CHARACTERIZATION OF COURTSHIP SOUNDS OF SPECIES OF THE SUBGROUP *fasciola* (DIPTERA, DROSOPHILIDAE, *Drosophila repleta* GROUP): INTERSPECIFIC AND INTERPOPULATIONAL ANALYSES

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(With 8 figures)

ABSTRACT

The aim of this work was to characterize the male courtship song pattern of various species of the *fasciola* subgroup and to determine the level of variation both within and among species. The parameters analyzed were intrapulse interval (PI), interpulse interval (IPI), and intrapulse frequency (IF). Six different species were analyzed: *D. coroica* (three populations), *D. ellisoni*, *D. fascioloides*, *D. moju*, *D. onca,* and *D. rosinae* (one population each). There were significant differences among the six species for these three courtship song parameters. The IPI was the most variable parameter among these species, suggesting that this parameter is important for female discrimination. Four different hypotheses could explain this variation: 1. different selection pressures with absence of flow gene; 2. intraspecific sexual selection; 3. sympatric effects on song evolution; and 4. genetic drift. The PI was the only parameter that was significantly different among the three population of *D. coroica*. Low variability among populations within the same species was already observed for other subgroups and could be explained by the following hypotheses: strong selection acting on the song parameters, gene flow, or recent colonization from a common source. Additional studies of the courtship song of other species of the *fasciola* subgroup, as well as for other subgroups of the *repleta* group, and studies, using molecular makers, that focus on the genetic basis of the differences among these species in courtship song would allow us to evaluate the association of courtship song and sexual isolation in these species, and would also help us to understand the evolution of these behavioural differences.

Key words: *drosophila*, *fasciola* subgroup, courtship song, evolution, behavior.

RESUMO

Caracterização do som da corte sexual de espécies do subgrupo *fasciola* (Diptera, Drosophilidae, grupo *repleta*): análises interespecíficas e interpopulacionais

Com o objetivo inicial de caracterizar o padrão sonoro da corte sexual de machos de diferentes espécies do subgrupo *fasciola* e determinar a possível ocorrência de variação e o grau de diferenciação tanto inter como intraespecífico, foram analisados os parâmetros intervalo intrapulso (PI), intervalo interpulso (IPI) e freqüência intrapulso (IF) de 6 diferentes espécies: *D. coroica*, *D. ellisoni*, *D. fascioloides*, *D. moju*, *D. onca* e *D. rosinae*, e três populações de diferentes origens geográficas da espécie *D. corioca*. Comparações estatísticas entre as 6 espécies mostraram diferenças significativas em relação aos 3 parâmetros analisados. O IPI foi o parâmetro de maior diferenciação interespecífica, mostrando ser importante durante o reconhecimento da fêmea. As hipóteses que podem explicar essas diferenças são: 1. pressões seletivas diferentes com ausência de fluxo gênico, 2. seleção sexual intraespecífica;

3. efeito de simpatria; e 4. deriva genética. As análises realizadas entre as 3 populações da espécie *D. coroica* mostraram diferenças estatísticas somente em relação ao PI. Essa baixa variabilidade entre populações de uma mesma espécie também já foi encontrada em outros subgrupos e pode ser explicada pelas seguintes hipóteses: forte seleção sobre os parâmetros sonoros, ocorrência de fluxo gênico entre as populações ou colonizações recentes a partir de um ancestral comum. Estudos da corte sonora de outras espécies do subgrupo *fasciola* e mesmo de espécies de outros subgrupos do grupo *repleta,* e também estudos relacionados à base genética das diferenças sonoras entre as espécies, utilizando marcadores moleculares, ajudariam a avaliar a relação dos parâmetros sonoros com o processo de isolamento sexual e também a entender a evolução dessas diferenças comportamentais.

Palavras-chave: *drosophila*, subgrupo *fasciola*, corte sonora, evolução, comportamento.

