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

second could go from Primitive I to *repleta-mercatorum* subgroups; and a third could connect directly the Primitive I with *hydei* subgroup (Wasserman, 1982, 1992).

In Ecuador, there are three species that make up the subgroup *inca* proposed by Rafael and Arcos (1989). This sixth subgroup of *repleta* group clusters *Drosophila inca* Dobzhansky and Pavan, 1943 (this subgroup was considered in a miscellaneous group of no clustered species by Wasserman until 1982) with *Drosophila huancavilcae,* an Ecuadorian species belonging to *repleta* group, discovered in 1989; and with *D. yangana* Rafael and Vela, 2003, discovered in another exploration of Ecuadorian drosofauna.

We asked ourselves as follows: Which is the position of *inca* subgroup in the evolutionary scheme of the species of the *repleta* group? Do the three species form a phylogenetic unit?

Studies of species characterization allow us to determine that these species live in arid regions, they are cactophilics, and they have a restricted distribution: *D. huancavilcae* was recorded in three coastal provinces of Ecuadorian Pacific: Manabí, Guayas and El Oro; *D. inca* was recorded in two interandean provinces: Pichincha and Loja; and *D. yangana* was recorded only in Loja. For cytological characterization of these species we worked with individuals of *D. yangana* and *D. inca*, species which were collected in sympatry, in Loja province (4° 23 'S, 79° 11' W), and *D. huancavilcae,* that is allopatric to the previous species, was collected in the Manabí province (1° 2 '46"S 80° 40' 4"W 450m). The three species had been found in nature in the fruits and cladodes of cactus *Opuntia ficus indica, Opuntia soederstromiana* and some species of genus *Armatocerus* (Rafael and Arcos, 1989; Rafael and Vela, 2003), while in the laboratory these species have the best development enriching their cultures with chunks of fruit. Their biological cycles were studied in these conditions during 22 days in *D. yangana* and *D. inca,* and 23 days in *D. huancavilcae*. The values of the fitness components are in ascending order from 68% in *D. huancavilcae* to 88.7% in *D. yangana* and to 92.7% in *D. inca.* The fertility as well as the viability percentages were 64%, 81.2% and 86.6%. We had recently described the mitotic karyotypes of three species of *inca* subgroup (Mafla, 2005a, 2005b, 2008).

Traditionally the phylogenetic studies with species of diptera have used the advantages of exceptionally characteristic polytenic chromosomes, and they had relegated the mitotic chromosomes, because they have considered these as of limited information; however, White (1978) noted that in *Drosophila* the metaphasic chromosomes allow the detection of the heterochromatin rearrangements. Many authors call attention to analyze these sections of genetic material for different functions that it redeem: nucleolar organizer regions are heterochromatic parts; the NORs that not only form the nucleolus but also play a promoter role of meiotic mating; also fertility factors that are in the heterochromatic Y chromosome; as well as the indirect effect of Darwinian aptitude that has repetitive DNA of heterochromatin, and it indicates its importance in the evolutionary process (Hartl and Lozovskaya, 1995; Powell, 1997).

These reasons have conducted us to do a comparative analysis of percentage content of heterochromatin in mitotic chromosomes, with the goal to approach an explanation about the relationships between species subgroup as well as advance hypotheses about the position of the *inca* subgroup within the scheme of phylogeny proposed by Wasserman.

The cultures of species from *inca* subgroup were created with virgin females from nature. The types of these populations are codified with catalogue number QCAZ-1760 and QCAZ-1761 for *D. huancavilcae*; QCAZ-1757 and QCAZ-1758 for *D. yangana*; QCAZ-7808 (♀) for *D. inca* in the Invertebrate Section of the Museum of Zoology of the Pontificia Universidad Católica del Ecuador (QCAZ).

