



Chromosomal phylogeny of the *Drosophila fasciola* species subgroup revisited (Diptera, Drosophilidae)

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Abstract

The analysis of polytene chromosomes in 26 strains of seven species in the *Drosophila fasciola* subgroup, from several locations in Brazil, in addition to strains of two species belonging to the *Drosophila mulleri* subgroup (*D. aldrichi* and *D. mulleri*), enabled us to determine that the 3c inversion found in the latter species differ in one of its break points from that present in the species of the *fasciola* subgroup. Therefore, a change in the *mulleri* complex denomination from inversion 3c to inversion 3u is proposed. Accordingly, the *fasciola* subgroup is no longer a lesser phylogenetic part within the *mulleri* subgroup. Rather, it is directly related to the likely ancestor of the *repleta* group, called Primitive I. This information removes the main obstacle to considering the *Drosophila fasciola* subgroup as an ancestral group within the *Drosophila repleta* species group, according to the hypothesis of Throckmorton. Our data also support the conclusion that *D. onca* and *D. carolinae* are closely related species based on one new inversion in chromosome 4 (4^f), in both species. *D. fascioloides* and *D. ellisoni* also form a pair of sister species based on the presence of fusions of chromosomes 2-4 and 3-5. *D. rosinae* is related only to the likely ancestor of the *fasciola* subgroup, where the 3c inversion was fixed.

Key words: *Drosophila*, chromosome phylogeny, *repleta* group, *fasciola* subgroup, chromosome inversions.

Received: March 28, 2003; Accepted: October 13, 2004.

Introduction

The *repleta* group of the genus *Drosophila* is endemic to the Americas. This group comprises more than 95 nominal species (Sturtevant, 1942; Vilela, 1983; Rafael and Arcos, 1989; Vilela and Bächli, 1990; Tidon-Sklorz and Sene, 1995a, b, 2001; Bächli and Vilela, 2002), and is divided into six subgroups: *fasciola*, *hydei*, *inca*, *mercatorum*, *mulleri* and *repleta*.

Their species are widely distributed in the New World and mostly found in semiarid regions with open vegetation (Pavan, 1959; Sene *et al.*, 1980; Vilela, 1983; Vilela *et al.*, 1983; Tidon-Sklorz and Sene, 1995c; Tidon-Sklorz *et al.*, 1994). The species in the *hydei*, *mercatorum* and *repleta* subgroups are mostly generalists, while those in the *mulleri* and *inca* subgroups use cacti as breeding sites (Pereira *et al.*, 1983; Rafael and Arcos, 1989).

The *fasciola* subgroup comprises an assemblage of 21 nominal species (Wasserman, 1962a; Vilela, 1983; Vilela and Bächli, 1990; Bächli and Vilela, 2002), which inhabit

mostly forests. They are associated with various substrates: for instance, *D. fulvalineata* was collected on fungi (Patterson and Wheeler, 1942); *D. fasciola* emerged from flowers and fruits such as *Aphelandra micans* (Acanthaceae), *Erythrina berteroana* (Fabaceae), *Heliconia latispatha* (Musaceae) and aroid (Araceae) (Pipkin *et al.*, 1966). In forest environments, besides these substrates, these flies use epiphytic cacti (*Rhypsalis* sp.) as breeding sites (Sene *et al.*, 1977, Morais *et al.*, 1995). Moreover, in open vegetation, *D. rosinae* emerged from columnar cacti (*Cereus* sp.) (Wasserman, 1962a; Pereira *et al.*, 1983; Tidon-Sklorz and Sene, 1995c). Apparently, the morphology of the testicles and of the seminal receptacle of the species in this subgroup is intermediate between that in the *mulleri* and *repleta* subgroups (Wasserman, 1962a).

The origin and adaptive radiation of the cactophilic species in the *repleta* group probably occurred in the Oligocene and Miocene (Throckmorton, 1975, 1982). This group is likely to have originated in the transition zone between the Nearctic and Neotropical biogeographic regions in Mexico (Wasserman, 1954).

Cytologically, the ancestral species of all *fasciola* subgroup would have evolved from the Primitive I, a hypo-

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thetical sequence of polytene chromosomal bands, suggested by Wharton (1942), that differs from the standard arrangement of the *Drosophila repleta* by the presence of the Xabc;2ab;3b inversions, and by the fixation of the 2o³, 2e³ and 2l³ inversions. Accordingly, the basic chromosomal composition of the subgroup would be the Primitive VII (Xabc; 2abo²e³l³; 3b; Wasserman 1960, 1962a, 1992).

