duration could enhance the fitness at 12ºC. Therefore, this confirms 22ºC is the optimum temperature for sexual activity of *Drosophila* flies.

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Spontaneous melanic mutant found in a *Drosophila neocardini* natural population.

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Introduction

The *Drosophila cardini* group is a Neotropical polymorphic group of species of the genus *Drosophila* (Heed, 1962; Heed and Russell, 1971). This group consists of 16 species inhabiting different areas of the Neotropical Region (Heed, 1962; Heed and Russel, 1971; Vilela *et al.*, 2002; De Toni *et al.*, 2005). Seven of these species (*D. antillea*, *D. arawakana*, *D. belladunni*, *D. caribiana*, *D. dunni*, *D. nigrodunni*, and *D. similis*) belong to the *dunni* subgroup, distributed in the Caribbean islands, while the other nine species (*D. acutilabella*, *D. bedichecki*, *D. cardini*, *D. cardinoides*, *D. neocardini*, *D. neomorpha*, *D. parthenogenetica*, *D. polymorpha*, and *D. procardinoides*) belong to the *cardini* subgroup, and are observed in an area that starts in Mexico and stretches to south Brazil, covering also the north of Argentina and Chile (Heed and Russell, 1971; Vilela *et al.*, 2002; De Toni *et al.*, 2005).

A series of previous studies reported the monomorphic or the polymorphic abdominal pigmentation state of the species of the *D. cardini* group. All but one of the species of the *D. cardini* subgroup are characterized by a highly polymorphic intraspecific abdominal pigmentation pattern that varies from almost completely pigmented to nearly unpigmented flies (Da Cunha, 1949; Da Cunha *et al*., 1953; Heed and Krishnamurthy, 1959; Heed and Blake, 1963; Martinez and Cordeiro, 1970). The exception is *D. procardinoides*, apparently restricted to the higher elevations in the Andes of Bolivia and Peru (Heed and Russell, 1971). The developmental control of abdominal pigmentation is variable in this subgroup as well (Da Cunha, 1949; Heed, 1963; Martinez and Cordeiro, 1970). *Drosophila neocardini* is one of the flies that displays this type of variation (Da Cunha, 1955). Its distribution covers Mexico, Panama, Colombia, Ecuador, Peru, and Brazil (Stalker, 1953; Heed and Russell, 1971), and occupies several kinds of environments with low abundance, except *cerrado* and *caatinga* Brazilian Biomes (Sene *et al.*, 1980). The abdominal pigmentation of *D. neocardini* is very similar to that of *D. neomorpha*, *D. parthenogenetica*, and *D. polymorpha*, but it is different in respect to the pattern of the abdominal black bands. In the middle of the sixth tergite

in *D. neocardini* there is a black square, which is absent in the other species (Da Cunha, 1955; De Toni *et al.*, 2001, 2005). Here, we report the discovery of a body melanic form in *D. neocardini* that has never been documented before for other *D. cardini* species.

Materials and Methods

Sample

The darker *D. neocardini* strain here analyzed was obtained from a collection performed in autumn of 2006 in a locality of Porto Alegre city, Brazil (30°07'S 51°10'W) (Garcia *et al.*, unpublished data) subject to a humid subtropical climate (Cfa according to Köppen) with average temperatures of 25°C in the summer and 14°C in the winter. The collection site is classified as an area of low urbanization level (over 40% vegetation cover) based on a classification system defined by Ruszczyk (1986) that considers the ratio between the green and built areas to differentiate the urbanization level.

Maintenance of color pattern test

To test whether the darker body color of the *D. neocardini* collected was influenced or not by the environmental conditions, we established two sub-strains from the darker F4 isofemale line. These two new strains were kept in chambers of controlled temperature and humidity, one of them at 17°C (± 1°C, 60% r.h.) and the other at 25°C (± 1°C, 60% r.h.) for 15 generations.