We reiterate that the plates of mitotic chromosomes of species belonging to the *inca* subgroup were tinged with Giemsa 5%, and in description of the karyotypes of the three species we used the nomenclature for the morphology of chromosomes recommended by (Levan *et al*., 1964). The

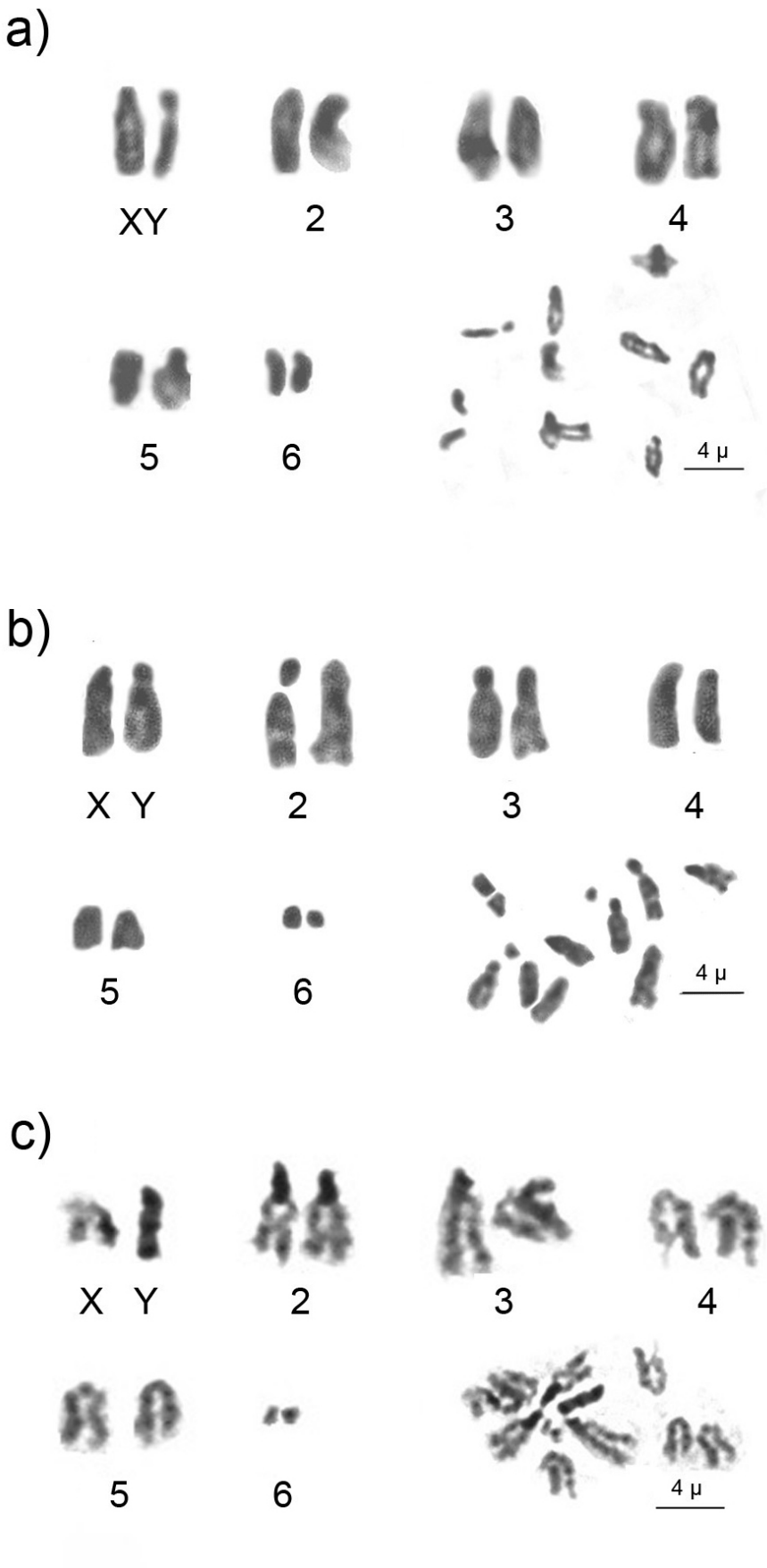

Figure 1. Karyotypes of: a) *Drosophila inca* with the par 6 sm, b) *D. yangana*, c) *D. huancavilcae* with the 6 chromosomes T and the Y chromosome bearer of secondary constriction.

Table 1. Relative length (RL) and morphology of chromosomes of the species of the *inca* subgroup.

