



## Short Communication

## A spontaneous body color mutation in *Drosophila nappae* (Diptera, Drosophilidae)



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## ABSTRACT

A yellow-bodied male appeared spontaneously in an isofemale line of *Drosophila nappae* established from a wild-caught female collected at the Forest Reserve of the Instituto de Biociências da Universidade de São Paulo, Cidade Universitária “Armando de Salles Oliveira”, São Paulo city, state of São Paulo, Brazil. This is the first mutation found in *D. nappae*, a species belonging to the *tripunctata* group. The yellow male was isolated and individually crossed to two wild-type (brown-colored) virgin females from the same generation, yielding numerous offspring. All F<sub>1</sub> individuals were wild-type, but the phenotypes yielded in the F<sub>2</sub> generation were wild-type females, and both wild-type and yellow-bodied males. The latter yellow male mutants backcrossed with virgin wild-type F<sub>1</sub> females yielded four phenotypes (brown-colored and yellow-colored flies of both sexes), indicating an inheritance pattern of X-linked recessive. Chi-square goodness of fit tests ( $\alpha = 5\%$ ) detected no significant differences among the number of flies per phenotype. The new mutation is hereby named yellow, due to its probable homology to a similar mutation with an identical inheritance pattern found in *Drosophila melanogaster*.

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*Drosophila nappae* Vilela, Basso-da-Siva and Valente, 2004 is a member of the *Drosophila tripunctata* species group. It is the second largest group within the *Drosophila* subgenus in the Neotropical region (Vilela, 1992), currently with 86 described species (Bächli, 2016), only surpassed by the *repleta* group. Among those species, one in particular has been extensively studied. Aiming to transform *Drosophila mediopunctata* into a model organism to study Ecological and Evolutionary Genetics, Dr. L.B. Klaczko's research group has been investigating numerous aspects of its biology for over 30 years (Klaczko, 2006; Batista et al., 2014). So far, they were able to obtain nine mutations (most of them presenting allelic variation), using both X-ray treatments and inbreeding of flies collected in the field (Marques et al., 1989, 1991; Klaczko, 2006; Batista et al., 2014).

*D. nappae* was misidentified as *Drosophila angustibucca* Duda, 1925 for decades. Wheeler (1963), while analyzing the material that Frota-Pessoa (1954) used to redescribe the species, suspected those flies could in fact belong to an undescribed species. This suspicion was later confirmed by Vilela and Bächli (1990), who performed a redescription of *D. angustibucca* based on the only male paralectotype, which had not been analyzed by Frota-Pessoa. *D. angustibucca* Duda, sensu Frota-Pessoa, 1954, was later described by Vilela et al. (2004) under the binomial name *D. nappae*, upon

studying a multifemale strain established from specimens collected between 1994 and 1995 at Morro Santana, in Porto Alegre (RS), by Luciano Basso da Silva.

Assuming that all specimens identified by Frota-Pessoa (1954) as *D. angustibucca* actually belong to *D. nappae*, the distribution of the latter may be considered well-known (Vilela et al., 2004). Thus, *D. angustibucca* Duda sensu Frota-Pessoa had been initially collected in the following Brazilian states: Rio de Janeiro (Rio de Janeiro city [then Distrito Federal]), Rio Grande do Sul (Feliz and Porto Alegre), and São Paulo (Campos do Jordão, Mogi das Cruzes, Mongaguá [referred to as Vila Atlântica], and Pirassununga); located in southeastern and southern Brazil. Later on, the occurrence of this species (yet under the name *D. angustibucca*) in the state of Santa Catarina and at additional sites of the states of Rio Grande do Sul and São Paulo has been recorded by different authors (Araújo and Valente, 1981; Franck et al., 1984; Franck and Valente, 1985; Val and Kaneshiro, 1988; Valente and Araújo, 1991; De Toni and Hofmann, 1995; Saavedra et al., 1995), who probably based their identifications on the key proposed by Frota-Pessoa (*op. cit.*). Additional collection sites are Serra do Cipó (state of Minas Gerais, Brazil) [Tidon et al., 1994, as species R3] and Hohenau (Paraguay) [Bächli et al., 2000, as *D. paraguayensis* Duda, 1927].