INTRODUCTION

Courtship sounds

Courtship behavior of flies of the genus *Drosophila* is important for species recognition. After Shorey (1962) showed that *D. melanogaster* males emitted sounds during sexual courtship, many studies were made with the various groups and subgroups of the genus *Drosophila*, describing the specific nature of the sound types. These studies have provided evidence that sound has a function both as a sexual stimulant and in sexual isolation. These sounds vary significantly among the various species of the *affinis* (Chang & Miller, 1978), *melanogaster* (Cowling & Burnet, 1981), *virilis* (Hoikkala *et al.*, 1982; Hoikkala & Isoherranen, 1997), *mercatorum* (Ikeda & Maruo, 1982), *repleta* (Ewing & Miyan, 1986), *auraria* (Tomaru & Oguma, 1994), *planitibia* (Hoikkala *et al.*, 1994), *willistoni* (Ritchie & Gleason, 1995), *quinaria* (Neems *et al.*, 1997), and *obscura* (Noor & Aquadro, 1998) groups.

The interspecific differences in *Drosophila* courtship sounds are characterized by one or more sound parameters such as: interpulse interval (IPI) which is the time from the beginning of one pulse to the beginning of the next; intrapulse interval (PI) which is the pulse duration; intrapulse frequency (IF) which is acoustically the strongest frequency within the pulse; number of pulses per series; and duration of each interval within a series (Hoikkala & Lumme, 1987; Wheeler *et al.*, 1988). Although it has not been firmly established which sound parameters confer specificity, there is evidence that IPI is important. According to Ritchie & Kyriacou (1996), the IPI of *Drosophila* courtship sounds varies very little within natural populations and is important for male reproductive success. However, it is clear that many *Drosophila* species produce complex sounds with various characteristics, one or more of which can be of value in identifying species. This specificity in *Drosophila* courtship sounds can be explained by selection for discrimination between species (Tomaru & Oguma, 1994) of the different types of sounds, which could have evolved through intraspecific sexual selection (Ritchie & Gleason, 1995). Also, the different types of sounds found in the various *Drosophila* species could simply reflect phylogenetic divergences.

The fasciola subgroup

The species of the subgroup *fasciola* are found in humid forests, principally those of Central and South America and the Caribbean islands. In Brazil they have been collected from the Atlantic rain forest, as well as from forests in the states of São Paulo, Paraná, Mato Grosso do Sul, and Bahia (Vilela, 1983; Vilela *et al.*, 1983; Tidon-Sklorz & Sene, 1992).

Most work on this subgroup has been cytological and was done in the beginning of the 60s, showing that the species in this group differ from all of the rest of the *repleta* group in having the homozygotic inversions: $2o^2$, $2e^3$, and $2l^3$. Wasserman (1982) proposed a common ancestry for the subgroups *fasciola* and *mulleri*, because they have the 3c inversion in common. Recently however, Diniz (1998) showed that the 3c inversion of the subgroup *fasciola* is different from that found in the subgroup *mulleri* and proposed that the subgroup *fasciola* was derived directly from primitive I, and can no longer be considered linked to the *mulleri* subgroup. Kuhn *et al.* (1995) made a karyotypic study of some species of the *fasciola* subgroup and found interspecific variation in the size of the microchromosome and in chromosome numbers.

According to Wasserman (1992), the *fasciola* subgroup consists of nine species: *D. pictilis* and *D. pictura*, forming the pictilis complex; *D. paraguttata; D. mojuoides* and *D. moju*, forming the moju complex; and *D. fulvalineata, D. fasciola, D. coroica,* and *D. fascioloides* which are not found in any complex. Vilela (1983), studying the morphology of male genitalia, determined that the species *D. fascioloides* studied by Wasserman (1962) actually did not belong to this species, and renamed it *D. ellisoni*. He also added the species *D. linearepleta* and *D. onca* to the subgroup *fasciola* and described *D. carolinae, D. hermionae, D. ivai, D. querubimae, D. rosinae,* and *D. senei* as new species, increasing to 18 the number of species in this subgroup.

In this work we describe the courtship sound patterns of six species of the subgroup *fasciola*: *D. ellisoni*, *D. fascioloides*, *D. moju*, *D. onca*, *D. rosinae,* and *D. coroica*. Besides the interspecific analyses, we also examined the differences in the sound patterns of three different populations of *D. coroica*.

MATERIALS AND METHODS

Establishment and maintenance of isolines

Isolines were produced from individual females already mated in the wild. These isolines were identified through morphological analysis of the genitalia of F1 males (Vilela, 1983). Third instar larvae of the subgroup *fasciola* normally attempt to crawl out of the culture vial. To avoid this problem, the culture vials were opened and placed on moist sand within plastic bottles so that the larvae could pupate in the sand. Ecloded flies were removed with an aspirator and transferred to vials filled with culture media, where they matured, mated, and laid eggs, reinitiating the cycle.

