Acoustical Comparisons of Calling Songs from *Anastrepha* Species in Brazil

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INTRODUCTION

Anastrepha is an economically important genus of the family Tephritidae that infests over 180 plant species from southern United States to northern Argentina (Norrbom and Kim 1988). Some species, including *A. fraterculus* (Wiedemann), *A. obliqua* (Macquart), *A. sororcula* Zucchi (1979), and *A. grandis* (Macquart) are highly destructive pests of tropical and temperate fruits. The first three are cryptic species. Accurate identification requires examination of ovipositor morphology. *A. grandis* is easily distinguished from the other species by its larger size. *A. fraterculus* shows considerable heterogeneity over its geographical range, with differences in morphology, karyotype and isozyme pattern (Stone 1942, Solferini and Morgante 1987, Morgante et al. 1980, Steck 1991). *A. sororcula* occurs sympatrically with *A. fraterculus* in much of Brazil and overlaps considerably in its host range. The other species have different host ranges but occur sympatrically in many areas of Mexico, Central and South America.

The typical sexual behavior in the genus includes lek formation (mating aggregations) by 3-15 males in an arena in the host or occasionally a nearby non-host tree (Burk 1983, Morgante et al. 1983). Males usually occupy a leaf bottom or pedicel. They call by everting their rectal pheromone glands and fanning their wings (Burk 1991, Sivinski and Burk 1989). Lekking behavior may have evolved through female preference for males in groups (Bradbury 1981) or through aggregation of males in microenvironments where female density is likely to be high (Sivinski 1989).

In choosing potential mates, females of some *Anastrepha* species discriminate in favor of larger conspecific males, which produce calling songs of lower fundamental (wing-beat) frequency and shorter intervals between bursts (pulse trains) (Burk and Webb 1983, Sivinski et al. 1984, Webb et al. 1984). If significant differences exist among calling songs of cryptic *Anastrepha* species, quantification of such differences could assist in species identification. Also, it is conceivable that reproductive isolation between two or more of these populations is mediated by differences in acoustical signals or the timing of calling (e.g. Alexander 1967). Perhaps, displacement in one or more acoustical characters evolved through sexual selection, with a secondary effect of enhancing reproductive isolation among sympatric *Anastrepha* species. Similar hypotheses have been proposed for other species complexes (West-Eberhard 1984, Krebs and Markow 1989, Hoy 1990).

We recorded and analyzed male songs of *A. obliqua*, *A. sororcula*, *A. grandis*, and two geographically separated populations of *A. fraterculus* to determine if they contain group-specific acoustical features, and if so, to place them in an appropriate context as potential reproductive isolation mechanisms.

METHODS AND MATERIALS

Insects were collected from the following locations and hosts: *A. fraterculus* from São Paulo, Itaquera and Cruz das Almas, Bahia on guava (*Psidium guajava*); *A. obliqua* from Bebedouro, São Paulo, on carambola (*Averroa carambola*); *A. sororcula* from Nova Soure, Bahia on guava; *A. grandis* from Laranjal Paulista, São Paulo on pumpkin (*Cucurbita maxima*). Recordings were done at 18-27°C, 250-600 lux in a screen cage with a LeSon MC-22AB electret microphone 0.5-1.0 cm from the calling male. The Cygnus Model AC 300 amplifier had a 40-18,000 Hz frequency range.

Cassette recordings of the calling songs were digitized at 25 kHz by a 12-bit MetraByte (Keithley/Metrabyte Inc., Taunton, MA) DAS-16G A/D board installed in a 80486 microcomputer. The digitized signal was filtered and analyzed as needed to remove background noise by DAVIS, a custom-written signal processing and spectral analysis computer program (Mankin, unpublished).

Digitized files of recordings from individual males were analyzed by standard digital signal processing techniques. Each file consisted of approximately 25 consecutive wing-fanning bursts (pulse trains) separated by pauses. Calling was considered to have ended when a pause exceeded the average interval between pulse trains (PTI) by greater than two standard deviations. Analysis of variance and discriminant analysis (SAS Institute Inc. 1988) were performed on mean wing-beat frequency (fundamental frequency = FF), mean time from the beginning of one pulse train to the beginning of the next (period = PP) mean pulse-train duration (PD), and mean interval between pulse trains (PTI) (Figure 1). The Generalized Squared Distance between two populations *i, j* was calculated as:

$$D^2_{ij} = (\underline{M}_i - \underline{M}_j)' \; \underline{Cov}^{-1} \; (\underline{M}_i - \underline{M}_j),$$

where $\underline{M}_i$ or $\underline{M}_j$ is a 4-element matrix, [FF, PP, PD, PTI], for population *i* or *j*, the prime (′) indicates a matrix transpose operation, $\underline{Cov}$ is the covariance matrix, and the superscript ($^{-1}$) indicates the matrix inverse operation (SAS Institute Inc. 1988).