The Drosophilidae Stock Center of the Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo currently keeps an isofemale *D. nappae* strain (coded M29F5) established from a fertilized female collected by one of us (CRV)

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between 22.VII and 03.VIII.2007 at the forest reserve on the main campus of the referred university, known as *Cidade Universitária “Armando de Salles Oliveira”*, São Paulo city, state of São Paulo, Brazil (23°33'54.6"S, 46°43'42.4"W; 766 m). In the Eighties, it was a challenging task to establish a strain of this species, since wild caught flies initially do not breed well in corn meal or banana-agar culture media, as commonly noted for most species within the *tripunctata* group. As posteriorly determined, the use of powdered milk-agar culture medium produces better results (Bächli et al., 2000). However, one important detail was unintentionally omitted in the cited paper: considering the sexual maturation of the species belonging to the *tripunctata* group is a slow process, during this phase it is essential to keep flies in a modified banana-agar culture medium (Goñi and Vilela, 2016) and only two weeks after emergence they should be placed in that special culture medium. However, it should be stressed that after successfully establishing the strain, it may be kept exclusively in modified banana-agar culture medium indefinitely.

Currently, the *D. nappae* stock is maintained at constant temperature ( $18 \pm 1^\circ\text{C}$ ) and photoperiod (13 h: 11 h, L:D). According to Goñi and Vilela (2016), after emergence, the flies are transferred weekly (for three weeks) to new vials containing a modified banana-agar culture medium, with a small ball of fresh bakers' yeast (*Saccharomyces cerevisiae*). It is worth noting that specimens of *D. nappae* can display catalepsy (feigning death) (Frota-Pessoa, 1954), meaning adults get paralyzed after suffering a mechanical impact. Therefore, the transference to new vials and the consequent collision with the culture medium often makes flies get stuck into its surface while paralyzed, and frequently are not able to detach themselves after recovering their movements. To overcome the high mortality rate, the vials with adult flies are kept upside down for the first three weeks, when adults are discarded. After discarding the mature flies, the vials from the third week, with numerous eggs and first instar larvae, are stored in the regular position. If the upper layer of the culture medium is too wet, V-shaped strips of filter paper (usually 4 per vial) are inserted into the culture medium.

On 20.V.2015, while one of us (ASR) was transferring the 1–7 days old wild-type (brown-colored) strain to new vials, noticed a conspicuously contrasting yellow specimen (Figs. 1 and 2). After determining it was a male, it was left isolated until reaching sexual maturity (circa 14 days), but transferred to new vials once a week. On 03.VI.2015 it was transferred once again to a new vial together with a wild-type virgin female. Even though they were kept together under observation for 2 h, and the male had displayed courtship behavior, the female was not receptive, so the flies did not mate. After this period of time, the female was removed from the vial. On the next day we added a different wild virgin female, and crossing was observed. In sequence, the male was transferred again to another vial, with a different wild virgin female, and the couple was kept together until 08.VI.2015. The original mutant male died on 13.VI.2015, was double-mounted and, together with samples from the strains M29F5 and M29F5F1 (see below), will be housed in the collections of the *Museu de Zoologia da Universidade de São Paulo*.

