. When the *brown* mutation produces white eye color with the *cinnabar* (*cn*), as observed in *D. melanogaster* (Lindsley and Zimm, 1992), it was interpreted as resulting from the occurrence of crossing over within the *bw-cn* interval in the previous generation(s). Considering the usefulness of these mutations as genetic markers for genetic mapping studies in *D. willistoni*, we present the chromosome gene arrangement of several eye color mutants and their hybrids.

Material and Methods

For the description of the eye color mutations and the experimental conditions of the mutant strains and genetic crosses, refer to Soler and Goñi (2012). Mutants were isolated from isofemale lines collected at the Faculty of Agronomy (34º 53´ S; 56º 16´ W), Montevideo City, Uruguay. The mutant strains used here are as follow:

- $bw^{SG23.00}$, $bw^{Sy11.03}$, bw^{Q51F13}, 2000, 2003, and 2009, respectively,
- $cd^{SG12.00}$, $cd^{SG4.01}$, 2000, and 2001, respectively, and
- $cn^{SM35.00}$, cn^{Q51F13}, 2000, and 2009, respectively.

The polytene chromosome preparation technique of Ashburner (1989, Protocol 18) was applied to obtain well-extended chromosomes of the eye color mutants and their hybrids. For chromosome identification and the description of inversions in *D. willistoni*, two important revisions were consulted. First, the research article of Schaeffer *et al.* (2008) presenting the genetic and physical maps of 11 *Drosophila* species, including the photomap of *D. willistoni.* As used in the previous article, we refer the chromosome (arms) to a single Müller chromosome element (A to F) as Müller (1940) and Sturtevant and Novitski (1941). Second, a recent revision by Rodhe and Valente (2012) presenting an exhaustive description of the arrangements in all five chromosome arms of 30 natural populations. Chromosomes were registered with Zeiss photomicroscope and phase contrast at 1000× magnification. Photomicrographs were edited using Adobe Photoshop 5.0.

Results and Discussion

Table 1 summarizes data on the chromosome arrangements found in the eye color mutant strains and their hybrids. Only inversions in the left arm of chromosome II (IIL) and in the chromosome III (acrocentric) were detected. The XL, XR, and IIR chromosomal arms are free of inversions in all strains and slides analyzed, presenting the standard order shown in Schaeffer *et al.* (2008) and in Rohde and Valente (2012).

Four segregating inversions in the IIL chromosome (Müller C) and four in the chromosome III (Müller E/F) were detected in the mutant strains analyzed (Table 1; Figures 1 and 2). Unlike previous studies on chromosome polymorphism in Uruguayan populations of *D. Willistoni* that include data on heterozygous inversions (Valente *et al.*, 2001, 2003), here we identify the homozygous arrangement for the IIL H inversion in the $cd^{SG12.00}$ mutant strain. Most relevant is that until now IIL H inversion homozygote was not observed in natural populations (Valente, pers. comm.); its fixation in the $cd^{SG12.00}$ strain may be related by a chance event during the isolation of this mutation. Apparently, individuals that are IIL H inversion homozygotes show good viability under laboratory conditions.

Table 1. Chromosome gene arrangements observed in eye color mutant strains and hybrids of *D. willistoni*.

Mutations, mutant strains and hybrids		Chromosome arm		Total larvae examined
		IIL	III	
brown	$bw^{SG23.00}$	F, D+E	J, B	15
	$bw^{Sy11.03}$	---	J	7
	$bw^{Q51.F13}$	---	J, B, C	10
cardinal	$cd^{SG12.00}$	H*	J, B, A	19
	$cd^{SG4.01}$	H, D+E	B	6
cinnabar	$cn^{SM35.00}$	F, D+E	B	6
Double mutant	$bw^{Q51.F13}$ $cn^{Q51.F13}$	---	J, B	5
F_1 hybrids	$bw^{SG23.00}/+$; $cd^{SG12.00}/+$	H, F, D+E	J, B, A	6
	$bw^{Q51.F13}/+$; $cd^{SG12.00}/+$	H, D+E	J, B, C, A	2
	$bw^{Q51.F13}/+$; $cd^{SG4.01}/+$	H, D+E	J, B,C	16

*****Homozygous for IIL H inversion. In all mutant strains and hybrids, the IIR arm has the standard gene arrangement. The double mutant strain, $bw^{Q51.F13}$ $cn^{Q51.F13}$, was isolated from the recombinant progeny between the segregating mutations of the same isofemale line.