RESULTS

None of the acoustical features (FF, PP, PD, PTI) successfully discriminated among all of the *Anastrepha* populations, but each feature was significantly different for at least two groups (Table 1). *A. grandis* had the longest pulse train period, duration, and interpulse interval and the lowest fundamental frequency. The two *A. fraterculus* groups were not significantly different for any acoustical feature. As a single species, *A. fraterculus* had the shortest PP, PD and PTI, all of which were significantly different from both *A. obliqua* and *A. grandis*. *A. sororcula* had the highest FF, significantly different from all other groups.

These calling differences permitted discriminant analysis to separate *A. sororcula* from all other groups, and *A. fraterculus* from *A. grandis* (Table 2). Two types of error were calculated: %GERR, the percentage from one population misclassified into a different population; and %SERR, the percentage from one species misclassified into a different species. The calculations of Generalized Squared Distance, D^2 (Table 2), indicated a close similarity between *A. grandis* and *A. obliqua*, as well as between the São Paulo and Bahia populations of *A. fraterculus*. *Anastrepha obliqua* was
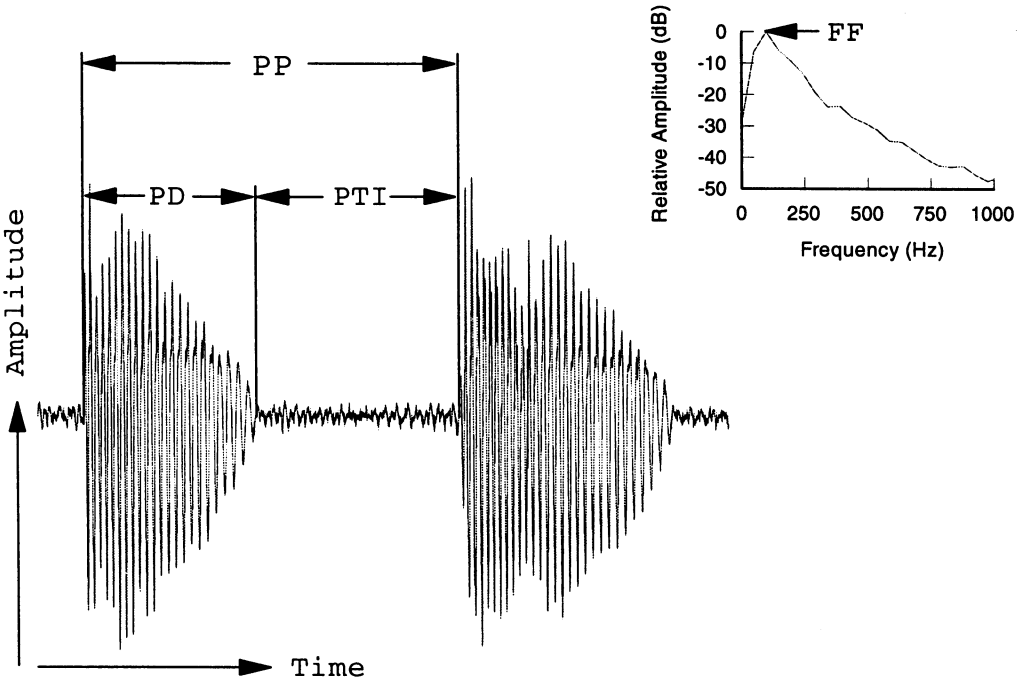
Figure 1. Section of *A. fraterculus* calling song showing two pulse trains of ca. 0.3 sec duration separated by a pause of ca. 0.4 sec. The fundamental frequency in the spectrum insert is ca. 90 Hz (wingbeats/sec). PP = period; PD = pulse train duration; PTI = pulse train interval; FF = fundamental frequency.

