

Reproductive isolation between *Anastrepha bistrigata* and *A. striata* (Diptera, Tephritidae)

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ABSTRACT

The reproductive isolation between two closely related species, *Anastrepha bistrigata* and *A. striata*, was studied in the laboratory. Interspecific copulation attempts were observed, but examination of the spermathecae showed that sperm transference did not occur, even after a prolonged period of contact between the mating pairs. These results indicate prezygotic isolation. The analysis of the hourly distribution of mating activities under laboratory conditions, here described for the first time for *A. bistrigata*, clearly showed differences for the two species, the activities being concentrated in the afternoon period for *A. striata* and in the morning for *A. bistrigata*.

INTRODUCTION

Many species of flies belonging to the Tephritidae are considered fruit pests since their larval development occurs in fruit pulp. The genus *Anastrepha* is endemic of neotropical regions, and up to the moment comprises 183 described species (Aluja, 1994), 80 of which occur in Brazil (Zucchi, 1988). Because of their economic importance, there is wide interest in these flies and, in fact, a great number of studies on their biology have been performed. However, there have been few studies on the reproductive isolation.

A. bistrigata and *A. striata* are two closely related species belonging to the taxonomic group "*striata*" (Norrbon and Kim, 1988). They have similar karyotypes (Solferini and Morgante, 1987), but there are substantial genetic differences (Morgante *et al.*, 1980). However, no information about reproductive isolation between them is available. Also, daily mating activities are only known for *A. striata* under field-cage conditions (Aluja, 1993).

MATERIAL AND METHODS

The colony of *A. bistrigata* was established from infested guavas (*Psidium guajava*, Myrtaceae) collected in Jundiaí, in the State of São Paulo, in 1990 and *A. striata* from guavas collected in Belém, in the State of Pará, in 1989. The flies were maintained in population cages (30 x 30 x 60 cm) under a natural photoperiod, at a temperature of $25 \pm 3^\circ\text{C}$ and $60 \pm 10\%$ relative humidity. They were fed with water and a 3:1 mixture of sugar and corn protein hydrolysate. Guavas were furnished as a substrate for oviposition and larval development.

Virgin individuals 20-30 days old were used for the experimental assay of reproductive isolation between *A. bistrigata* and *A. striata*. Four population cages consisting of six mating pairs each were made, two containing the intraspecific crosses and the other two composed of interspecific pairs (one cage with *bistrigata* females x *striata* males, and one cage with *bistrigata* males x *striata* females).

Observations of adult behavior were made during two consecutive days between 9:00 and 18:00 h, the number of copulations being noted. After the period of observation the flies were taken from the cages and dissected. The testes and the spermathecae were individually transferred to a drop of Ringer solution on

a microscope slide, burst and covered with a coverslip. The preparations were examined for the presence of spermatozoa.

In another test, the crosses were made in the same manner described above. However, the mating pairs were maintained in the cages for 20 days. During this period, the guavas were furnished for oviposition, were replaced every five days, and transferred to cages with a layer of vermiculite, where the larvae fall to pupate. After 20 days, the vermiculite was examined for the presence of pupae.

For the study of the diel pattern of mating activities of *A. bistrigata* and *A. striata*, five males and five virgin females (20 days old) were placed in a population cage and observed during one day. Five such cages were made for each species, using different flies in each one. Observations were done from 6:00 to 19:00 h. The number of mating attempts and the time they occurred were registered.

RESULTS AND DISCUSSION

In the control crosses seven copulations of *A. bistrigata* and 12 of *A. striata* were observed. Some attempted copulations in the crosses between *A. striata* and *A. bistrigata* were observed, most of them between *bistrigata* males and *striata* females (N = 13), while in the reciprocal cross just four attempted copulations occurred. Examination of the testes showed that all males involved in the crosses presented mature spermatozoa. As expected, sperm was present in the spermathecae of females of the intraspecific crosses. However, no spermatozoa were detected in the spermathecae of females from the interspecific crosses. The absence of spermatozoa indicates prezygotic isolation.

