

EVALUATION OF THE COURTSHIP AND OF THE HYBRID MALE STERILITY AMONG *Drosophila buzzatii* CLUSTER SPECIES (DIPTERA, DROSOPHILIDAE)

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(With 1 figure)

ABSTRACT

In the *Drosophila repleta* group the establishment of subgroups and complexes made on the basis of morphological and cytological evidences is supported by tests of reproductive isolation. Among species in the *repleta* group, the *buzzatii* cluster, due to its polymorphism and polytypism, is an excellent material for ecological and speciation studies. Some interspecific crosses involving *Drosophila seriema*, *Drosophila* sp. B, *D. koepferae* and *D. buzzatii* strains were completely sterile while others involving strains from these species produced F1 hybrids that did not yield F2. In the present work, data on courtship duration and copula occurrence obtained in the analysis of flies from parental sterile crosses and on spermatozoon mobility observed in F1 hybrids that did not yield F2 are presented. Copula did not occur during one hour of observation and the spermatozoon also did not show mobility at any of the analyzed stages (3, 7, 9 and 10 days old). There was a high variation in courtship average duration and in the percentage of males that courted the females. The reproductive isolation mechanisms indicated by these observations were pre and post-zygotic, as supported by the absence of copula and male sterility. Data obtained also showed the occurrence of different degrees of reproductive compatibility among the strains classified as the same species but from distinct geographic localities.

Key words: courtship, hybrids, male sterility in *Drosophila*.

RESUMO

Avaliação da corte e da esterilidade do macho híbrido entre espécies do cluster de *Drosophila buzzatii* (Diptera, Drosophilidae)

No grupo *repleta* de *Drosophila*, o estabelecimento de subgrupos e complexos realizado com base em evidências citológicas e morfológicas é suportado por testes de isolamento reprodutivo. Entre as espécies do grupo *repleta*, o cluster *buzzatii*, devido a seu politipismo e polimorfismo, é um excelente material para estudos ecológicos e de especiação. Alguns cruzamentos interespecíficos envolvendo linhagens de *Drosophila seriema*, *Drosophila* sp. B, *D. koepferae* e *D. buzzatii* foram completamente estéreis, enquanto outros produziram híbridos F1 que não deixaram F2. No presente trabalho são apresentados dados sobre a duração da corte e ocorrência de cópula dos intercruzamentos estéreis e dados sobre a mobilidade dos espermatozoides dos híbridos F1 que não deixaram F2. Não houve ocorrência de cópula no período de 1 hora de observação e os espermatozoides dos híbridos analisados

não foram móveis em qualquer das idades testadas (3, 7, 9 e 10 dias de idade). Houve alta variação na duração média da corte e na porcentagem dos machos que cortejaram as fêmeas. A ausência de cópula e esterilidade dos machos F1 indica mecanismos pré e pós-zigótico operando entre as espécies desse cluster. Os dados também mostram diferentes níveis de compatibilidade reprodutiva entre linhagens classificadas como da mesma espécie mas de diferentes localidades geográficas.

Palavras-chave: corte, híbridos, esterilidade do macho em *Drosophila*.

INTRODUCTION

A good correlation between reproductive isolation and phylogenetic relationships has been found when groups of species are considered. In the *Drosophila repleta* group, the subgroups and their complex divisions established on the basis of morphological and cytogenetics evidence, have been supported by reproductive isolation tests (Crow, 1942; Wharton, 1944; Patterson, 1947; Wasserman, 1982, 1992).

Naveira & Fontdevila (1991) studying hybrids in the *repleta* group showed that throughout the autosomes chromosomes of *D. buzzatii* and *D. koepferae* there are many non-allelic, minor sterility genes, whose individual segregation cannot be recognized phenotypically, and which act cumulatively on the same characteristics of spermatogenesis, each contributing a small effect to their disturbance in the hybrid male. Accordingly, these genes should be considered as polygenes, and the type of sterility they bring about ('minimum size effect') should be designated as polygenic sterility.