Sound recordings

Recently ecloded virgin flies were isolated in 35 ml culture vials and left for 7-10 days to allow time for sexual maturation. Subsequently, a male and a female of the same lineage were introduced into a sound recording chamber described by Sene & Manfrin (1998). To record the sound, the chamber containing the pair of flies was placed on the diaphragm of an ultrasensitive microphone, as described by Manfrin *et al.* (1997). The flies were observed for a maximum of 10 minutes, or until mating. All recordings were made at 25 ± 1°C.

Sound analysis

The electromagnetic signals of the analogical recordings were converted into digital signals at a frequency of 5 KHz, with an analogical-digital converter circuit CAD 12/36-60 k (Lynx) and the AqDADOS 4 (Lynx) program.

The courtship sound patterns of six different species of the subgroup *fasciola* are described in Table 1. The sound patterns of *D. coroica* males from three different locations were also analyzed in order to study interpopulational differences.

TABLE 1

Locality, habitat and collection date of the different isolines of the species of the subgroup *fasciola* used in this study.

Isoline	Species	Locality	Habitat	Collection date
D83M1	*D. ellisoni*	São Sebastião-SP	Atlantic rain forest	February-1992
D86N17	*D. fascioloides*	Nova Friburgo-RJ	Continental forest	March-1992
F34M2	*D. moju*	Panamá	?	August-1989
J16CM1	*D. onca*	Tibaji-PR	Continental forest	November-1997
D63M1	*D. rosinae*	Mucugê-BA	Caatinga	July-1990
D96N74	*D. coroica*	São Carlos-SP	Continental forest	August-1993
D88M1	*D. coroica*	Sertãozinho-SP	Continental forest	September-1992
J7C1	*D. coroica*	Itirapina-SP	Continental forest	November-1997

The parameters used for comparative studies of the sound patterns were the intrapulse interval (PI), interpulse interval (IPI) and intrapulse frequency (IF). The PI and IPI values were obtained directly from the figures. The IFs were obtained with the help of MATLAB software (MathWorks, Inc.) using Fast Fourier Transforms (FFT). This analysis breaks down the sound signal into a series of waves (sines and cosines) of different frequencies and amplitudes. The IF of a sound is defined as the frequency of the highest amplitude wave in the sound signal.

The FFT can analyze sounds containing thousands of points per millisecond, but maximum analysis efficiency is obtained by restricting the number of points to base two potentials. For this reason, all intervals used for analysis of IF were selected so that they had a number of points equal to base two potentials. Therefore, intervals of 64 points were used for *D. moju*; 128 points for *D. rosinae* and *D. coroica*; 256 points for *D. ellissoni*; and 512 points for *D. onca* and *D. fascioloides*.

Ten measures were made of the parameters PI, IPI, and IF for each of 10 males from each of the isolines.

Statistical analysis

The lines described in Table 1 were analyzed with the objective of investigating interspecific differences. Isoline D96N74 was chosen as representative of *D. coroica*. The three lines (D96N74, D88M1, and J7C1) of *D. coroica* were tested separately for interpopulational differences. The values of the parameters intrapulse interval (PI), interpulse interval (IPI), and intrapulse frequency (IF) were analyzed separately by variance analysis (ONE-WAY ANOVA) at a 0.05 significance level.

RESULTS

Figs. 1 to 6 show the basic sonograms of the various species analyzed. Isoline D96N74 was chosen to represent the species *D. coroica*. These sonograms and the analysis of sound pulses permit a division of the courtship sounds into three distinct groups: 1. courtship sounds of the species *D. onca, D. fascioloides,* and *D. ellisoni*, in which each sound sequence is composed of a sequence of short pulses, followed by a trill, similar to that found in some species of the subgroup planitibia, denominated by Hoikkala *et al.* (1994) as phrase song. In the species *D. fascioloides* and *D. onca*, the sequence of pulses is made up of four pulses, followed by a relatively long trill, while in *D. ellisoni*, there are only two pulses, followed by a shorter trill; 2. the type typical of the sonogram of *D. rosinae*, with a courtship sound formed by simple but duplicated pulses, a pattern also observed by Ewing & Miyan (1986) in some species of the *repleta* group; 3. a type based on the courtship sounds of the species *D. moju* and *D. coroica*, with short pulses in sequence, similar to the pulse song of *D. melanogaster* (Schilcher, 1976) and sound A of *D. mercatorum* (Ikeda & Maruo, 1982; Manfrin *et al*, 1997).