Shortly after mating, it was possible to observe eggs on the surface of the culture medium and 3 days later the first hatched larvae were noticed. This fact suggested the mutant male was fertile. All emerged adults were phenotypically wild, indicating this mutation, if hereditary, was recessive. Flies from the  $F_1$  generation were allowed to freely mate. Some pupae from the  $F_2$  generation were isolated and imagoes were sexed upon emergence, since this species does not exhibit a reliable pupal secondary sexual dimorphism. Only females were selected and kept isolated and, therefore, were virgins. In the  $F_2$  generation, emerged flies were analyzed on a daily basis, and three phenotypic classes were observed: wild-type females, wild-type males, and mutant males

**Table 1**

Total number of  $F_2$  offspring of two virgin wild-type (brown-colored) females ( $\varnothing$  1 and  $\varnothing$  2) from isofemale line M29F5 of *Drosophila nappae* crossed to one spontaneous yellow mutant male from the same strain. Emerged imagoes were analyzed daily, between 10.VIII and 16.X.2015. All adult males and females of  $F_1$  generation were phenotypically wild type.

$F_2$ phenotype	$\varnothing$ 1	$\varnothing$ 2
Wild (brown-colored) $\varnothing$	585	815
Wild (brown-colored) $\sigma$	255	377
Mutant (yellow-colored) $\sigma$	274	345
Total	1114	1537

**Table 2**

Total offspring of backcross [ $F_2$  (yellow-colored males)  $\times$   $F_1$  (brown-colored females)] from *Drosophila nappae* isofemale line M29F5. Four out of the eight performed crosses yielded offspring: three of them (females a, d and e) involving descendants of the  $\varnothing$  1 parental female and one (female g) descendants of the  $\varnothing$  2 parental female. Emerged adults were analyzed on a daily basis, between 20.IX and 31.X.2015.

Phenotypes	$\varnothing$ 1			$\varnothing$ 2	Pooled offspring
	a	d	e	g	
Wild (brown-colored) $\varnothing$	30	24	12	21	87
Wild (brown-colored) $\sigma$	29	27	15	16	87
Mutant (yellow-colored) $\varnothing$	26	30	9	17	82
Mutant (yellow-colored) $\sigma$	22	21	9	12	64
Total	107	102	45	66	320

(Table 1). The absence of mutant females suggested this mutation was X-linked. Chi-square goodness of fit test (Zar, 1996) at 5% significance level (with the use of Yates correction for continuity), accepted the null hypothesis that there were no significant differences between the number of adult wild-type and mutant yellow males in the  $F_2$  generation (calculated  $\chi^2 = 0.61$ ; critical  $\chi^2$  value = 3.84;  $0.30 < p < 0.50$ ). In sequence,  $F_2$  mutant males were backcrossed with  $F_1$  virgin wild females. Emerged flies of the next generation were analyzed daily as well, and four phenotypic classes were observed: wild and mutant females and wild and mutant males (Table 2). Even though there were more wild-type than mutant individuals in both sexes, a chi-square test accepted the null hypothesis that there were no significant differences among the number of wild-type and mutant yellow  $F_2$  flies pooled according to phenotype (calculated  $\chi^2 = 4.48$ ; critical  $\chi^2$  value = 7.82;  $0.20 < p < 0.30$ ). Additional studies are necessary to clarify this question, since it has been demonstrated that *Drosophila melanogaster* yellow mutant males present reduced level of locomotion and abnormal courtship, which causes mating disadvantage when paired with wild-type females (Bastock, 1956; Wilson et al., 1976; Burnet and Wilson, 1980). However, it should be pointed out that, in our experiments, the backcrosses of  $F_2$  yellow males to  $F_1$  wild males were performed with no choice for the females.

After crossing mutant virgin females and mutant males, we successfully established two yellow mutant isofemale strains of *D. nappae*, coded M29F5F1 and M29F5F2, but just the former is currently being maintained in our laboratory.

### Conflicts of interest

The authors declare no conflicts of interest.

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**Figs. 1, 2.** *Drosophila nappae*, habitus of males in right lateral views; to the same scale. 1, wild-type (brown-colored), from isofemale line M29F5. 2, mutant (yellow-colored), from isofemale line M29F5F1. Note the somewhat reticulate clouds of the wing crossveins r-m and dm-cu of the yellow mutant (2) contrasting with the conspicuous clouds of the brown-colored wild-type (1). Scale bar = 1 mm.

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