ABSTRACT

The process of speciation occurs through the evolution of any of several forms of reproductive isolation between taxa, including inviability of hybrids. In this work, strains derived from allopatric populations of Drosophila buzzatii cluster species were experimentally crossed in order to evaluate their reproductive and cytogenetic relationships, and to contribute toward understanding the reproductive isolation in this group of sibling species. Although intrastrain crosses were highly fertile, we consider it relevant to discuss the differences in intra- and interspecific fertility and fecundity here. Among 30 interspecific crosses, about 63% were partially or completely sterile. Fifty three percent of interspecific F1 crosses (female and male F1 crossed) were also partially or completely sterile, in contrast to only one out of 24 intraspecific F1 crosses that was partially sterile. An analysis of hybrid polytene chromosomes revealed complete synapsis, except in the microchromosomes (VI) and in the proximal region of the X chromosome. The intraspecific divergence observed in this study and the variable degree of chromosome pairing shown here reveal part of the complexity of the speciation process pertinent to Drosophila buzzatii cluster, which is consistent with different traits studied in this cluster.

Keywords: Drosophila buzzatii cluster, reproductive isolation, polytene chromosome synapse.

RESUMO

Relações reprodutivas e grau de sinapse nos cromossomos polítenicos de espécies de Drosophila do cluster buzzatii

O processo de especiação ocorre pela evolução de qualquer uma das diversas formas de isolamento reprodutivo entre táxons, incluindo inviabilidade de híbridos. Neste trabalho, linhagens provenientes de populações alopátricas de espécies de Drosophila do cluster buzzatii foram cruzadas experimentalmente com o objetivo de avaliar suas relações reprodutivas e citogenéticas, e contribuir para o entendimento do isolamento reprodutivo neste grupo de espécies intimamente relacionadas. Os cruzamentos dentro de uma mesma linhagem foram altamente férteis, contudo as diferenças na fertilidade e fecundidade encontradas intra e interespécificamente são discutidas neste artigo. Dos 30 cruzamentos interespecíficos, 63% foram parcialmente ou completamente estéreis. Cinquenta e três por cento dos cruzamentos de F1 interespecíficos (fêmeas e machos F1 cruzados entre si) também foram parcialmente ou completamente estéreis, em contraste com apenas um, em 24 cruzamentos de F1 intra-específicos, que foi parcialmente estéril. A análise dos cromossomos polítenicos nos híbridos revelou sinapse completa, exceto nos microcromossomos (VI) e na região proximal do cromossomo X. A divergência intra-específica e o grau variável de pareamento...
INTRODUCTION

The study of reproductive isolation patterns regarding species divergence is critical for understanding the speciation process. Such comparative analyses have been conducted in several animal groups (see Lijtmaer et al., 2003 for an example on birds group). Similarities among some studies suggest that the patterns of the evolution of postzygotic isolation, and the process of speciation in general, are shared among animal groups. The genus *Drosophila* of Diptera has already been studied in terms of reproductive relationships among allopatric and sympatric populations (for example, see Prakash, 1972 and Dobzhansky, 1975). Several studies have demonstrated that interspecific hybrids of *Drosophila* showed incomplete chromosomal synapsis in comparison with chromosomal synapsis inside the species (Cordeiro, 1968; Evgen’ev, 1971; Bicudo, 1979; Madi-Ravazzi & Bicudo, 1992, Madi-Ravazzi et al., 1997, for examples). Madi-Ravazzi & Bicudo (1992) and Madi-Ravazzi et al. (1997) demonstrated a parallel between hybrid asynapsis and postzygotic reproductive isolation in species of the *Drosophila repleta* group. The divergence in reproductive traits among incipient species has been well documented in this group (Markow, 1981, 1991; Bizzo, 1983; Madi-Ravazzi & Bicudo, 1992; Marin et al., 1993; Madi-Ravazzi et al., 1997; Machado et al., 2002; Markow et al., 2002; Pitnick et al., 2003).