The sonograms of each of the species analyzed were a sequence of one type of sound pulse. This finding is different from those of studies made on the species *D. melanogaster* (Schilcher, 1976), *D. mercatorum* (Ikeda & Maruo, 1982; Manfrin *et al.*, 1997), and some species of the subgroup *repleta* (Ewing & Miyan, 1986), in which two different types of sounds were found.

Table 2 show the mean values for PI (intrapulse interval), IPI (interpulse interval), and IF (intrapulse frequency) of the isolines described in Table 1. A comparison of the standard deviations (± sd) among the various species shows that *D. ellisoni* and *D. fascioloides* have the least variation for all three parameters: PI (27.470 ± 2.73 and 69.859 ± 6.12), IPI (97.473 ± 5.76 and 125.757 ± 7.87), and IF (251.358 ± 19.36 and 362.901 ± 17.04), indicating that the courtship sounds of these species are uniform with little intraspecific variation. On the other hand, *D. onca* had considerable variation in PI (93.662 ± 31.44) and IPI (508.091 ± 73.06) and moderately high variation in IF (289.260 ± 32.71) when compared to the other species, indicating that the differences in PI and IPI, though large, were not sufficient to significantly alter the pulse wave form. The standard deviations were relatively low for PI (7.363 ± 7.36 and 14.673 ± 1.98) and IPI (50.859 ± 5.47 and 72.340 ± 5.68) in *D. moju* and *D. rosinae*, but the IF values were relatively high (412.501 ± 55.63 and 306.624 ± 57.80). In this case, though the pulse duration and the time from one pulse to the next were relatively uniform within each of these species, the sound pulse waveform varied considerably.

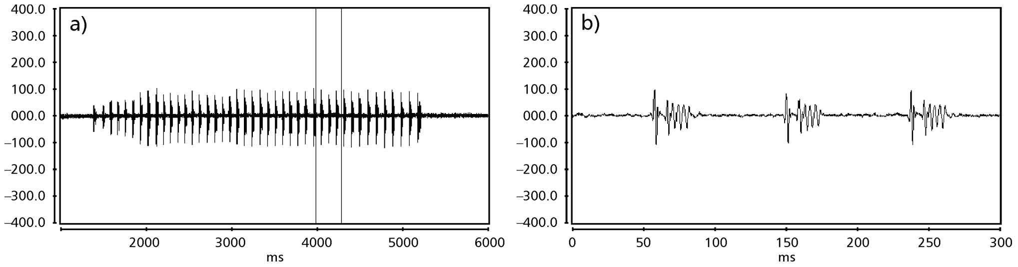

Fig. 1 — Courtship sound pattern of *D. ellisoni* (D83M1). (a) 5 second interval, (b) 300 millisecond interval. The X-axis represents the time in milliseconds and the Y-axis indicates arbitrary (relative) intensity units.

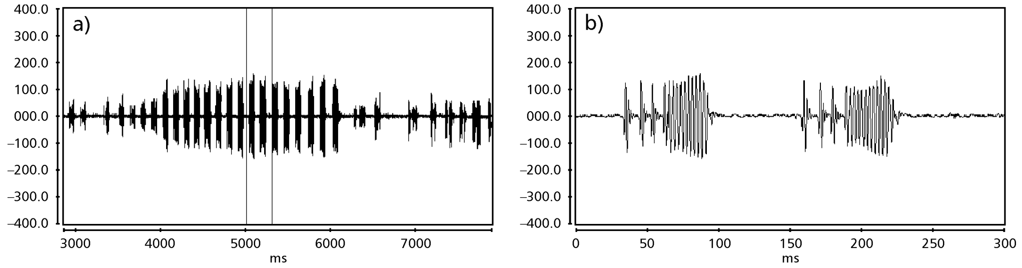

Fig. 2 — Courtship sound pattern of *D. fascioloides* (D86N17). (a) 5 second interval, (b) 300 millisecond interval. The X-axis represents the time in milliseconds and the Y-axis indicates arbitrary (relative) intensity units.