Species	X	2	3	4	5	6	Y
Drosophila inca	11.35 st	9.92 St	9.07 st	8.32 sm	7.08 sm	5.08 Sm	9.21 sm
D. yangana [1]	10.19 sm	10.94 Sm	9.58 sm	8.90 sm	7.73 sm	2.58 T	10.06 sm
D. huancavilcae [2]	9.83 sm	11.89 St	10.08 st	8.64 st	7.41 st	2.21 T	9.31 st

[1]It can have one or two supernumerary chromosomes of RL 2.14.
[2]It can have one supernumerary chromosome of RL 3.25.

Table 2. Comparison of the heterochromatin percentages in the species of the *inca* subgroup.

Chromosome	*D. inca*	*D. yangana*	*D. huancavilcae*
X	2.46	2.66	2.64
2	4.30	5.64	5.76
3	4.40	4.74	3.68
4	4.36	4.62	3.40
5	3.86	4.22	3.32
6	10.16	5.16	4.42
Y	9.21	10.06	9.31
Total	38.75%	[1] 37.10%	[2] 32.53%

[1,2] No se considera la heterocromatina de los supernmerarios.

mitotic chromosomes were named as st, s, m or T according to the centromere position in subterminal, submedial regions, or at the end point, respectively (Mafla, 2005b).

The idiograms were built standardizing the values of 20 karyotypes (15 ♂♂, 5 ♀♀) of *D. inca*, 32 (27 ♂♂, 5 ♀♀) of *D. yangana*, and 27 (18 ♂♂, 9 ♀♀) of *D. huancavilcae* (Mafla, 2008). The percentage values of heterochromatin were obtained considering the relative lengths of short arms of chromosomes X, 2, 3, 4, 5 as well as the total length of the Y chromosome and chromosome 6.

In the three species of *inca* subgroup the most notable is that they coincide in their chromosome number 2n = 12 (Figure 1, Table 1). This fact allows us to consider these species like carrying the ancestral karyotype of genus (five bars and one dot), and this fact could guide us to relate the *inca* subgroup with the phyletic line that connects the Primitive I with *hydei* subgroup or *mulleri* subgroup, clusters where species with this karyotype are recorded.

However, the coincidence in chromosome number is only superficial, because each species has different amounts of heterochromatin in the short arms (See Table 2).

These rearragements are summarized by describing karyotypes: *D. inca* has three pairs of chromosomes st and three sm; the heterochromosome Y is sm, and it is completely heterochromatic.

The karyotype of *D. yangana* presents five pairs sm, the smallest couple is T; chromosome Y is sm, but it has a secondary constriction in its long arm. The karyotypes with one or two supernumerary chromosomes were found. The karyotype of *D. huancavilcae* has four couples st, one sm y one T; the chromosome Y is st, and highlighted by its heteropicnosis. In well extended nuclei is visible a secondary constriction in the long arm of Y chromosome; also karyotypes with one supernumerary chromosome had been registered.