The male hybrid sterility is the most common post-zygotic reproductive isolation among related animal species, and, therefore, it has received special attention in the speciation studies (Zeng & Singh, 1995). In the last decade, the genetic basis of reproductive isolation has been shown to be surprisingly polygenic and recently several studies of reproductive isolation have been realized using molecular analysis. Extending the discovery of fertility rescue between *Drosophila melanogaster* and *Drosophila simulans*, Sawamura *et al.* (2000) showed that this hybridization could permit systematic and precise delineation of the genetic and molecular basis of speciation. These authors discovered at least six genes of hybrid male sterility and none for female sterility in a region of 5% of

the *D. simulans* genome introgressed into *D. melanogaster* genome by deficiency mapping. Another work considering molecular data in the hybrid male sterility is the one developed by Dermitzakis *et al.* (2000). These authors introgressed regions of the *Drosophila sechellia* genome into *D. simulans* genome to identify and map genetic defects in interspecific hybrids. The data indicated that the sex ratio phenotype results from an epistatic interaction between at least two factors.

The differentiation among populations of geographically distinct species of *buzzatii* cluster has already been estimated using several forms of genetic variation: karyotype, reproductive isolation, chromosome inversions, aedeagus morphometry, fertility and fecundity, synapse degree in the polytene chromosomes, sexual isolation, esterase patterns, mitochondrial DNA, RAPD and satellite DNA (Vilela & Sene, 1977; Baimai *et al.*, 1983; Bizzo, 1983; Naveira *et al.*, 1986; Tosi & Sene, 1989; Naveira & Fontdevila, 1991; Silva & Sene, 1991; Madi-Ravazzi & Bicudo, 1992; Moraes, 1992; Lapenta *et al.*, 1995; Tidon-Sklorz & Sene, 1995; Kuhn *et al.*, 1996; Madi-Ravazzi *et al.*, 1997; Manfrin *et al.*, 2001; Monteiro, 1997; Lapenta *et al.*, 1998; Castro & Madi-Ravazzi, 2000; Kuhn *et al.*, 1999; Machado *et al.*, 2001, *submitted*).

This work intended to obtain additional data on the differentiation within the *buzzatii* cluster species, now focusing pre and post-mechanisms acting among the species.

MATERIAL AND METHODS

The species, strains and geographic regions used in this work are in Table 1.

In the text and tables the symbols of strains were shortened to the first letter plus the two following numbers, except for *D. buzzatii* strains for which R2 and R5 were used.

TABLE 1

Species, strains, and geographic locality of strains utilized in the present study.

Species	Strains	Locality
<i>Drosophila</i> sp. B	A55F11	Bela Vista (MS, Brazil)
	B50Q3	Ibotirama (BA, Brazil)
<i>Drosophila seriema</i>	A95F3	Serra do Cipó (MG, Brazil)
	D40F1	
	D62C4BM	Mucugê (BA, Brazil)
	D63M	
	D71C1BM	Morro do Chapéu (BA, Brazil)
	D72M	
	D73C5BM	Cachoeira do Ferro Doido (BA, Brazil)
<i>Drosophila koepferae</i>	B20D2	Tapia (Tucuman, Argentina)
	B25D7	Famatina (La Rioja, Argentina)
<i>Drosophila buzzatii</i>	D69R2	Locality without name (BA, Brazil)
	D69R5	

For the courtship study, 25 couples (with 8 days old) of each sterile cross were analyzed (Table 2). Each couple was put separately in tubes containing culture medium and was observed during one hour. The beginning and the end of the courtship were computed. The tubes were stored during a month for detecting the presence or absence of eggs and larvae, and then they were discarded. Control experiments were realized involving the observation of the courtship in intracrosses of all strains showed in Table 1 (Table 3). Mobility of the hybrid spermatozoon from sterile F1 intercroses was tested using the technique described by Orr (1992), with modifications. Testes preparations of three, seven, nine and 10 days old males from the intracrosses and intercroses were used as control (Table 4). Males were dissected in DEMEREC physiologic solution, and the testes were transferred to a slide containing a drop of the same solution. The preparations were covered with a coverslip, squashed and immediately examined in the light microscope.

In order to verify if the sterility of the F1 intercroses was due to the hybrid male, hybrid males and females were retrocrossed with the parental.