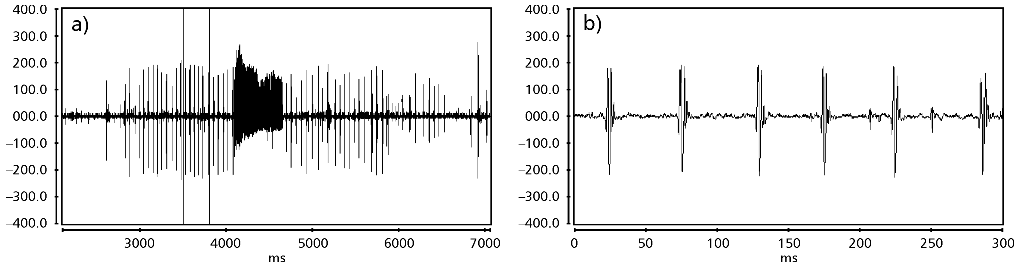

Fig. 3 — Courtship sound pattern of *D. moju* (F34M2). (a) 5 second interval, (b) 300 millisecond interval. The X-axis represents the time in milliseconds and the Y-axis indicates arbitrary (relative) intensity units.

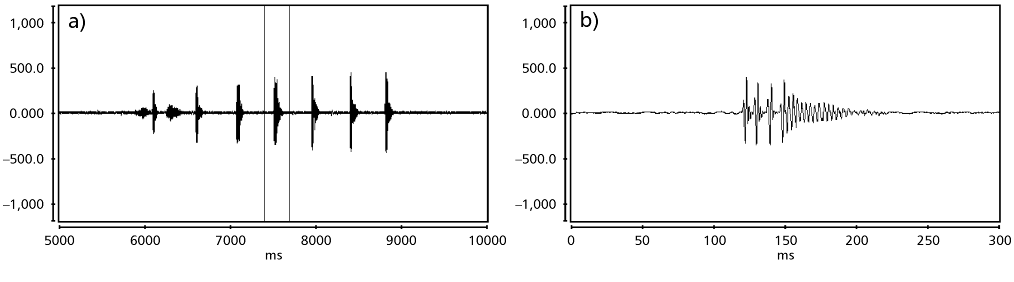

Fig. 4 — Courtship sound pattern of *D. onca* (J16CM1). (a) 5 second interval, (b) 300 millisecond interval. The X-axis represents the time in milliseconds and the Y-axis indicates arbitrary (relative) intensity units.

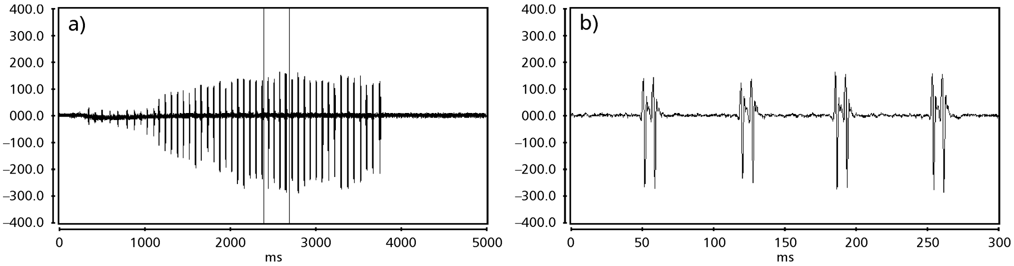

Fig. 5 — Courtship sound pattern of *D. rosinae* (D63M1). (a) 5 second interval, (b) 300 millisecond interval. The X-axis represents the time in milliseconds and the Y-axis indicates arbitrary (relative) intensity units.