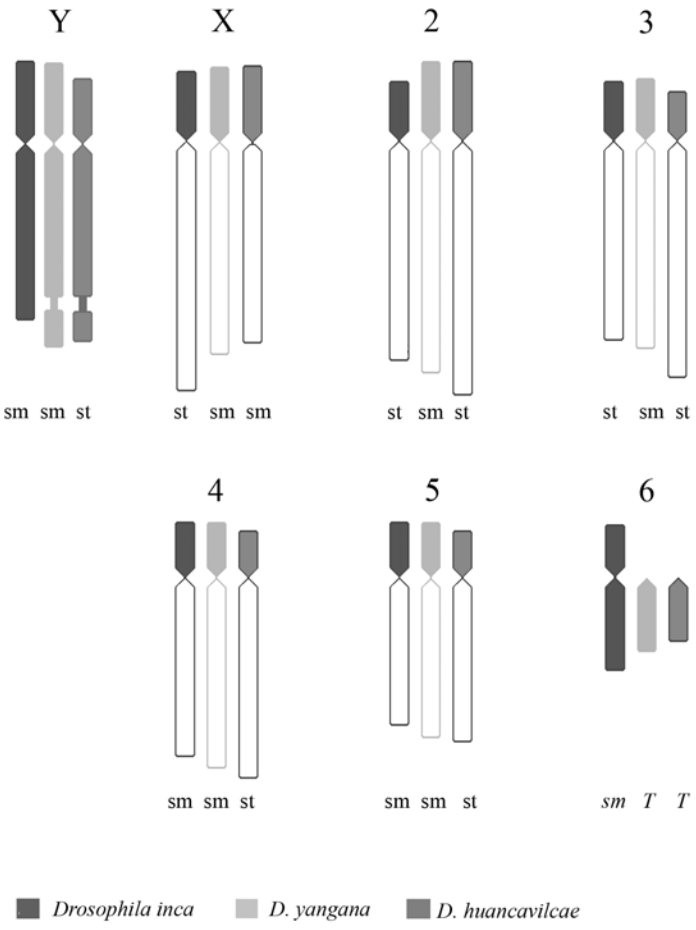

Figure 2. Idiograms showing the difference of morphology, relative length, and heterochromatin distribution.

The coincidence about the Y chromosome of *D. yangana* and *D. huancavilcae* having a secondary constriction, it could mean that they are more related between them than *D. inca*, whose Y chromosome lacks secondary constriction. But also the similarity in this marker with the reports in *D. uniseta* and *D. starmeri*, members of *mulleri* subgroup (Wasserman and Koepfer, 1979), provides a pathway that links the *inca* subgroup with phyletic line that connects the Primitive I with *mulleri* - *fasciola* subgroups rather than the *hydei* subgroup.

The presence of supernumerary chromosomes in both species, in *D. yangana* as in *D. huancavilcae*, is striking, because the extra chromosomes are considered exceptional in the genus, previously found only in two species: *D. nasuta albomicans* and *D. subsilvestris* (Powell, 1997). This isolated fact also could be unusual argument for proposing the closest relationship in this duo species.

The third aspect about similarity between *D. yangana* and *D. huancavilcae* is the presence of small telocentric 6, a characteristic that separates them from *D. inca*, which has metacentric 6 with double length.

The idiograms (Figure 2) simplify the morphological characteristics of chromosomes of these species and allow us to appreciate the heterochromatic portions. We can clearly discern that the short arms of subtelocentric chromosomes have less proportion than submetacentric chromosomes. As well as the great accumulation of heterochromatin in the Y chromosome and 6 chromosome, this latest property suggests a possible link between the origin of chromosome 6 metacentric of *D. inca* with supernumerary chromosomes of the other two species.

In Table 2 we compared the heterochromatin percentages of the three species of *inca* subgroup, highlighting the accumulation referred and the upward trend from *D. huancavilcae* to *D. yangana* and to *D. inca.* This gradient is similar to what was observed in the values mentioned in the introduction. This fact could be a confirmation of the correlation between accumulation of heterochromatin and acquiring greater biological fitness during the speciation process. Likewise, the extensive range of distribution that we had evidenced in *D. inca* could be interpreted like a stroke of major fitness of this species in comparison with their sisters that recently we had registered in the interandean province of Imbabura in the north of Ecuador Rafael, V., and Acurio, A. (2008, personal communication).

Consequently *D. inca* could be a derivative species and *D. huancavilcae* probably more ancient, and it could be related with *D. yangana.*

Polytene chromosomes of *D. huancavilcae* were analyzed, and we identified a new inversion called $2y^5$ and inversions: Xabc, 2ab, 3b of Hypothetic Primitive I sequence (Romero and Mafla, 2008 in press), while we are cultivating the two others species: *D. inca* and *D. yangana* to continue with the cytologic analysis of giant chromosomes.

Acknowledgments: We thank Dr. Clifford Keil, who helped in the revision of this article with appropriate comments about the content and wording, and also Dr. Violeta Rafael for her constant encouragement.

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Response to light and distribution of *Drosophila* larvae in a feeding environment.

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Introduction

Photoresponses are widespread between invertebrates. The sign of the photoresponse may adjust to variations in the external stimulus situation. In this way, the response to illumination