The *Drosophila repleta* species group (Diptera, Drosophilidae) occurs in different habitats, although one notable characteristic of this group is the capacity of many species to use cactus tissues for breeding and larval development (Wasserman, 1992). This adaptation has allowed the group to occupy the deserts and arid zones of the New World (Ruiz & Fontdevila, 1981). The *Drosophila buzzatii* cluster of the *D. repleta* group (*D. mulleri* subgroup) is widely distributed in different types of vegetation in South America, and the polymorphism and polytypism found in geographical populations of this cluster make it very useful for ecological adaptation and speciation studies. Pioneer studies of Brazilian populations of *Drosophila buzzatii* found a group composed of cryptic species characterized by different polytene chromosome fixed inversions, aedeagus morphology and geographic distribution (Sene et al., 1982; Sene et al., 1988). Further studies (such as Vilela & Sene, 1977; Silva & Sene, 1991; Tidon-Sklorz & Sene, 1995a,b and 2001) allowed for the description of seven species in the *Drosophila buzzatii* cluster.

*Drosophila buzzatii* has an aedeagus morphology that differs from the other six species of the cluster and presents the 5 g polytene chromosome fixed inversion. *Drosophila buzzatii* is a native of South America, but has also become cosmopolitan with the introduction of its host cactus around the world (Wasserman, 1962). The morphological aedeagus type of *Drosophila borborema*, which has 2e8 polytene chromosome fixed inversion, is unlike that of *D. buzzatii* but very similar to those of the other species of the *buzzatii* cluster. *Drosophila borborema* is distributed in northeastern Brazil and in Grão Mongol, state of Minas Gerais (Vilela & Sene, 1977; Tidon-Sklorz & Sene, 1995a). *Drosophila koepferae*, which occurs on the slopes of the Andes from Argentina to Comarapa, Bolivia, on the western side of the Chaco (Fontdevila et al., 1988), has 2j9 fixed inversion and aedeagus type E. *Drosophila seriema* occurs in northeastern Brazil, on the eastern side of the Espinhaço mountain range and along Brazil’s northeastern Atlantic coast down to the state of Rio Grande do Sul, has 2x7 fixed inversion and aedeagus type A (Vilela & Sene, 1977). *Drosophila gouveai* shares 2e8 fixed inversion with *D. borborema* and *D. seriema* and presents aedeagus type B. It is distributed in Brazil’s western Caatinga region and in the center and southeast of the country (Tidon-Sklorz & Sene, 2001). *Drosophila seriema* is limited to the Espinhaço mountain range (Tidon-Sklorz & Sene, 1995b) and presents...
and interspecific crosses, and the respective F1 cross boxplot graphs, were done for all the crosses described in Table 1; however, we illustrate here only the boxplot which allows for a comparison of reciprocal intra- and interspecific crosses with their respective F1 crosses (Figs. 2-5). Not all the fecundity data of crosses were included on boxplot graphs because many F1 crosses could not be done due to sterility or low productivity of some interspecific crosses. Moreover, some interspecific F1 crosses were found to be sterile (Table 2).

Virgin males and females seven to nine days old were used in all mass crossing experiments. Four crosses (1 to 4, Table 2) were done concomitantly, with 20 couples each, for all the experiments analyzed in this study. Two transfers to fresh culture medium were done at a one-week interval; the parental flies were discarded one week after the second transfer. Thus, three different age groups for each mass cross were obtained and named 1st, 2nd and 3rd oviposition periods. The mean age of the flies in these periods were 11, 18 and 25 days old, respectively. The sex and number of individuals emerging from the crosses were computed twice a week for two weeks, making a total of four counts for each oviposition period. Fertility was also compared along the four crosses and the three oviposition periods involving the same strains. Experiments that failed to present adult progeny in any of the crosses during the various oviposition periods were considered completely sterile. On the other hand, experiments producing adult progeny in all the crosses were considered completely fertile. Experiments resulting in a few sterile crosses were regarded as partially sterile.

Fertility and fecundity were analyzed in 69 mass crosses (15 intrastrain crosses, 24 intraspecific and 30 interspecific crosses). Fertility was evaluated as larval presence or absence and fecundity or productivity as the average and total number of descendants produced in each oviposition period. The fertility and fecundity of F1 hybrids were also studied in 39 mass crosses, as described for parental crosses; however, a single transfer to fresh culture medium was done. All the flies were kept at 20 °C ± 1 °C.

Polytene chromosomes extracted from the salivary gland of 3rd instar larvae were cytogenetically analyzed through slides prepared by squashing and staining with 2% lacto-acetic
Our main interest in these analyses was to observe the degree of synapsis and occurrence of heterozygote inversions in hybrid polytene chromosomes. We analyzed about 450 slides (15 nuclei/slide) and photographed some nuclei with a Zeiss II photomicroscope.