According to Wasserman (1982), the existence of the 3c inversion in species of the *mulleri* complex (included in the *mulleri* subgroup) and of the *fasciola* subgroup sustains the hypothesis of a common ancestry, with the *fasciola* subgroup stemming from the *mulleri* subgroup. The fact that the species of the *fasciola* subgroup are forest dwellers could be an indicator of reinvasion of the forests by desert-adapted species.

Throckmorton (1982), and in earlier works (Throckmorton 1962, 1975), discusses the problem of the origin of the *repleta* group and states: "whether the ancestor of the *repleta* group itself was a forest species which "became" a "*repleta*", began diversifying in the forest, and subsequently moved into arid habitats, or whether it first moved into arid habitats and became a *repleta* there, is difficult to determine. Its closest relatives, the *castanea*, *canalina*, *dreyfusi* and *mesophragmatica* groups, are forest forms, for the most part, and apparently primitive members of the *repleta* group that are at least facultative forest forms breeding in fallen fruit. Parsimoniously, this permits

the inference that the founder of the *repleta* group was a forest form, not necessarily of the wet forest, which "became" a *repleta* while still associated with forest habitats. At the present time, and on anatomical grounds especially, the major separation within the *repleta* group is between the *hydei* subgroup on the one hand and the remaining subgroups on the other, with the *fasciola* subgroup being the most primitive among the latter forms". Supporting this idea, Morais et al. (1995) proposed the possibility of the *repleta* group ancestor having inhabited the forests, and, based in composition studies of yeasts, associated to these flies, suggesting that the *fasciola* subgroup represents the oldest lineage from which the South American species of the *repleta* group may have evolved. This statement is in agreement to the ecological data mentioned above.

Even though Throckmorton's hypothesis (1962, 1975 and 1982) rests upon morphologic and ecological data, it does not explain the presence of the 3c inversion in both *fasciola* and *mulleri* subgroups, favoring the hypothesis proposed by Wasserman (1962a and b; 1963;1982).

Material and Methods

We analyzed 26 isofemale strains of seven species in the *fasciola* subgroup established from specimens collected (Tidon and Sene, 1988; Tidon-Sklorz and Sene, 1992) in different locations (Table 1).

Table 1 - List of strains, collection localities and chromosomal arrangements of seven species of *Drosophila* in the *fasciola* subgroup.

Species	Strain	Locality	Chromosome standard sequence of PRIMITIVE VII				
			Xabc	2abo ² e ³ l ³	3b	4	5
<i>D. carolinae</i>	J16C2	Tibagi, PR	+		c	l ² *,n ² *	+
<i>D. coroica</i>	D88N1	Sertãozinho, SP	+	+	c, p	+	+
	D88N19	"	+		c, p	+	+
	D88N24	"	+	+	c, p	+	+
	D88N27	"	+	+	c, p	+	+
	D88N53	"	+	+	c, p	+	+
	D88N58	"	+	+	c, p	+	+
	D88N60	"	+	+	c, p	+	+
	D88N64	"	+	+	c, p	+	+
	D88N69	"	+	+	c, p	+	+
	D93N3	Cianorte, PR	+	v	c, p	+	+
<i>D. ellisoni</i>	D83N5	Camburi, SP	+	d ³ p ²	c	+	+
	D83N8	"	+	d ³ p ²	c	+	+
	D84N11	"	+	d ³ p ²	c	+	+
<i>D. fascioloides</i>	D84N2	Camburi, SP	+	d ³ p ²	c	+	+
	D86N6	Nova Friburgo, RJ	+	d ³ p ²	c	+	+
	D86M	"	+	d ³ p ²	c	+	+
	D86N17	"	+	d ³ p ²	c	+	+

Table 1 (cont.)

Species	Strain	Locality	Chromosome standard sequence of PRIMITIVE VII				
			Xabc	2abo ² e ³ l ³	3b	4	5
<i>D. moju</i>	F34M2	Panamá, Panama	+	p ² ,r ² ,n ²	c, m	+	h,i,j
<i>D. onca</i>	D93N1	Cianorte, PR	+	+	c	f ²	+
	H61C30	Arroio Teixeira, RS	+	+	c	f ²	+
	H61C19	"	+	+	c	f ²	+
	J14C2	Salto S. Rosa, PR	+	+	c	f ²	+
	D63C1	Mucugê, BA	+	+	c	+	+

* - New inversions described in this paper.