RESULTS AND DISCUSSION

Copula did not occur during the observation period of the different intercroses. A high variation in the percentage of males that courted the females was observed in the different intercroses, ranging from 4% in the intercroses of the *D. buzzatii* x *D. koepferae* (F D69 x M B20) to 44% in the crosses of females of the *D. seriema* (A95 and/or D73) versus males of the *D. buzzatii* R2 (Table 1). The males courted females more than once. The average of the courtship duration also showed variation, from 30 seconds up to 2 minutes, in the different intercroses analyzed (Table 2). In the control intracrosses, after a brief period (few seconds, Table 3), the couples had already copulated.

TABLE 2
Average duration in seconds and standard error of courtship between sterile cross couples, and couple percentage in which courtship occurred. F = female, M = male.

Strains and crosses		Courtship average duration			Males percentage that courted the females
F	M		±		
A95	R2	130.00	±	46.10	44%
R2	A95	46.43	±	8.05	28%
D62	R2	30.30	±	13.60	8%
R2	D62	43.00	±	12.10	12%
D71	R2	39.25	±	3.20	20%
R2	D71	38.00	±	6.72	8%
D72	R2	59.50	±	22.70	12%
R2	D72	34.00	±	6.21	16%
D73	R2	49.90	±	12.00	44%
R2	D73	25.75	±	7.75	8%
B50	R2	53.41	±	9.92	24%
R5	B20	52.00	±	0.00	4%
B20	R5	28.80	±	5.62	12%

The absence of copula was observed in sterile couples indicating the pre-zygotic reproductive isolation. The importance of natural selection in reinforcing the isolation is evidenced by the fact that among the allopatric species or allopatric strains of different species the isolation is less strong than among sympatric strains or species (e.g. Dobzhansky *et al.*, 1964; Grant, 1966). Our results relative to crosses of different strains of *D. seriema* with *D. buzzatii* are an example of this situation: the strains intercrossed are all allopatric in nature, but *D. buzzatii* is the only one among this species cluster that became cosmopolitan and can be found in sympatry in many regions with the others from the same cluster.

Thus, it may be supposed that the genetic divergence between *D. seriema* and *D. buzzatii* took place in allopatric populations that became sympatric and, when this happened, natural selection favored the reproductively isolated individuals, fixing, in this way, the isolation between the species.

The only asymmetric pre-zygotic isolation observed was between *Drosophila* sp. B (B50) and *D. buzzatii* (R2). However, the crosses among A55 (another strain of *Drosophila* sp. B) and the strains R2 and R5 of the *D. buzzatii* were fertile, showing that the females of this strain, differently of B50, did not discriminate males of strains of the *D. buzzatii*. This indicates variation between these strains of the same species, in this aspect Sureau & Ferveur (1999) observed the male courtship behavior among *Drosophila melanogaster* strains of different geographical localities and noticed that the male courtship behavior is genetically controlled and influenced by sex pheromones [7-tricosene (7-T) induces a dose-dependent inhibition of male-male courtship, whereas 7,11-dienes stimulate male courtship of females]. They observed that there was a geographical quantitative variation in the production of two predominant male hydrocarbons, 7-T and 7-pentacosene. These geographical differences in the hormone quantity could be responsible for the variation in the courtship between these two strains.

TABLE 3
Intracrosses and average period and standard error in seconds that the couples copulated after put male and female together.

Strains	Average period ± SE		
		±	
A55	20.00	±	0.00
B50	15.75	±	3.02
A95	18.90	±	1.60
D40	21.20	±	2.30
D62	23.10	±	3.01
D63	15.50	±	1.03
D71	16.92	±	3.72
D72	19.80	±	2.30
D73	17.95	±	2.95
B20	19.00	±	1.72
B25	20.14	±	2.20
R2	19.90	±	3.62
R5	17.93	±	1.08

TABLE 4
Control intracrosses (≡), control intercrosses (||||) and sterile F1 intercrosses (■) used to analyse the spermatozoon mobility. F = female, M = male.