Data were analyzed by MINITAB Release, version 10.1 for Microsoft Windows, using

Fig. 1 — Map of the vegetation showing the distribution of species and locations of the analyzed strains (adapted from Monteiro, 1997). 1) A55F11* (Bela Vista/MS); 2) B50Q3* (Ibotirama/BA) are Drosophila gouveai strains; 3) D69R2 and 4) D69R5 (BA) are D. buzzatii strains; 5) A95F3* and 6) D40F1 (Serra do Cipó/MG). 7) D62C4BM and 8) D63M (Mucugê/BA), 9) D71C1BM and 10) D72M (Morro do Chapéu/BA) and 11) D73CSBM (Cachoeira do Ferro Doido/BA) are D. seriema strains; 12) B31D1* (Puerto Tirol/Argentina) is D. antonietae strain; 13) B20D2* (Tapia-Tucuman/Argentina) and 14) B25D7* (Famatina-La Rioja/Argentina) are D. koepferae strains. In this study, we also analyzed 3B* strain from BA not shown on this map. Strains ending with M derive from mass cultures; the others are isofemales.

* indicates strains kept in the laboratory since 1982, and the others since 1990. Prof. Dr. Fábio de Melo Sene and collaborators collected all the strains.
TABLE 1
Cross experiments; F = female; M = male; S = intrastrain crosses; A = intraspecific crosses; E = interspecific crosses; CS = completely sterile interspecific crosses; GOU = Drosophila gouveai; SER = D. seriema; ANT = D. antonietae; KOE = D. koepferae; and BUZ = D. buzzatii.

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Student’s t-test to measure the significance of the differences between male and female progenies. Productivity boxplot graphs were drawn using Statistica, version 5.0 for Microsoft Windows. The results presented here were also based on qualitative analyses of the graphs.

RESULTS
We observed no statistical differences between male and female progenies. Most of the intrastrain crosses were completely fertile, except the D72 (Drosophila seriema) and B20 (D. koepferae) strains, which yielded no progeny in the 3rd oviposition period nor in one of the four crosses, respectively (Fig. 2 and Table 2). Considering intraspecific crosses, only one in 24, F D63 x M A95 (Drosophila seriema strains) had two sterile crosses and was not fertile in the 2nd oviposition period (Table 2). Considering interspecific crosses, only one in 24, F D63 x M A95 (Drosophila seriema strains) had two sterile crosses and was not fertile in the 2nd oviposition period (Table 2 and Fig. 3). Therefore, among 30 interspecific crosses, 13 (= 43%) were completely sterile (Table 1). Ten of these crosses involved Drosophila buzzatii (R2) and D. seriema strains while two were between D. buzzatii (R5) and D. koepferae (B20) and one was between D. gouveai (B50) females and D. buzzatii (R2) males. Among the remaining 17 interspecific crosses, about 35% were partially sterile. Considering only the interspecific F1 crosses, 33% were partially sterile and 20% completely sterile (Table 2). We were unable to observe eggs or larvae in crosses that produced no adult progeny.

Intrastrain crosses involving B50 (Drosophila gouveai), D63 (D. seriema) and B31 (D. antonietae) showed low fecundity. D73 (Drosophila seriema) was the most productive strain, as clearly evidenced in the 1st oviposition period (see Fig. 2a). The fecundity of R2 (Drosophila buzzatii) intrastrain crosses, which was similar in the three oviposition periods, was revealed by the low standard error (SE); this was not observed in any other strain. The greatest intraspecific variation and the highest SE values were obtained in D62 (Drosophila seriema) and B20 (D. koepferae) intrastrain crosses, presenting different reproductive patterns in both intra- and interspecific comparisons (Fig. 2). Regarding fecundity in the three oviposition periods, the first two periods were more productive for both intra- and interspecific crosses than the third period. As for F1 crosses, the most productive period was the
Fig. 2 — Box-plot of intracrosses productivity in three oviposition period. a) 1st oviposition period; b) 2nd oviposition period; and c) 3rd oviposition period. □ = maximum and minimum range. ■ = mean ± standard error.
Fig. 3 — Box-plot of reciprocal intra- and interspecific crosses productivity in three oviposition period. The cross direction is always females $\times$ males. a) 1$^{st}$ oviposition period; b) 2$^{nd}$ oviposition period; and c) 3$^{rd}$ oviposition period. $\square$ = maximum and minimum range. $\blacksquare$ = mean $\pm$ standard error.
A comparison of the fecundity of Drosophila seriema intraspecific crosses with the respective intrastrain crosses revealed a high percentage (40.91%) of intraspecific crosses with productivity very similar to intrastrain crosses. Similarly, the majority of intraspecific F1 crosses presented productivity close to their respective parental intraspecific crosses (Fig. 4 and 5). In a comparison
of the fecundity of *Drosophila seriema* reciprocal crosses, we found that the majority (63.64%) showed a similar productivity, indicating that the cross direction does not interfere with the number of progeny (Fig. 3).