Polytene chromosomes from the salivary glands of third instar larvae were prepared by squashing techniques in 2% lacto-aceto-orcein, fixed in acetic acid and perchloric acid. They were then compared with the maps depicted by Wharton (1942) and Wasserman (1962a).

In order to define the presence of the 3c inversion, the polytene chromosomes of two species of *Drosophila* belonging to the *mulleri* complex of the *mulleri* subgroup (*D. aldrichi* and *D. mulleri*) were also analyzed.

Results

Of the seven *Drosophila* species in the *fasciola* subgroup under study, five came from humid coastal and inland Brazilian forests, one from Panamanian forests and the remaining one from the Caatinga domain (Table 1).

By comparing chromosome 3 of species in the *mulleri* (*D. aldrichi* and *D. mulleri*) and *fasciola* subgroups, we verified that one of the breakpoints of the 3c inversion present in the species of the *fasciola* subgroup is not the same as determined for the 3c inversion present in the species of the *mulleri* group. That is, there are two overlapped inversions sharing one breakpoint, and not one single inversion as previously thought. The 3c inversion was described by Wasserman (1962a), by analyzing species from the *fasciola* subgroup, as having the E4a and G1c breakpoints. However, we observed that the inversion present in the species of the *mulleri* subgroup differs by one of the two breakpoints: E5b - G1c. Thus, we suggest that this inversion in the *mulleri* subgroup, as it is still undescribed, should be renamed as 3u. (Figures 1 and 2; Table 1). In addition, two new inversions fixed in chromosome 4 were observed along with the inversions reported in the literature (Table 1; Figure 1). Their breakpoints are shown in Figure 3.

The dozen lineages of *D. coroica* ($2n = 12$) that were analyzed have the previously described sequence 3p (Wasserman, 1962a).

In *D. ellisoni* and *D. fascioloides* ($2n = 8$), chromosomes 2 and 4 (2-4F) and 3 and 5 (3-5F) are fused. These fusions were described earlier by Dobzhansky and Pavan (1943), Wasserman (1962a) and Kuhn *et al.* (1995), studying metaphase chromosomes. The presence of the 2p² and

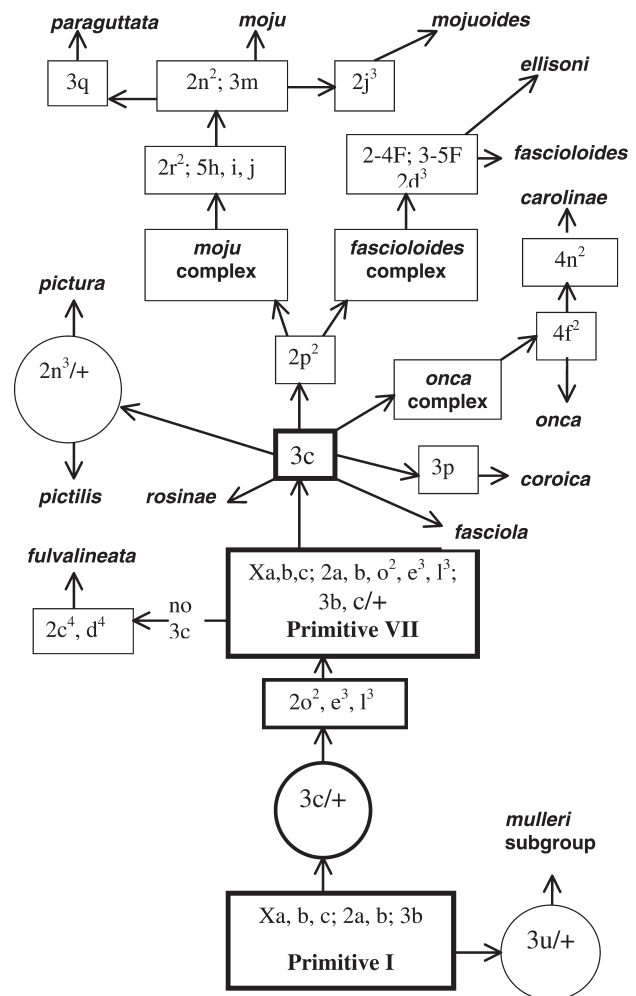


Figure 1 - The diagram illustrates the revised phylogenetic relationships among some species within the *fasciola* subgroup and between the *fasciola* subgroup and the *mulleri* subgroup in the *repleta* group, as proposed in this study (modified from Wasserman, 1992:522).