F \ M	A55	A95	B25	B50	D40	D62	D63	D71	R2
A55	≡							■	
A95			■						
B25				■					
B50									
D40					≡				
D62						≡			
D63									
D71									
R2									

Monteiro & Sene (1995) had observed that each population of *Drosophila* sp. B can be discriminated, suggesting absence of gene flow between them. This could occur because these populations are found mainly in hills, forming true isolation. This fact could explain the differences found in the present work between the two strains of *Drosophila* sp. B. Moreover, Madi-Ravazzi *et al.* (1997) observed that the strain B50 showed a different behavior from the other *Drosophila* sp. B strains. This strain presented a low degree of reproductive compatibility with *D. seriema*.

In a review of the genus *Drosophila*, Bock (1984) listed 266 cases of interspecific hybridization in the laboratory and also 8 reported cases of hybridization in nature. These data show that interspecific hybridization in this genus is not at all a rare phenomenon, at least under the artificial conditions of the laboratory. But, this and other articles (Madi-Ravazzi & Bicudo, 1992; Marin *et al.*, 1993; Madi-Ravazzi *et al.*, 1997; Machado *et al.*, 2001) also show clearly that the production of viable hybrids is restricted to the most closely related species. Our results agree with Marin *et al.* (1993) regarding reproductive differences between *D. koepferae* and *D. seriema*. Even the intercrosses between *D. buzzatii* and *D. seriema* strains and *D. koepferae* were sterile in both directions of crosses. Besides, the intercrosses between, *Drosophila* sp. B females (B50) and *D. buzzatii* males (R2) also were sterile, but, the reciprocal crosses were fertile. However data obtained by Marin *et al.* (1993) showed inverse fertility, that is, *D. buzzatii* females with *Drosophila* sp. B males, using different strains of that used in this work.

In the present study the spermatozoon mobility analysis of males derived from control intra- and intercrosses showed little or no mobility in most of the 5-day old males, a relative mobility in the 7-day old males, and a high mobility in 9-day old males. The sterile hybrid males resultant of three intercrosses did not show mobile spermatozoa in all tested ages. Another fact that indicates the male sterility is the backcrosses carried out between these males and the parental females. These crosses were also sterile, while the backcrosses between the F1 females and parental males were fertile.

Bizzo (1983) also showed post-zygotic isolation between allopatric populations of species

included in the *D. buzzatii* cluster. He showed that mass intercrosses between A95 isofemale strain and B53 strain (Milagres, BA) produced sterile males and fertile females in both cross directions. Wasserman & Richardson (1987), also using species of *buzzatii* cluster, found another example of allopatric species that showed post-zygotic isolation. The reciprocal crosses between allopatric populations of *D. serido* and *D. koepferae* produced fertile females. However, when *D. serido* females were used, the male offspring was sterile, while in the reciprocal cross the males were fertile.

The rule of Haldane considers that in cases of unisexual inviability and sterility, the heterogametic sex is more affected. Several authors had considered models to explain the rule of Haldane (Charlesworth *et al.*, 1987; Coyne & Orr, 1989; Hollocher & Chung-I, 1996; Turelli & Orr, 2000, for example). Several studies have shown that X chromosome, by itself, has the greatest role in the sterility of hybrid male, exerting its effect by the interaction with genes of other species. The genes that interact with heterospecific X chromosome can be in the autosomes, the Y chromosome or cytoplasm (when the females are heterogametic) (Coyne & Orr, 1989; Zouros, 1989).

Dobzhansky (1936), who studied hybrid sterility in *Drosophila pseudoobscura* had already observed that the causes of hybrid sterility remained one of the unsolved problems of biology. It seems that this problem, besides advances in the molecular area, still has many questions to be answered.

The hybrid sterility, by itself, is not an adaptive phenotype, but a pleiotropic effect of several unknown factors. Thus, the genetic of the sexual isolation would be more representative of divergent species, and the sexual isolation characteristics are most probably products of adaptive evolution rather than of hybrid sterility (Hollocher *et al.*, 1997).

In the present study the reproductive behavior showed that distinct strains classified as same species presented differences in female discrimination regarding the males courtship. However, the finding of this intraspecifically achievement needs the support of other markers to distinguish between intraspecific or interspecific differences. The hybrid male sterility is an important subject and studies focusing on the genetic and cellular aspects of spermatogenesis must be the next step to improve our analysis.

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