Comparing the productivity of interspecific crosses with the respective parental intrastrain crosses, we found that less than 15% of intraspecific crosses were more productive than intrastrain crosses. Similar fecundity values were
TABLE 2

Sterile degree of crosses along the three oviposition periods. SD = sterility degree; PS = partially sterile crosses; CS = completely sterile crosses; S = intrastrains crosses; AF1 = intraspecific F1 crosses; E = interspecific crosses; EF1 = interspecific F1 crosses; F = female and M = male; (V) = Drosophila buzzatii; (f) = D. gouveai; (°) = D. seriema; (Δ) = D. antonietae; and () = D. koepferae.

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found between: – reciprocal interspecific crosses; – interspecific crosses and their respective intrastrain crosses; and – interspecific F1 crosses and their respective parental interspecific crosses.

The productivity of intrastrain crosses declined more markedly along the different oviposition periods than did the intra- and interspecific crosses (Fig. 2 and 3). Drosophila seriema intraspecific experiments F A95 x M D71 and F D63 x M D40 showed the highest range of oviposition periods over the four crosses. The first cross, F A95 x M D71, presented the highest SE values in the 1st oviposition period while, in the F D63 x M D40 crosses, the 2nd oviposition period was more heterogeneous (Fig. 3). The lowest fecundity among oviposition periods occurred in the Drosophila seriema intraspecific crosses F D72x M A95, and in the interspecific crosses F R2 (D. buzzatii) x M B50 (D. gouveai) and F A55 (D. gouveai) x M R2 (Fig. 3).

The degree of polytene chromosome synapsis in the progeny of intra- and interspecific crosses did not differ from that of intrastrain crosses, except for the proximal region of the X chromosomes and the microchromosomes (VI). These chromosomal regions showed a high degree of asynapsis in most of the analyzed cells (Fig. 6). A low rate of asynapsis was observed in the hybrid chromosomes of some intra- and interspecific crosses, e.g., in intermediate regions of the II and IV chromosomes and in the proximal region of II and V chromosomes of hybrids of Drosophila seriema strains (see Fig. 7).

We were unable to find heterozygote polymorphic inversions in hybrid polytene chromosomes, possibly due to the significant number of sterile interspecific crosses, which have a higher probability of producing inversions loops. The fertile interspecific crosses presented low fecundity, making it hard to find 3rd instar larvae to prepare slides, even when the interspecific crosses were repeated specifically to prepare polytene chromosomes slides. No heterozygote inversions were found in the few polytene chromosome slides obtained from interspecific crosses.
Fig. 6 — Synapsis in hybrids between F A95 x M D72 (Drosophila seriema strains). Double arrows show slightly despained in a and b and completely despained microchromosomes in c and d. Single arrows show heterochromatic aspect of the third proximal portion of chromosome X. In d, the same region is highly despained. (X4,270). Scale = 2 μm.

Fig. 7 — Variables degrees of asynapsis observed in intrastrains crosses and intraspecific crosses. a) Chromosome II intermediate regions of D63; b) Chromosome IV intermediate region of F D73 x M D40 hybrid; c) Chromosome X proximal region of F D72 x M A95 female hybrid; d) Chromosome V of F D72 x M A95 female hybrid; e) Chromosome V proximal region of F D73 x M D40 male hybrid; and f) Chromosome II proximal region of F D73 x M D40 male hybrid. Arrows indicate chromosomes despained regions. a to d X2,760, scale in e = 3 μm refers to Figs. a to d; e and f X6,900, scale in e = 1.5 μm refers to Figs. e and f.)
DISCUSSION

Before discussing our findings, it should be noted that experimental conditions in the laboratory differ considerably from those occurring in nature; indeed, they may not even occur in nature at all. Therefore, the findings of this paper do not necessarily imply that hybridism occurs in nature. Nevertheless, our results suggest the presence or absence and the degree of genetic affinity among *Drosophila buzzatii* cluster species.

