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## Excitable ancestors and functionally referential communication: effect of food quality and location on recruitment sounds and success in two stingless bees, *Melipona mandacaia* and *Melipona bicolor*

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### ABSTRACT

It is unclear whether stingless bees in the genus *Melipona* (Hymenoptera, Apidae, Meliponini) can reliably encode the distance to a food source through recruitment sounds produced inside the nest, in part because the sound features correlated with distance also vary with food quality. We therefore trained marked foragers of two species, *Melipona mandacaia* and *M. bicolor*, to feeders at different distances and to different sucrose concentrations at the same distance. In both species, foragers successfully recruited to a rich 2.5 M food source and produced pulsed recruitment sounds in which pulse duration was significantly and positively correlated with distance to the rich food source. When returning from poorer food sources (0.6-1.5 M), foragers of both species decreased sound production, producing shorter sound pulses and longer sound inter-pulses than they did for 2.5 M food located at the same distance. Thus the temporal structure of *M. mandacaia* and *M. bicolor* recruitment sounds varies with distance and food quality. However, nestmates were not recruited by performances for poorer food sources (0.6-1.5 M) whose sucrose concentration was sufficiently low to affect recruitment sounds. Surprisingly, the interphase (the time between behavioral phases that communicate location) also increases with decreasing food quality in the closely related honeybees (*Apis*), suggesting a potential homology in the effect of food quality on the recruitment systems of *Apis* and *Melipona*. We explore the evolutionary implications of these similarities.

**Keywords:** distance encoding, food quality, stingless bees, recruitment, sound

### INTRODUCTION

Successful foragers of highly social insects commonly communicate food source location to other colony members. In honeybees (*Apis*), such location communication can occur through the waggle dance (von Frisch 1967; Gould 1975; Dyer 2002). Aside from *Apis*, the tropical stingless bees (Meliponini) are the only highly social bees, and both groups are closely related (Michener 2000, Cameron and Mardulyn 2001). Several authors have therefore studied the diverse communication systems used (or not used) by various species of Meliponini with the objective of discovering their functions as well as possible homologies with the *Apis* communication system. However, many aspects of meliponine communication remain to be clarified. In particular, it is unclear whether stingless bees in the genus *Melipona* can use recruitment sounds to communicate the distance to a food source (Hrcir et al. 2000; Dyer 2002; Jarau et al. 2002).

Correlations between the duration of recruitment sound pulses and the distance to the food source have been found in four species of stingless bees, *M. quadrifasciata*, *M. merillae*, *M. panamica*, and *M. costaricensis* (Esch 1967; Nieh and Roubik 1998; Aguilar and Briceño 2002). Debate over the existence of such distance encoding has arisen over three points: motivational vs. distance encoding, replication, and signal variance (see Nieh et al. 2003b). Different species may possess different communication systems, but different methodologies may also have led to different results. We therefore address aspects of these issues in our experiments with *M. mandacaia* and *M. bicolor*—species whose recruitment sounds had not been previously studied, live in quite different habitats, and can recruit nestmates to a specific distance

and direction (Nieh et al. in press). *Melipona mandacaia* Smith, 1863, is endemic to the southern portion of the State of Bahia, in the semi-arid Caatinga ecosystem (Rizzini 1997). *Melipona bicolor bicolor* Lepetelier 1836 is found in the Atlantic Rainforest in the Brazilian states of São Paulo, Rio de Janeiro, Espírito Santo, and Minas Gerais (Hilário 1999). Our goals were to determine the effect of food quality and distance on recruitment sounds and successful recruitment.

## METHODS

**Study site and bee colonies.** We conducted all studies on a farm, the Fazenda Aretuzina, in the state of São Paulo, Brazil. We used one colony of *M. mandacaia* (approximately 300-400 workers) from southern Bahia and one colony of *M. bicolor* (approximately 800-1100 workers) from Cunha in coastal southern Brazil. We trained and marked bees at sucrose solution feeders following the methods of von Frisch (1967).