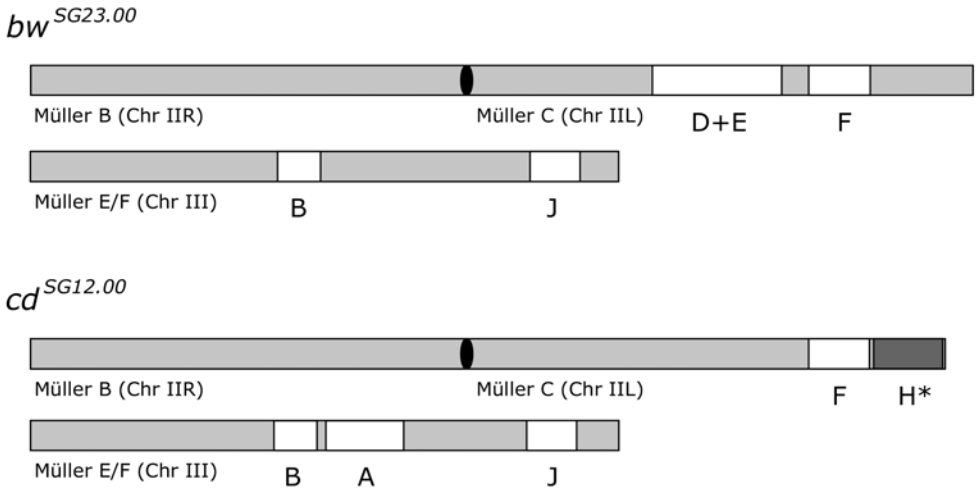

Figure 1. Genetic and chromosomal organization of the $bw^{SG23.00}$ and $cd^{SG12.00}$ mutant strains of *D. willistoni.*

The chromosome inversions observed in the mutants and their hybrids reveal that the *brown* and the *cinnabar* loci are unlinked to the IIL segregating inversions (F, D+E). Similarly, the *cardinal* locus is unlinked to any to the chromosome III segregating inversions (J, B, A). Spassky and Dobzhansky (1950) reported the eye color mutations *brown, orange, pink-wing,* and *purple* linked to chromosome 2, and the *claret* and *karmoisin* mutations linked to chromosome 3. These authors