2d³ inversions was confirmed. The 2d³ inversion described by Wasserman (1962a) as polymorphic in *D. ellisoni* (cited as *fascioloides*) was found to be fixed in our strains.

D. moju, from Panama, has the same sequence previously described by Wasserman (1962a).

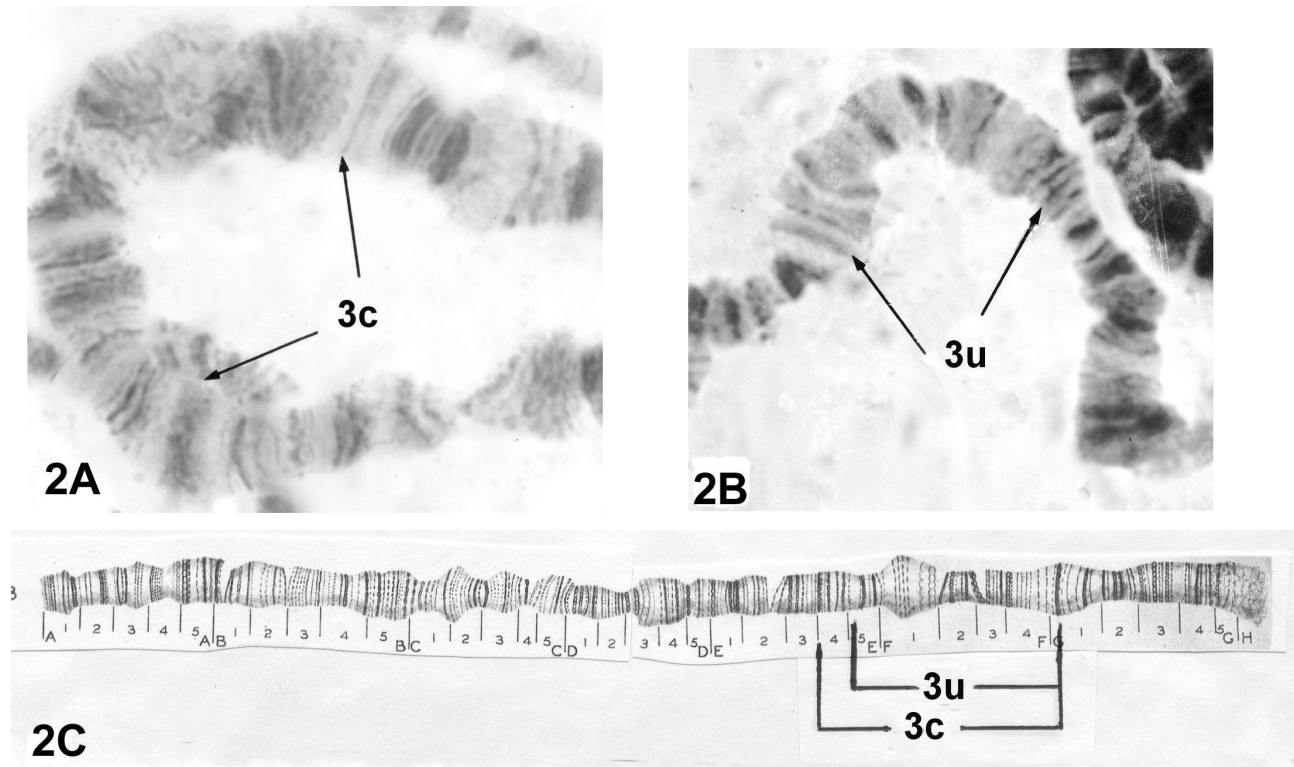


Figure 2 - (A) inversion 3c (*D. ellisoni*) and (B) inversion 3u (*D. aldrichi*). The breakpoints are marked in the chromosome map of *Drosophila repleta*, as depicted by Wharton (1942) (C).