**Video and sound analysis.** We illuminated the food-unloading platform with a 20 W halogen light and videotaped the motions and sounds of recruiting foragers with a Canon XL1 digital camcorder and a Radio Shack electret condenser microphone (catalog no. 33-1052) connected to a Teflon tube inserted onto the unloading platform. We imported digital video sequences into an Apple iBook computer with iMovie v2.0 and used VideoPoint v2.1.2 and Canary v1.2.4 software respectively for motion and sound analysis. We analyzed all sounds during a *performance*, a complete visit of a recruiting forager inside the nest (for further details, please see Nieh et al. 2003b).

## RESULTS

### Effect of food quality

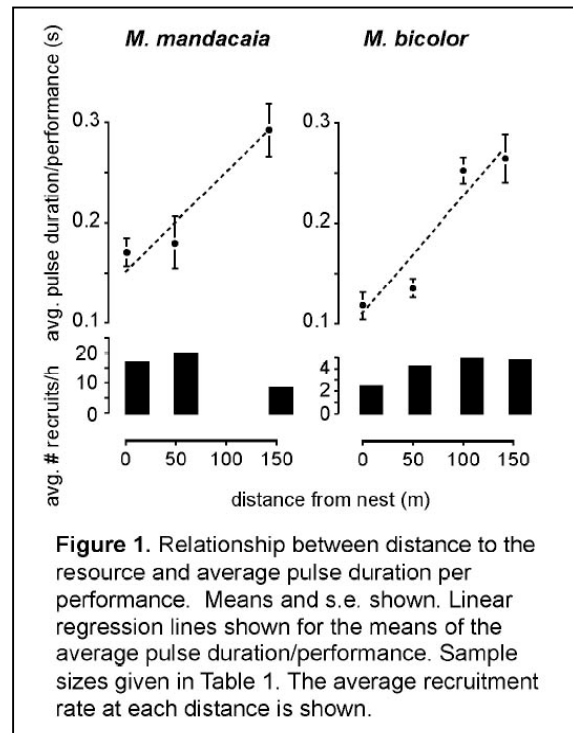
Sucrose concentration influenced the recruitment sounds of *M. mandacaia* and *M. bicolor* foragers in similar ways. There is no significant effect of sucrose concentration on fundamental frequency in *M. mandacaia* (ANOVA,  $F_{1,58}=0.4$ ,  $P=0.55$ ) or in *M. bicolor* (ANOVA,  $F_{1,61}=0.8$ ,  $P=0.38$ ). In both species, overall sound production decreased as sucrose concentration decreased (*all* pulse and interpulse duration data). Moreover, recruitment behavior for lower sucrose concentrations (0.6-1.5 M) was ineffective at recruiting nestmates to the food source.

In *M. mandacaia* and *M. bicolor*, the *average pulse* duration per performance decreased significantly with decreasing sucrose concentration (*M. mandacaia*, ANOVA,  $F_{2,25}=9.4$ ,  $P=0.0009$ ; *M. bicolor*, ANOVA,  $F_{1,39}=4.7$ ,  $P=0.036$ ). In both species, the *average interpulse* duration per performance increased significantly with decreasing sucrose concentration (*M. mandacaia*, ANOVA,  $F_{2,25}=5.5$ ,  $P=0.011$ ; *M. bicolor*, ANOVA,  $F_{1,39}=23.2$ ,  $P<0.0001$ ).

### Effect of distance

In both species, (Fig. 1) *all pulse* durations during an entire performance significantly increased with increasing distance to the food source (*M. mandacaia*: ANOVA,  $F_{2,3329}=74.9$ ,  $P<0.0001$ ; *M. bicolor*: ANOVA,  $F_{3,3984}=151.4$ ,  $P<0.0001$ ), but *all interpulse* durations did not significantly change with distance (*M. mandacaia*: ANOVA,  $F_{1,3264}=1.3$ ,  $P=0.27$ ; *M. bicolor*: ANOVA,  $F_{3,3892}=0.8$ ,  $P=0.50$ ). Similarly, the *average pulse* duration per performance significantly increased, but the *average interpulse* duration per performance did *not* significantly change with increasing distance to the food source. Foragers successfully recruited nestmates at all tested distances.