Crossing tests

The crossing tests were performed using the *D. neocardini* darker strain and a *D. neocardini* strain with light body color from Joinville city, Brazil (26º17'S 49º00'W). We carried out two experiments: (1) Cross 01: 10 virgin melanic females were crossed with 10 light-colored males, and (2) Cross 02: 10 virgin light-colored females were crossed with 10 melanic males, always with two vials for each cross. Control crosses were also done using 10 virgin melanic females and males, and 10 virgin light-colored females and males. All crosses were maintained in a controlled chamber at 25°C (± 1°C, 60% r.h.) in corn/flour culture medium. Photos of the specimens were taken with an Olympus stereophotomicroscope.

Cytological preparations

The salivary glands of third instar female larvae of each strain (dark and light) were prepared according to Ashburner (1989, p. 30) using Ringer's solution for dissection. Three larvae from each strain and three larvae from the F1 hybrid crosses were analyzed. The slides were examined under phase contrast Zeiss photomicroscope at 100× objective magnification.

Results and Discussion

In the analysis of the external characters of the three *D. neocardini* collected flies, we observed that they were darker than this species usually is, showing a shining light brownish-brown abdomen, mesonotum and scutellum (Figure 1). So, to confirm the initial identification we analyzed the terminalia of F1 offspring males, focusing on *aedeagus*. Using the available literature about the *cardini* group species (Val, 1982; Vilela and Bächli, 1990; Vilela *et al*., 2002; De Toni *et al.*, 2005), all the morphological characters indicated that the flies collected were conspecific of *D. neocardini*, but with darker body pigmentation, *i.e*., a melanic form.

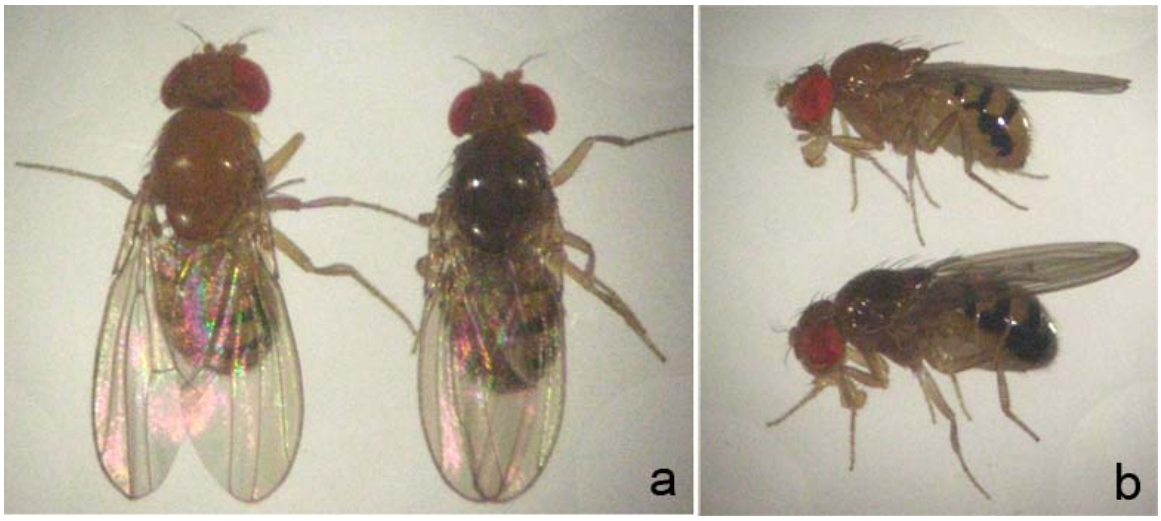

Figure 1. a, Dorsal view of the melanic (right) and light-colored (left) forms of *D. neocardini* individuals. b, Lateral view of the melanic (below) and light-colored

Confirming that the polymorphism detected is heritable, after 15 generations reared in different temperatures (17°C and 25°C) we verified that no different body color pattern became visible. This result indicates that: (1) this character is not environmentally controlled, and (2) all dark flies were possibly homozygous for the alleles that control body color pigmentation. Until now, after approximately 40 generations, we have not noticed any different pigmentation pattern in the melanic strain. We also observed that the lineages were better developed in the 25°C controlled chamber; this is why we used this temperature to conduct the crossing tests.