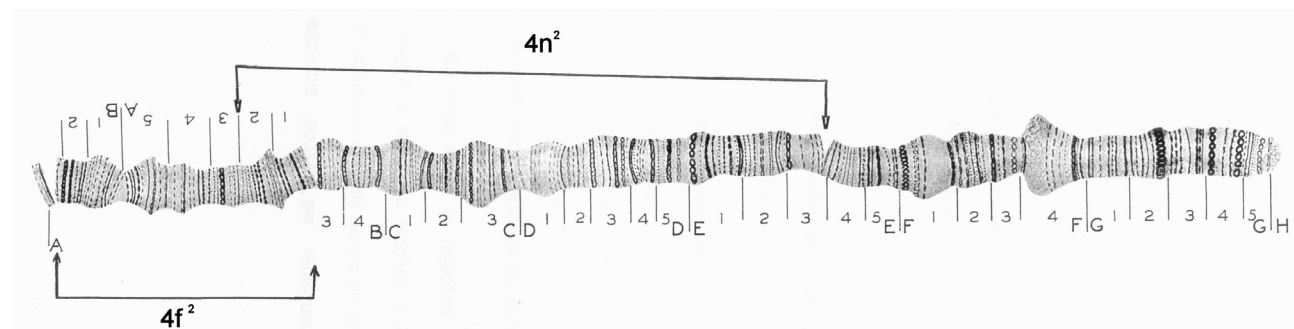


Figure 3 - Breakpoints of inversion 4f², in *Drosophila onca*, and inversion 4n² overlapping inversion 4f², in *D. carolinae*, in the chromosome map of *Drosophila repleta* as depicted by Wharton (1942).

D. onca ($2n = 12$) has a fixed inversion on chromosome 4 (4f², breakpoints A1c - B3b). *D. carolinae* ($2n = 12$) presents the 4n² inversion (break points A3a - E3i) overlapping the 4f² arrangement (breakpoints A1c - B3b) (Figure 3).

D. rosinae ($2n = 12$) shows the standard primitive sequence of the *fasciola* subgroup.

Discussion

Most of the information obtained in this study is in accordance with the literature (review in Wasserman, 1992). Fixed inversions on chromosome 4 were found in *D. onca* as well as in *D. carolinae*.

What does not match previous findings is the fact that the 3c inversion found in *fasciola* does not have the same 3c breakpoints as described in the *mulleri* complex by Wasserman (1962a, b). Accordingly, we propose that the denomination of the inversion present in the species of the *mulleri* complex be changed from 3c to 3u. This observation changes the previously proposed phylogenetic relationships among the species in the *mulleri* and *fasciola* subgroups as well as the relationships of the species in the *fasciola* subgroup within the *repleta* group. The *fasciola* subgroup becomes derivative of Primitive I in the *repleta* group and is no longer a derivative of the *mulleri* complex, as proposed by Wasserman (63) (Figure 1). The present hy-

pothesis was required for the subgroup *fasciola* to be considered ancestral of the *repleta* group with a forest origin, as proposed by Throckmorton (1975, 1982) and “supported” by morphological and ecological data (Pipkin, 1965; Pipkin *et al.*, 1966; Sene *et al.*, 1977; Pereira *et al.*, 1983; Morais *et al.*, 1995). This new phylogenetic hypothesis, based on chromosomal inversions, offers a better perspective to understanding the relationships within the subgroup to be inferred from other markers as in Costa and Sene (2002).

Furthermore, based on cytological data, we propose two new species complexes within the *fasciola* subgroup: the *fascioloides* complex, comprising *D. ellisoni* and *D. fascioloides* which share one inversion ($2d^3$), two centric fusions (2-4F and 3-5F), and a great karyotype similarity regarding the X chromosomes (Kuhn *et al.*, 1995); and the *onca* complex, comprising *D. carolinae* and *D. onca*, which share one inversion ($4f^2$) in addition to the similarity in the morphology of their aedeagi as shown by Vilela (1983). The species *D. rosinae* directly derives from Primitive VII, the hypothetic ancestral sequence to the *fasciola* subgroup (Figure 1), after fixation of the 3c inversion.

Acknowledgments

The authors wish to thank Prof Marwin Wasserman for the time NM Diniz spent in his laboratory, CR Vilela for providing the stock of *D. moju*, MH Manfrin for a critical reading of the manuscript, MAR Alves for assistance in obtaining the chromosome preparations, and PR Epifânio for assistance in the maintenance of stocks. Financial support from CAPES, CNPq, FAPESP, FINEP and USP made this study possible.

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Associate Editor: Angela Maria Vianna-Morgante