Between species, there are significant differences in the *average pulse* duration per performance at 0 m (ANOVA,  $F_{1,28}=7.3$ ,  $P=0.012$ ) and 50 m (ANOVA,  $F_{1,56}=5.5$ ,  $P=0.022$ ), but not at 140 m (ANOVA,  $F_{1,51}=0.3$ ,  $P=0.57$ ). Within each species, the *average interpulse* duration per performance did not significantly change with changing distance to the food source. However, there were significant differences between the species: the average interpulse duration per performance for all distances was  $396\pm 32$  ms ( $N_m=N_p=59$ ,  $N_i=20$ ) for *M. mandacaia* and  $286\pm 18$  ms ( $N_m=N_p=109$ ,  $N_i=20$ ) for *M. bicolor* (Kruskal-Wallis test,  $c^2=11.5$ , 1 d.f.,  $P=0.0007$ ).



**Figure 1.** Relationship between distance to the resource and average pulse duration per performance. Means and s.e. shown. Linear regression lines shown for the means of the average pulse duration/performance. Sample sizes given in Table 1. The average recruitment rate at each distance is shown.

## DISCUSSION

We studied the effect of food location on recruitment sounds in two species of stingless bees, *M. mandacaia* and *M. bicolor*, species whose recruitment communication sounds had not been previously been studied. Two main parameters of the sounds emitted by foragers inside the nest, pulse duration and interpulse duration, were modulated by the concentration of the nectar source and its distance from the nest. Pulse duration increased with increasing distance from the nest. However, interpulse duration increased when nectar concentration decreased (0.6-1.5 M sucrose solution) and remained fairly constant within a range of different distances. We therefore suggest that pulse duration signals food distance and interpulse duration may provide an indicator of food quality. No newcomers were recruited to a food source of sufficiently low quality (0.6-1.5 M) to affect recruitment sounds. Intriguingly, the influence of food quality upon *M. mandacaia* and *M. bicolor* recruitment sounds parallels the relationship between food quality and the return phase of the honeybee waggle dance (Seeley et al. 2000).

### Effect of food quality

Food quality affected the motivation of *M. mandacaia* and *M. bicolor* foragers to produce sound. Sound production decreased as food quality decreased (decreased pulse duration and increased interpulse duration). *M. costaricensis* (Aguilar and Briceño 2002) and *M. seminigra* (Jarau et al. 2002) recruiters also decreased sound pulse durations and increased interpulse durations with decreasing sucrose concentration. However, it is unclear if foragers of these species successfully recruited newcomers to lower quality food sources. We found that *M. mandacaia* and *M. bicolor* foragers did not successfully recruit nestmates to poor food sources (0.6-1.5 M) of sufficiently low quality to elicit changes in sound production. Thus potential recruits may not be attracted to performances in which the interpulse durations exceed a threshold value. In addition, other sources of information such as trophallactic contact (as in *Apis*, De Marco and Farina 2003) and excitatory jostling motions (Hrncir et al. 2000) may provide information on food quality.

One may wonder why recruitment sounds change with sucrose concentration and why foragers produce sounds for lower quality food sources. Changes in receiver attention with changing food quality can facilitate optimal group foraging in bees and ants (Seeley 1985; Seeley 1987; Seeley 1989; Hölldobler and Wilson 1990). Moreover, many animal behaviors are graded in response to motivation levels (Morton 1977; Hauser 1996; Greenfield 2002). Interestingly, such behavioral changes are predicted by the signal continuum model, which proposes that referential signals (signals that abstractly encode environmental information) often contain components reflecting the sender's motivational state (Marler et al. 1992).

### Effect of distance

*M. mandacaia* and *M. bicolor* foragers significantly increased the duration of recruitment sound pulses with increasing distance to the food source during the entire recruitment performance. Thus pulse duration may reliably encode food distance if potential recruits distinguish between performances for high and poor quality food sources.