The high fertility observed in intrastrain crosses was expected due to the methodology of mass crosses employed here, which increases the probability of females being receptive to male courtship. Even so, one intrastrain experiment (B20, *Drosophila koepferae*) presented a sterile cross (Table 2), and another (D72, *Drosophila seriema*) had a sterile oviposition period (Fig. 2). The differences in fecundity among distinct geographical populations possibly reflect differences accumulated as a result of a long period of isolation or a bottleneck or genetic drift effect.

More than 50% of the interspecific crosses were fertile, confirming the close relation of the studied strains. Some variations in fertility and fecundity among species had already been detected earlier in the *Drosophila buzzatii* cluster (Bizzo, 1983; Moraes, 1992; Marin et al., 1993; Madi-Ravazzi et al., 1997). In addition, isolation barriers have been broken in laboratory experiments on other *Drosophila* species groups (Singh & Chartterjee, 1987; Carracedo et al., 1998).

The greatest number of sterility cases and two cases of low fecundity in interspecific crosses were attained among *Drosophila buzzatii* with *D. seriema* and *D. gouveai* strains. Machado et al. (2002) detected premating isolation in these cases of interspecific cross sterility, probably resulting from the females’ nonacceptance of male courtship. These findings reinforce previous observations that *Drosophila buzzatii* is, at some level, genetically isolated in the laboratory conditions from *D. borborema, D. serido, D. gouveai*, *D. seriema* and *D. antonietae*. *Drosophila koepferae* is capable of gene exchange with the Brazilian allopatric species in laboratory experiments (Madi-Ravazzi et al., 1997) and potentially with *D. borborema* (Wasserman, 1992), confirming the *D. serido* subcluster proposed by Ruiz et al. (2000). However, *Drosophila koepferae* also seems to diverge to some degree from the other species of the *D. serido* subcluster. This conclusion is based on the fact that some interspecific F1 crosses carried out in this study, involving *Drosophila koepferae* with *D. gouveai* and *D. seriema*, were sterile, probably because of immobility of the hybrid male spermatozoa (Machado et al., 2002).

An impressive result was the hybridization that occurred between the D63 (*Drosophila seriema*) and R2 (*D. buzzatii*) strains. The same was not the case when R2 strain was crossed with any other *Drosophila seriema* strain, possibly as a result of the greater degree of geographic differentiation of this particular *Drosophila seriema* strain from Mucugê (BA). Kuhn et al. (1996) observed that strains from the same locality of D63 exhibited a basic metaphase karyotype unlike that of *Drosophila seriema*, with a smaller and telocentric (dot-like) 6th chromosome.

In an evaluation of the reproductive compatibility among species of the *Drosophila buzzatii* cluster, Madi-Ravazzi et al. (1997) observed variable degrees of fertility, depending on the strains used, and also complete sterility when *Drosophila buzzatii* was crossed with *D. serido* subcluster species. Marin et al. (1993), also studying reproductive compatibility among species of the *Drosophila buzzatii* cluster, obtained hybrids in 10 out of 12 interspecific combinations and, in 5 cases, F1 females were partially fertile. Some of our results are congruent with Madi-Ravazzi et al. (1997) and Marin et al. (1993) regarding the reproductive differences between *Drosophila buzzatii* and *D. serido* subcluster species. However, the results of our work differed from theirs depending on the strains and cross directions done.

Madi-Ravazzi et al. (1997) observed complete sterility when *Drosophila buzzatii* was crossed with *D. serido* subcluster species. On the other hand, we found fertile intercroses between *Drosophila gouveai* and *D. buzzatii*. The only asymmetric prezygotic isolation observed in this work was between *Drosophila gouveai* (B50) and *D. buzzatii* (R2). The cross between B50 females and R2 males was sterile, while the reciprocal cross was fertile. However, crosses between A55 (another *Drosophila gouveai* strain) with R2 and R5 were fertile, i.e., unlike B50, A55 females did not discriminate R2...
and R5 male courtship. Marin et al. (1993) obtained few hybrids when males of Drosophila buzzatii were crossed with species of D. serido subcluster and no descendants were produced when D. buzzatii females were tested. We found that intercrosses of both Drosophila buzzatii strains (R2 and R5) with most D. seriema strains and with D. koepferae were sterile in both cross directions.