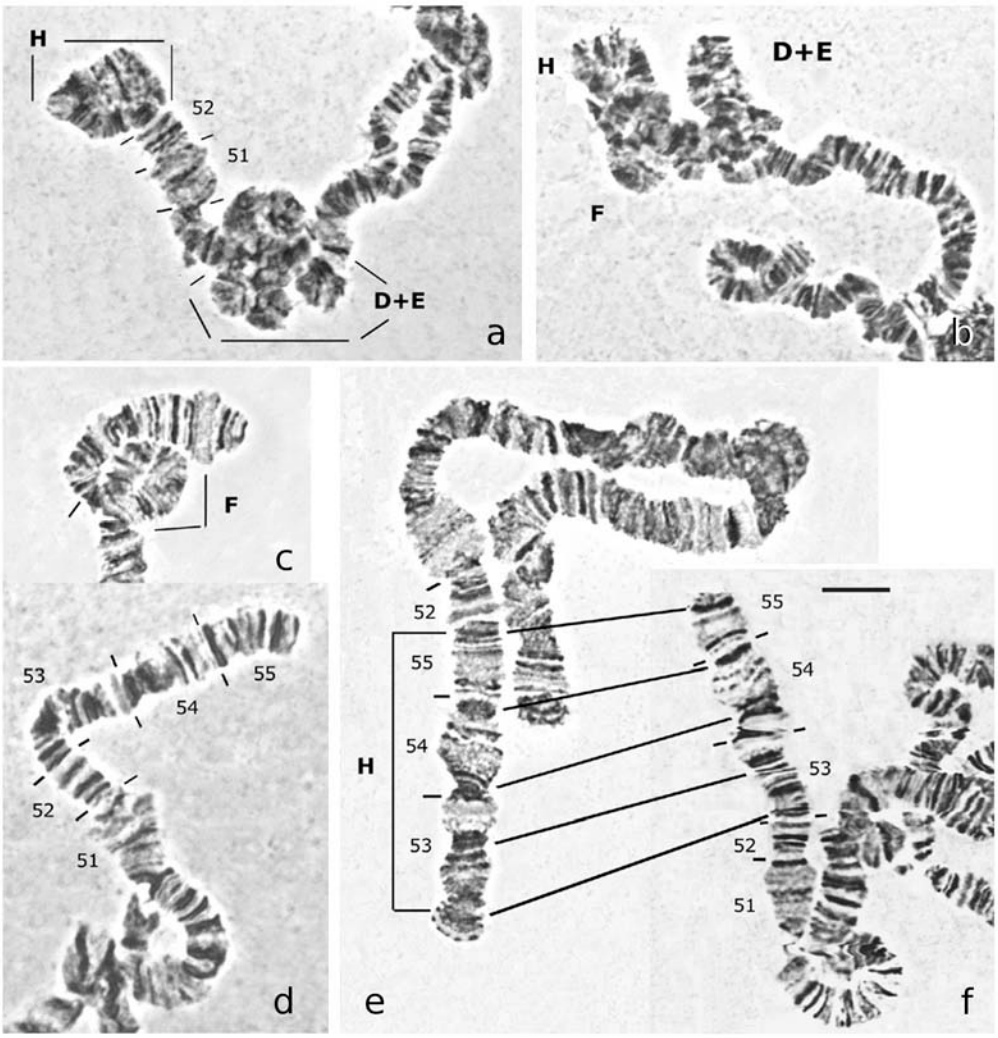

Figure 2. Chromosome gene arrangements at the IIL chromosome arm (Müller C) of *D. willistoni* described in this article. Complex gene arrangements in hybrids between the *bw* $^{SG23.00}$ and *cd* $^{SG12.00}$ eye color mutant strains (a, b). The tip of the chromosome IIL showing homozygous chromosome for the H inversion (e) in the *cd* $^{SG12.00}$ strain, and the standard chromosome gene arrangement (d, f) in the *bw* $^{SG23.00}$ strain. Characteristic IIL F heterozygous inversion in (c). IIL H: 52C – 55B; IIL F: 50A- 52C, and D+E: 42A-48B, as the cytogenetic map reference in Schaeffer *et al.* (2008) and in Rohde and Valente (2012).

mapped the *brown* locus on the distal region of the chromosome IIL, at 28 cM from *plexus*, and 70 cM from the *pink wing* locus. Mapping studies using new genetic and physical markers in *D. willistoni* will contribute to a better comprehension the genome of this species.

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References: Ashburner, M., 1989, Drosophila: *A Laboratory Manual*, pp. 434, Cold Spring Laboratory Press, New York; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster. 1133 pp, San Diego, Academic Press Inc; Müller, H.J., 1940, *The New Systematics*

(Huxley, J., ed.) pp. 185-268, Clarendon Press, Oxford; Rohde, C., and V.L.S. Valente 2012, Genet. Mol. Biol. 35: 966-979; Schaeffer, S.W., A. Bhutkar, B.F. McAllister, M. Matsuda, L.M. Matzkin, P.M. O'Grady, C. Rohde, V.L.S. Valente, M. Aguadé, W.W. Anderson, K. Edwards, A.C.L. Garcia, J. Goodman, J. Hartigan, E. Kataoka, R.T. Lapoint, E.R. Lozovsky, C.A. Machado, M.A.F. Noor, M. Papaceit, L.K. Reed, S. Richards, T.T. Rieger, S.M. Russo, H. Sato, C. Segarra, D.R. Smith, T.F. Smith, V. Strelets, Y.N. Tobari, Y. Tomimura, M. Wasserman, T. Watts, R. Wilson, K. Yoshida, T.A. Markow, W.M. Gelbart, and T.C. Kaufman 2008, Genetics 179: 1601-1655; Soler; A.M., and B. Goñi 2012, Dros. Inf. Serv. 95; Spassky, B., and T. Dobzhansky 1950, Heredity 4: 201-215; Sturtevant, A.H., and E. Novitski 1941, Genetics 26: 517-541; Valente, V.L.S., C. Rohde, V.H. Valiati, N.B. Morales, and B. Goñi 2001, Dros. Inf. Serv. 84: 55-59; Valente V.L.S., B. Goñi, V.H. Valiati, C. Rohde, and N.B. Morales 2003, Genet. Mol. Biol . 26: 163-173.

Effects of *α-synuclein* expression in the developing *Drosophila* eye.

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Introduction

As the fly brain has over 300,000 neurons and is organized into specialized areas for learning, olfaction, vision and memory (Wolf and Herbelein, 2003; Cauchi and Heuvel, 2006; Hardaway, 2010), *Drosophila* has become an important organism in which to model human neurodegenerative disorders. Furthermore, the *Drosophila* eye is tolerant to genetic manipulations and is dispensable for the survival of the fly (Chan and Bonini, 2000; Celotto and Palladino, 2005; Jeibman and Paulus, 2009). The directed expression of *α-synuclein* results in flies that are viable, accumulate aggregated α-synuclein in perinuclear and neuritic filamentous inclusions similar to Lewy bodies and Lewy neurites, age–dependent loss of dorsomedial DA neurons, neuronal degeneration, age-dependent loss of climbing ability, retinal degeneration (Feany and Bender, 2000; Auluck *et al.*, 2002), and ommatidial degeneration (Todd and Staveley, 2008). Using the bipartite UAS/GAL4 system (Brand and Perrimon, 1993) to overexpress *α-synuclein* in eyes of *Drosophila melanogaster* and performed biometric analysis, we investigated the possibility that developmental phenotypes become more severe with increased expression of α-synuclein.

Materials and Methods

Drosophila *stock and culture*

Dr. M. Feany of Harvard Medical School generously provided *UAS-α-synuclein* flies (Feany and Bender, 2000). The *GMR-GAL4*^{12} (Freeman, 1996) and *UAS-lacZ* were obtained from the Bloomington Drosophila Stock Center at Indiana University. The *GMR-GAL4 UAS-α-synuclein/CyO* line was generated using standard recombination, tested via PCR, and used to overexpress *α-synuclein* in the developing eye in the Glass Multiple Reporter (*GMR*) pattern. Stocks and crosses were maintained on standard medium containing cornmeal, molasses, yeast, and agar. Stocks were