In both species, foragers recruited nestmates to all tested distances and interpulse durations were fairly constant at all distances (with the exception of *M. bicolor* at 50 m, for which the interpulses were shorter than average and may reflect forager perceptions of unusually high quality). Moreover, interpulse durations measured in the distance experiment were shorter or of comparable magnitude to interpulse durations (given the high variances) independently measured during the sucrose concentration experiment.

Significant correlations between the temporal structure of recruitment sounds and distance to a good food source have now been reported in *M. quadrifasciata*, *M. merillae*, *M. costaricensis*, *M. panamica*, *M. mandacaia*, and *M. bicolor* (Esch 1967; Nieh and Roubik 1998; Aguilar and Briceño 2002). However, the work of Hrncir et al. (2000) suggested that no correlations exist between distance and the recruitment sounds of *M. quadrifasciata* and *M. scutellaris*. This differs from the results of Esch (1967), who found significant correlations between distance and recruitment sound pulse durations in *M. quadrifasciata*. Given the substantial variation in *Melipona* habitats (Roubik 1989; Michener 2000) and variation in their ability to communicate food location (Nieh et al. 2003a), interspecific differences in the ability to encode and communicate distance may exist. However, the different results of Hrncir et al. (2000) may also have arisen from methodological differences (see discussion in Nieh et al. 2003b).

### *Melipona* and *Apis*: evolutionary homologies?

Honeybees produce a waggle sound pulse that is preceded and followed by interpulses of no sound production during the return phases (Wenner 1962). The duration of the waggle-phase sound pulse increases with increasing distance to the food source (von Frisch 1967), and the interpulse durations (*waggle dance return phases*) increase with decreasing food quality (Seeley et al. 2000). Thus an acoustic analysis of the honeybee waggle dance would reveal a pattern of increasing

interpulse duration with decreasing food quality and increasing pulse duration with increasing distance—the same relationships found in the *M. costaricensis* (Aguilar and Briceño 2002), *M. mandacaia*, and *M. bicolor*.

It is unclear whether these similarities are analogous or homologous. There is disagreement concerning the exact evolutionary relationship between stingless bees and honeybees in the Apidae, and eusociality and recruitment communication may have evolved independently in honeybees and stingless bees (Winston and Michener 1977; Cameron and Mardulyn 2001). If advanced eusociality has evolved twice, primitive eusociality may still have been the ancestral state for all four groups in the Apidae (Bombini, Euglossini, Meliponini, and Apini; Michener 2000). Recent work on the Bombini suggests that the Apidae may have shared a primitively eusocial ancestor that excited nestmates after discovering food. Upon returning from a rich food source, bumblebees can motivate nestmates to forage through food-alert runs, bouts of running through the nest and interacting with other bees (Dornhaus and Chittka 1999; Dornhaus and Chittka 2001; Dornhaus et al. 2003). This food-alert behavior is similar to the excitatory zigzag and jostling behaviors performed by foragers of several meliponine species, including species that do not communicate food location (Lindauer and Kerr 1958).

Thus the communication of food quality through a graded series of excitatory behaviors (movements and sounds) may be ancestral to the four tribes in the Apidae but was lost in the solitary Euglossini. Karl von Frisch (1967) proposed that foraging-related excitatory behaviors provided a source of behavioral variation for the evolution of the waggle dance. We offer a specific modification to this hypothesis and propose that the ancestor to the Apidae responded to decreasing food quality by increasing the interval between foraging-related excitatory behaviors. This *excitable ancestor hypothesis* leads to the following predictions. Decreasing food quality will increase the duration between food-alert runs in the Bombini and in the Meliponini, even in meliponine species that do not communicate food location.

We are currently conducting playback experiments that examine the role of vibrational communication of distance in the species *M. panamica*. Using laser vibrometry and a Microflown® USP particle-velocity sensor to measure natural recruitment sounds and to calibrate their playback, we have gathered initial data suggesting that vibrational playbacks can increase the recruitment of newcomers to a specific distance.

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