The differences in our fertility results from those obtained by Madi-Ravazzi et al. (1997) and Marin et al. (1993) may be due to variations in the reproductive pattern intrinsic to these strains, probably enhanced by geographic isolation. Strains from allopatric populations may present differences in fertility through the accumulation of genetic differences; however, one must keep in mind the real possibility of methodology-related interferences in the reproductive relation of the crosses.

Noor et al. (2001) examined the genetic bases of hybrid sterility and species preferences in Drosophila pseudoobscura and D. persimilis females and proposed a genetic model whereby inversions may contribute to the speciation process, thereby explaining the numerous different arrangements among closely related species that co-occur geographically. It was also suggested that inversions create linkage groups that cause sterility to persist between hybridization taxa. Some polytene chromosome inversion studies in hybrids of different groups of Drosophila (see Coyne et al., 2002, as a recent example) suggested that sterility could be related with interaction between chromosome X of one species and the genome of another species. In this study, we were unable to find loops of polymorphic heterozygote inversions, mostly because of the high sterility and low fecundity among interspecific crosses.

With regard to the degree of synopsis, the studies of Madi-Ravazzi & Bicudo (1992) and Madi-Ravazzi et al. (1997) showed differences in the banding pattern of polytene chromosomes 2, 3 and the proximal region of chromosome X, and a high degree of asynapsis in hybrids. They also showed the smaller degree of synopsis in hybrids of Drosophila koepferae and D. buzzatii, D. seriema and D. koepferae, and D. koepferae and D. serido. These species also exhibited the lowest degrees of reproductive compatibility. The Drosophila seriema and D. serido hybrids showed an intermediate degree of synopsis (only the proximal and distal ends were unpaired) and greater fertility than that found in other interspecific crosses.

Contrary to the findings of Madi-Ravazzi & Bicudo (1992) and Madi-Ravazzi et al. (1997), our analysis of the degree of asynapsis in polytene chromosomes of intra- and interspecific hybrids showed a degree of unpairing not very different from that of intrastain crosses, except for the high frequency of asynapsis in the proximal region of the X and microchromosomes of hybrids found here and by the aforementioned authors. Those differences may have been due to the distinct geographic strains used in these studies. The high frequency of asynapsis revealed in X and microchromosomes possibly indicates a homology and common origin for these two chromosomes. In fact, for Scaptodrosophila lebanensis from the victoria group, which has no microchromosomes, Papaceit & Juan (1998) suggest that a fusion X-micro chromosome is probably an ancestral trait in this group of species.

Populations of Drosophila buzzatii cluster species probably suffered, along with xerophytic vegetation, events of expansion during cold/dry periods of paleoclimatologic cycles (Bigarella et al., 1975; Ab’Saber, 1977; Vanzolini, 1981), and events of retraction during warm/wet periods. These changes probably contributed to events of population expansion, introgression and hybridism detected in some studies in this species cluster (Ruiz et al., 2000; Manfrin et al., 2001; de Brito et al., 2002). The last cold/dry period ended approximately 13,000 years ago. If the populations of Drosophila buzzatii cluster species suffered retraction since then, it has undergone about 156,000 generations of geographic isolation, considering one generation per month for these flies. Hence, sufficient time has elapsed for a certain degree of differentiation to have occurred in some populations, while others still conserve the ancestral genetic reproductive pattern. However, one must not disregard the possibility that some populations may have become reproductively differentiated from others of the same species through recurrent bottleneck and/or genetic drift effects during and after the retraction of populations caused by paleoclimatologic cycles.

The diagnostic characteristic among Drosophila buzzatii cluster species is its aedeagus
morphology as long as some species share fixed inversions in the polytene chromosome, despite their specific polymorphism inversions. Aedeagus morphology is an adequate character to define groups of sibling species. Kawano (2004) demonstrated, in beetles, that animal genitalia often show distinct developmental and evolutionary relationships with other parts of the body. Therefore, the intraspecific divergence of some strains as well as other findings, such as the reproductive compatibility and chromosome pairing obtained in this and other studies, indicates that several markers are important and must be considered for a good understanding of the complexity of the speciation process.

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