Table 1. Mean and standard error of pulse-train period, duration and interval, and fundamental frequency of four species of *Anastrepha*.

Group	Period (sec)	Duration (sec)	Interval (sec)	Fund. Freq. (Hz)
FRSP	0.733 c	0.321 b	0.407 c	89.1 b
	±0.064	±0.053	±0.034	± 4.5
FRBH	0.863 bc	0.261 b	0.599 bc	94.4 b
	±0.077	±0.026	±0.070	± 3.1
OBLQ	1.36 a	0.553 a	0.812 a	91.0 b
	±0.123	±0.064	±0.116	± 6.8
SORO	1.057 b	0.340 b	0.716 ab	185.6 a
	±0.064	±0.053	±0.034	± 4.5
GRND	1.488 a	0.628 a	0.856 a	82.2 b
	±0.079	±0.058	±0.080	± 2.7

Note: Anastrepha fraterculus São Paulo = FRSP, *A. fraterculus* Bahia = FRBH, *A. obliqua* = OBLQ, *A. sororcula* = SORO and *A. grandis* = GRND. Means in a column followed by the same letter are not significantly different by the Waller-Duncan K-ratio test (SAS Institute Inc. 1988).

Table 2. Discriminant analysis of pulse-train period, duration and interval, and fundamental frequency of 4 species of *Anastrepha*.

Number of Observations Classified Into:

		FRSP	FRBH	OBLQ	SORO	GRND	%GERR	%SERR
From:								
FRSP	#	7	2	1	0	0	30	10
	D^2_{FI}		*0.99*	*8.63*	*37.1*	*12.2*		
FRBH	#	4	6	0	0	0	40	0
	D^2_{FI}			*7.19*	*33.5*	*10.8*		
OBLQ	#	0	0	3	0	2	40	40
	D^2_{FI}				*34.1*	*0.66*		
SORO	#	0	0	0	10	0	0	0
	D^2_{FI}					*42.3*		
GRND	#	0	0	4	0	5	44	40
					mean %ERR =		31	16

Note: Anastrepha fraterculus São Paulo = FRSP, *A. fraterculus* Bahia = FRBH, *A. obliqua* = OBLQ, *A. sororcula* = SORO and *A. grandis* = GRND. %GERR is the percent misclassified out of group; %SERR is the percent misclassified out of species; # is the number of observations reclassified to *Into* group from *From* group; D^2_{FI} (values in italics) is the Generalized Squared Distance between *From* and *Into* group (SAS Institute Inc. 1988).

intermediately distant from *A. fraterculus* and *A. sororcula*. The latter species was distant from all other groups because of its high fundamental frequency. One *A. fraterculus* record was misclassified as *A. obliqua*. *A. grandis* could not be distinguished acoustically from *A. obliqua*.

DISCUSSION

Although the differences among calling song attributes are sufficient to permit pre-copulatory reproductive isolation among several of the species examined here, particularly *A. sororcula*, they are not necessarily the major isolating mechanism even if they play a role. All of these populations probably have species-specific sex pheromones. *A. fraterculus* males display in early morning while *A. obliqua* display at midday to midafternoon (Malavasi 1984). *A. grandis* is morphologically distinct from the other species and has a completely different host range.

The two populations of *A. fraterculus* represent a case where calling song attributes do not contribute to reproductive isolation, although they may nevertheless be cryptic species. The chromosomal studies carried out by Solferini and Morgante (1987) in the same two areas showed two

distinct karyotypes. Steck (1991) found large differences among isozymes in many populations of *A. fraterculus*, including the populations from Bahia and São Paulo; the populations labeled Braz-Bah 1 and Braz-S.P. in Steck (1991) are the same populations analyzed here. Perhaps because the two populations are widely separated, there is no selection pressure for their calling song attributes to diverge. *A. sororcula* may be under greater selection pressure for divergence of calling song attributes because it lives in sympatry with *A. fraterculus*. Unless it comes under strong selection pressure, sexual communication is usually expected to be relatively stable evolutionarily (Alexander 1967, Lundberg and Löfstedt 1987, Mankin 1991).

In conclusion, divergence in calling song attributes may contribute to reproductive isolation between *A. sororcula* and other *Anastrepha* species, and between *A. obliqua* and *A. fraterculus*. However, other mechanisms are responsible for reproductive isolation within *A. fraterculus* and between *A. grandis* and other *Anastrepha* species.

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