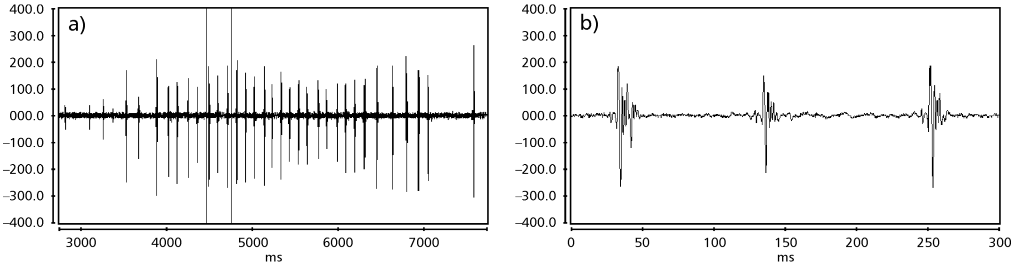

Fig. 6 — Courtship sound pattern of *D. coroica* (D96N74). (a) 5 second interval, (b) 300 millisecond interval. The-X axis represents the time in milliseconds and the Y-axis indicates arbitrary (relative) intensity units.

TABLE 2

Mean and standard deviation for the parameters intrapulse interval (PI), interpulse interval (IPI) and intrapulse frequency (IF) of the courtship sound of 10 males from each of the lines that were analyzed. The values for each individual included 10 measurements for each parameter.

Species (isoline)	PI (ms) ± sd	IPI (ms) ± sd	IF (Hz) ± sd
D. ellisoni (D83M1)	27.470 ± 2.728	97.473 ± 5.763	251.358 ± 19.358
D. fascioloides (D86N17)	69.859 ± 6.116	125.757 ± 7.871	362.901 ± 17.037
D. moju (F34M2)	7.363 ± 1.444	50.859 ± 5.473	412.501 ± 55.633
D. onca (J16CM1)	93.662 ± 31.436	508.091 ± 73.056	289.260 ± 32.711
D. rosinae (D63M1)	14.673 ± 1.979	72.340 ± 5.679	306.624 ± 57.804
D. coroica (D96N74)	17.271 ± 2.178	101.303 ± 12.604	318.353 ± 56.192
D. coroica (D88M1)	20.256 ± 3.900	109.101 ± 17.317	305.459 ± 61.180
D. coroica (J7C1)	22.819 ± 4.382	99.050 ± 18.576	314.364 ± 77.703

There was relatively little intrapopulational variation in the PIs of *D. coroica* lines D96N74, D88M1, and J7C1 (17.271 ± 2.18, 20.256 ± 3.90 and 22.819 ± 4.38, respectively), intermediate variability in IPI (101.303 ± 12.60, 109.101 ± 17.32 and 99.050 ± 18.58), and high variability in IF (318.353 ± 56.19, 305.459 ± 61.18 and 314.364 ± 77.70), indicating that even with low intrapopulational variation in the length of the sound pulse, there was considerable variation in the sound pulse waveform.

When mean values for parameters PI and IPI were compared, differences among the species of the subgroup *fasciola* could be found for at least one of these parameters. These differences in the courtship sounds can be seen in Fig. 7, in which the values for these two parameters are represented in the form of ellipses, which include 75% of the values for parameters PI and IPI. Significant differences were found in the PI (ANOVA, $F_{5,48}$ = 124.98, $p < 0.001$) and IPI (ANOVA, $F_{5,48}$ = 949.35, $p < 0.001$) values among species. The interspecific differences in IF are shown in Fig. 8. Significant difference were also found in the IF values (ANOVA, $F_{5,48}$ = 23.40, $p < 0.001$).

The three populations of *D. coroica* did differ significantly in the PI (ANOVA, $F_{2,24}$ = 8.75, $p < 0.05$) but not in the IPI (ANOVA, $F_{2,24}$ = 1.72, $p > 0.10$) and IF (ANOVA, $F_{2,24}$ = 0.15, $p > 0.25$).

DISCUSSION

Hoikkala *et al.* (1994) in their analysis of the courtship sound of various species of the subgroup *planitibia*, discovered that it is common to find the same types of sounds in phylogenetically close species. Also, Hoikkala & Kaneshiro (1993) observed that some closely related species in this subgroup, which are sympatric, use different types of sounds in courtship.

Noor & Aquadro (1998) found significant differences in courtship sounds in two closely related species of the subgroup *obscura*: *D. pseudoobscura* and *D. persimilis*. Some experiments involving crosses between these two species indicated that females of *D. pseudoobscura* probably increased their discrimination against *D. persimilis* males in sympatric populations. This could have occurred through selection against the production of badly adapted hybrids, resulting in a courtship sound alteration in these sympatric populations.