All the polytene chromosomes of the dark and light-colored strain, and also of the F1 hybrid offspring, presented the same banding pattern as in their reference photomaps (De Toni *et al*., 2001), and no rearrangements were detected in the hybrid chromosomes.

Table 1. Number of dark and light-colored offspring obtained in the crosses.

Offspring color	Cross 01		Cross 02	
	Vial A	Vial B	Vial A	Vial B
Light	58	41	24	24
Dark	11	05	06	09

In the crossing test all F1 offspring from crosses 01 and 02, resulted in a light color with yellowish-brown abdomen, mesonotum and scutellum. Table 1 shows the results obtained in the F2 offspring from crosses 01 and 02. In a first step, we tested our data for the χ^2 of heterogeneity for each cross, and it accepted to unite the data of the vials for each cross (cross 01 = 0.593 < $\chi^2_{0.05;1}$ = 3.84; cross 02 = 0.458 < $\chi^2_{0.05;1}$ = 3.84). Second, we tested our data for the hypothesis that the heritage pattern of the dark color observed behaves as a recessive autosomal characteristic; so, we tested the 3:1 proportion. The data from cross 01 did not confirm the H_0 hypothesis (cross 01 = 7.539 < $\chi^2_{0.05;1}$ = 3.84) that the data obtained did not differ statistically from the 3:1 proportion. However, the data from cross 02 supported the H_0 hypothesis (cross 02 = 0.047 < $\chi^2_{0.05;1}$ = 3.84). We observed that the melanic individuals had a less vigorous reproduction in the culture medium, sometimes resulting in the death of the larvae; in this way these individuals may have a less competitive ability when they are put together in the culture medium with the light-colored individuals. Considering this, we can assume that the dark individuals may have a reduced fitness, which would be reflected in the down deviations found in the statistical analysis of our crosses. Besides that, we also observed that the F2

melanic individuals produced a completely melanic F3 offspring, confirming that the dark *D. neocardini* form has a homozygous behavior.

Regarding the *D. neocardini* distribution (Heed and Russell, 1971), Porto Alegre city seems to be the southernmost limit of this species. At this latitude, the climate is subtropical with a mean temperature ranging from 2°C to 20.3°C in winter, although in this season temperatures as low as 0°C are common. In some *Drosophila* species, it is known that darker forms occur more commonly in samples collected in colder climates (Heed and Blake, 1963; Machado *et al.*, 2001).

So we can conclude that the *D. neocardini* melanic form corresponds to a recessive autosomal heritage pattern, and that the dark pattern of the individuals collected in Porto Alegre corresponds to a recessive homozygote condition for the dark allele.

Acknowledgments: The authors thank Dr. Marco Silva Gottschalk for the enthusiastic suggestion to begin this brief communication, B.Sc. Gisele de Souza da Silva for valuable help, to Dr. Ana Cristina Lauer Garcia by access to the collected material, and to Félix Nonnemacher for English revision. This work was supported by grants from CAPES, CNPq, and FAPERGS research funding.

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The heterochromatin of *Drosophila inca, D. yangana,* and *D. huancavilcae* of the *inca* subgroup, *repleta* group.

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In 1982, Wasserman extensively studied the chromosome phylogeny of the *repleta* group when he reported the results of the analysis of the sequence of bands of polytenic chromosomes in 70 species belonging to five subgroups that existed then. Considering the morphological, ecological, geographical, and genetic information and based on the presence of certain inversions, he proposed the existence of a sequence Primitive I that would occupy a central place in both the phylogeny of the *repleta* group as in the *Drosophila* genus and at least three phyletic lines connected to the different subgroups. One of these phyletic lines could go from Primitive I to *mulleri-fasciola* subgroups; a