In the second experiment, even though the *A. bistrigata* and *A. striata* pairs remained together for 20 days, thus favoring the occurrence of copulations, pupae were not obtained, indicating that matings did not occur, or if they did, no viable hybrids were produced. The first possibility is more probable, since transference of spermatozoa was not verified in the first experiment. In the control crosses ordinary production of pupae was observed, wherein 86 pupae of *A. striata* and 56 of *A. bistrigata* were obtained.

The hourly distribution of mating activities of the two species showed bell-shaped patterns, however, peak activities were clearly separated (Figure 1). According to Aluja (1993), the period of mating of *A. striata* in field-cage conditions is between 10:00 and 17:00 h, with a peak at 15:00-16:00 h. The present data, obtained under laboratory conditions, showed

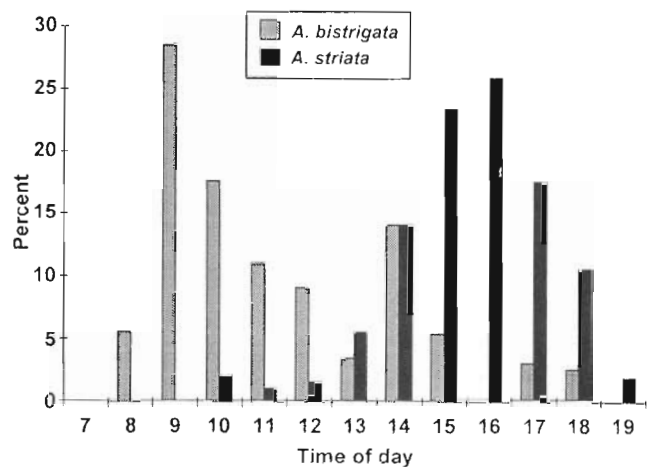


Figure 1 - Frequency distributions of mating activities of *Anastrepha bistrigata* and *A. striata* adults during the day.

essentially the same pattern, mating activities occurring from 10:00 to 19:00 h, with a peak between 15:00 and 17:00 h (Figure 1), the only difference being an extension (up to 17:00 h) of the periods of mating activities. This difference may be due to the different conditions in which the experiments were conducted rather than to a difference between the strains. Regardless of these differences, the results showed that the main peak of mating activity of *A. striata* is after 12:00 h. On the other hand, the mating activity of *A. bistrigata* occurred mainly in the morning (Figure 1). Matings occurred between 8:00 and 18:00 h, but were most frequent from 9:00 to 10:00 h.

A. striata and *A. bistrigata* also have different mating behaviors (Morgante *et al.*, 1993). The mating behavior of *A. striata* is more complex, with a courtship composed of several events while *A. bistrigata* has a more simple mating behavior, which usually occurs on the host fruit. Copulation is forced by the males, even during oviposition. There is no courtship and recognition of the partners seems not to be necessary, until physical contact is established. This behavior may explain the large number of copulation attempts in the interspecific crosses of *bistrigata* males with *striata* females. The *bistrigata* males attempt to copulate regardless of the type of female.

The reproductive isolation between these two species, characterized in the present study by the absence of progenies in the interspecific crosses, can be explained by differences in mating behavior and in timing of mating activities.

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RESUMO

Existem poucos estudos envolvendo testes de isolamento reprodutivo entre espécies do gênero *Anastrepha*. Neste trabalho apresentamos os resultados de testes feitos em laboratório com duas espécies relacionadas, *A. striata* e *A. bistrigata*. Nos cruzamentos interespecíficos foram observadas tentativas de cópula, mas o exame das espermatecas mostrou que não houve transferência de espermatozoides. Não ocorreram cruzamentos mesmo quando o período de contato entre as duas espécies foi prolongado. Os resultados sugerem a existência de isolamento pré-zigótico entre as espécies. A análise do padrão de atividade de cópula ao longo do dia, em condições de laboratório, pela primeira vez descrito para *A. bistrigata*, mostrou a existência de diferenças acentuadas, sendo que em *A. striata* as atividades estão concentradas no período da tarde, enquanto que, em *A. bistrigata*, no período da manhã. O isolamento reprodutivo entre estas duas espécies, aqui caracterizado, pode ser explicado pelas diferenças no comportamento e nos períodos de atividade de cópula.

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