Ewing & Miyan (1986), working with species of the *repleta* group, found no relationship between a phylogeny based on cytological data and another made from sound data. Nevertheless, some of the characteristics that most of the species studied have in common and which are also found in species of other groups allow us to propose an ancestral courtship sound for the *repleta* group. This sound would have been composed of two distinct components: the initial sound A, consisting of a maximum of 10 short and regular monocyclic pulses, with an interpulse interval of from 8 to 15 ms; and sound B, composed of a series of regular and monocyclic pulses, but with a larger number of pulses and a longer interpulse interval at the end of courtship. During the evolution of the *repleta* group, some species would have lost sound A, and others, sound B, and, in many, sound B would have become less regular and more complex.

Based on these suppositions and observing the sonograms obtained for the six species of the *fasciola* subgroup in this study, we can infer that in all of these species there was a loss of sound A, since the interpulse intervals are always much longer than 15 ms. *D. coroica* has irregular pulses, with a mean interpulse interval of 101.3 ms, while the courtship sounds of *D. moju* are made up of more regular pulses, with a mean interpulse interval of 50.9 ms. *D. rosinae* has a sonogram made up of duplicated pulses, a sound type also found by Ewing & Miyan (1986), with a mean interpulse interval of 72.3 ms. *D. ellisoni*, *D. fascioloides,* and *D. onca* have courtship sounds made up of polycyclic pulses, probably a result of a fusion of sounds A and B, with mean interpulse intervals of 97.4, 125.7, and 508.0 ms, respectively. These similarities in the types of sounds in these three species are not closely correlated to cytological data, except for *D. ellisoni* and *D. fascioloides* which were placed near each other in a phylogeny based on cytological characters, they have two chromosome fusions in common: 2-4F e 3-5F (Diniz, 1998). As is the case for morphological characters, a tendency for closely related species to share some sound parameters is expected. Nevertheless, even when the phylogenetic relationships among the species are known, the evolutionary tendencies based on sound characters have not been well established.

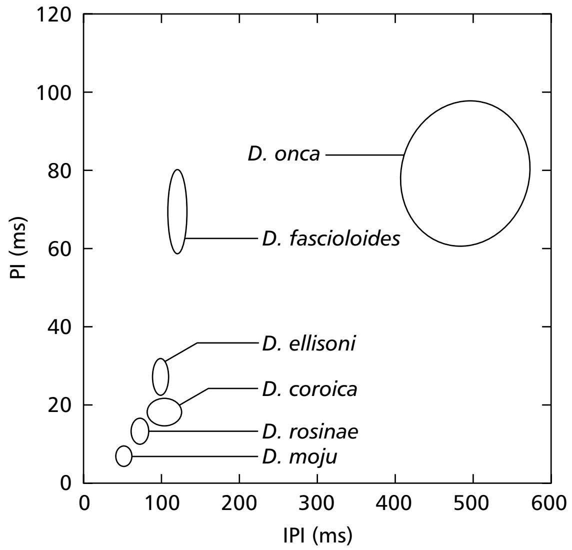

Fig. 7 — Representation of the ellipses that include 75% of the PI and IPI values of the six species of the subgroup *fasciola* that were analyzed: *D. ellisoni* (D83M1 – São Sebastião, SP), *D. fascioloides* (D86N17 – Nova Friburgo, RJ), *D. moju* (F34M2 – Panamá), *D. onca* (J16CM1 – Tibaji, PR), *D. rosinae* (D63M1 – Mucugê, BA) and *D. coroica* (D96N74 – São Carlos, SP).

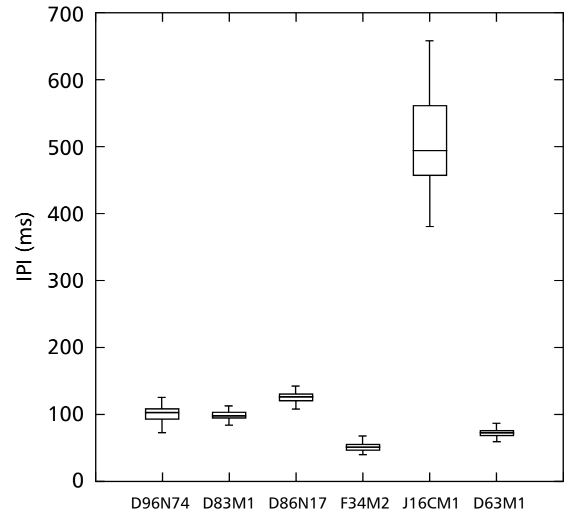

Fig. 8 — Distribution of the IFs of the six species of the subgroup *fasciola* that were analyzed: D96N74 (*D. coroica* – São Carlos, SP); D83M1 (*D. ellisoni* – São Sebastião, SP); D86N17 (*D. fascioloides* – Nova Friburgo, RJ); F34M2 (*D. moju* – Panamá); J16CM1 (*D. onca* – Tibaji, PR); D63M1 (*D. rosinae* – Mucugê, BA). The horizontal lines inside the boxes represent the medians. The boxes contain the values 25% above and below the median. The upper and lower portions contain the 25% highest and 25% lowest values, respectively.

Even though courtship sound is not an example of circadian behavior, research has related this behavior to the expression of the gene *period* (*per*) located on the X chromosome and well characterized at the molecular level. Mutations in this gene can alter the circadian rhythm of *Drosophila*. A genetic analysis made by Konopka *et al.* (1996) showed that the sound rhythm of the interpulse interval is also affected by mutations in the gene *per*. Kyriacou & Hall (1980) reported a rhythmic modulation of the interpulse interval with specific periods in *D. melanogaster* and *D. simulans* of 55 and 35 seconds respectively. These differences were shown to be related to some variations in the *per* gene. Mutations in this gene, that were initially studied by Konopka & Benzer (1971) in an analysis of abnormal circadian rhythms, provoked corresponding effects in the interpulse interval rhythms of the courtship sounds. The mutation per^{Short} (per^{S}), with a circadian rhythm of about 19-20 hours, had a mean period of 41.5 seconds; mutation per^{Long} (per^{L}) whose circadian rhythm of 28-30 hours had a mean period of 82.1 seconds; and mutation per^{0} had no interpulse interval rhythm.

In this research we found significant differences in the parameters intrapulse interval, interpulse interval, and intrapulse frequency in the *fasciola* species analyzed. Among these parameters, the interpulse interval was the one that differed most, with little superposition and little intraspecific variation, suggesting that this parameter could be part of a species recognition system. This high level of differentiation in the male sounds is consistent with the general consensus that courtship behavior can differ more than morphological characters or other characteristics in related species. However, it is impossible at the moment to know the direction of evolution of these different types of sounds in the *fasciola* subgroup. Genetic studies have shown that interspecific differences in interpulse intervals are frequently due to additive and polygenic factors. These differences can also be explained by adaptation to different environments in the absence of gene flow. It is known that there were alternating cycles between cold-dry and hot-humid in the Quartenary period. In a cold-dry climate cycle, vegetation adapted to the dry conditions expanded, while forest retracted. During the subsequent hot-humid cycle the situation inverted (Ab' Saber, 1977; Vanzolini, 1981). It is believed that the flies followed these processes of forest reduction and expansion, which would result in long periods of geographic isolation during the cold-dry cycles and some areas of sympatry during the hot-humid cycles.

Though the interpulse interval can vary widely among different species of the genus *Drosophila*, generally this is not observed when populations of the same species are analyzed (Costa *et al.*, 2000; Ritchie *et al.*, 1994). The three analyzed populations of *D. coroica* had significant differences only in the intrapulse interval, with no differences in the interpulse interval and intrapulse frequency. It is possible that a strong selection pressure is acting on the interpulse interval and intrapulse frequency. This low variability could also occur if geographically distinct populations were genetically homogeneous due to constant gene flow, or if a common ancestor had recently colonized these populations. However, the PI data show that this is not the case.

Little is known about the genetic mechanisms involved in the speciation processes, however it is first necessary to understand the genetics and evolution of the mechanisms impeding gene flow. Sterile and unstable hybrids are considered strong barriers to gene flow, but these types of post-zygotic mechanisms can result in high costs in the form of gametes and energy. Therefore, differences in courtship sounds can be an important isolating mechanism, as they would limit or prevent such losses (Welbergen *et al.*, 1992). Analysis of these signals, which are important for mating success, is of considerable interest for evolutionary biology and knowledge of the architecture of sounds could help prove various models of sexual selection and speciation.

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