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ORIGINAL ARTICLE

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Effect of food location and quality 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 interpulses 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

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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 (Hrncir et al. 2000, 2002; Dyer 2002).

Honeybees (*Apis*) encode the distance and direction to food sources in a waggle dance, a repeating figure-eight motion, consisting of a waggle phase in which the bee waggles her body medio-laterally while moving forwards, followed by a return phase during which the bee circles back without waggling in preparation for the next waggle phase (von Frisch 1967; Tautz et al. 1996). The angle of the waggle phase communicates food direction, and waggle-phase duration communicates food distance (von Frisch 1967; Dyer 2002). In addition, foragers produce a pulse of sound throughout the waggle phase, and the duration of this sound pulse is positively correlated with food distance (Wenner 1962). The return phase is therefore a location-communication interphase, a time period between location-communicating behavioral phases. Food quality also influences honeybee recruitment dances (Waddington and Kirchner 1992), and Seeley et al.

(2000) made the intriguing discovery that return-phase duration increases with decreasing food-source quality.

There has been controversy concerning whether foragers use the direction and distance information encoded in the waggle dance (Wenner et al. 1969; Wenner and Wells 1990; Wenner 2002); however, this has largely been resolved through experiments showing that honeybees can be predictably misdirected by manipulating the waggle dance (Gould 1975, 1976) and directed by a mechanical model of a dancing bee (Michelsen et al. 1989). In addition, experiments using flight tunnels have shown that bees measure the distance flown through optic flow and that the fictive distance experienced within the tunnel is effectively read and flown by followers that have never been to the food source (Esch et al. 2001). Recently, Sherman and Visscher (2002) showed that the honeybee waggle dance contributes significantly to colony fitness.

Correlations between the duration of recruitment sound pulses and the distance to the food source have been found in four species of stingless bees: *Melipona 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 versus distance encoding, replication, and signal variance.

First, the results of Aguilar and Briceño (2002) with *M. costaricensis* suggest that sound-pulse duration may not be a reliable measure of distance, because sound-pulse duration varies with food quality and distance to the food source. Hrncir et al. (2002) found that food quality exerted a similar effect on *M. seminigra* recruitment sounds. In both species, pulse duration decreased and interpulse duration increased as sucrose concentration decreased (Aguilar and Briceño 2002; Hrncir et al. 2002). However, it is unclear if recruitment behavior at lower-quality food sources was effective at successfully recruiting newcomers of these species.

Second, a recent study was not able to replicate earlier results with one species or find distance encoding in a newly studied species (Hrncir et al. 2000). Esch (1967) reported significant correlations in *M. quadrifasciata* and *M. merillae* between distance and pulse durations during the entire performance. However, Hrncir et al. (2000) did not find evidence for distance encoding in *M. quadrifasciata* or *M. scutellaris*, although they measured performance duration, the number of pulses per performance, pulse duration, interpulse duration, and the fundamental frequency of recruitment sounds.

Third, it is unclear if recruitment sounds contain sufficient distance information to account for spatial recruitment accuracy in some species. The temporal sound parameters correlated with distance to the food source have high variances (large standard deviations) in *M. costaricensis* (Aguilar and Briceño 2002) and *M. panamica* (Nieh and Roubik 1998). Esch (1967) reported relatively smaller standard deviations in *M. merillae* and *M. quadrifasciata*.

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). *M. mandacaia* Smith, 1863 is endemic to the southern portion of the State of Bahia, in the semi-arid Caatinga ecosystem (Rizzini 1997). *M. 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 (21°26.390′S, 47°34.810′W). We used 1 colony of *M. mandacaia* (approximately 300–400 workers) from southern Bahia and 1 colony of *M. bicolor* (approximately 800–1,100 workers) from Cunha (23°05′S, 44°55′W) in coastal southern Brazil. We studied *M. mandacaia* from 8 August to 5 September 2000 and *M. bicolor* from 1 July to 29 October 2001. Both colonies were housed in successive years at the Fazenda bee laboratory within observation nests consisting of plate glass covering three adjacent wood chambers of decreasing depth for respectively containing the brood comb, food-storage pots, and food recruitment activity (Nieh and Roubik 1998). We connected each nest to the laboratory exterior with a 25-cm-long, 1-cm-diameter vinyl tube. All recruitment behavior occurred on a flattened triangular area (30 cm long, 30 cm wide, 1 cm high) that narrowed to the nest entrance and modeled the entrance funnel in natural *Melipona* nests (Nieh 1998; Nieh and Roubik 1998).

Training and marking bees

We trained bees to anise-scented sucrose solutions (all containing 10 μl anise extract, McCormick, per liter of sucrose solution) following the method of von Frisch (1967). Paint-marking pens were used to uniquely mark each bee visiting the training feeder with different combinations of paint colors on the thorax and abdomen. In all experiments, we trained 20 individually marked foragers to a feeder and censused the visiting bees each 15 min to ensure a constant visitation rate and a fixed number of visiting foragers. Unmarked bees and additional marked foragers were captured in separate plastic aspirators, and marked foragers were released or captured as necessary to maintain a constant number of marked foragers visiting the feeder. We studied the effect of sucrose concentration by training *M. mandacaia* and *M. bicolor* foragers to a feeder 140 m west of the colonies and alternating sucrose concentrations each hour. We studied the effect of distance by training *M. mandacaia* and *M. bicolor* foragers to 2.5-M-sucrose feeders at different distances east of the nest.

Measuring recruitment rates

In order to study food-recruitment communication, a transfer of information that leads to an increase in the number of newcomers visiting a food source must occur. We therefore counted the number of newcomers (bees that had never previously visited any feeder), individually marked and released them at the end of each day, and verified that they were nestmates from the subject colony by observing their return inside the nest. We also conducted control experiments in which we captured all trained foragers and verified that newcomers arrived only when nestmates recruited (results in Nieh et al. in press). We provide recruitment rates (the average number of verified newcomers arriving per hour, as recruited by 20 experienced foragers) for each analysis.

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 (4.5 cm long, 1.7 mm inner diameter) inserted onto the unloading platform (overall system frequency response relatively flat from 50 Hz to 7,000 Hz, see Nieh and Roubik 1998). Foragers did not significantly change their behavior when illuminated by this white light as compared to filtered red light that they could not see (Roscolux medium red filter no. 27, excludes wavelengths <640 nm, see Hertel and Ventura 1985; Chittka et al. 1993).

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. When the transition from unloading to exiting was clearly visible, we also analyzed sounds in two separate phases: unloading and exiting, to facilitate comparisons with *M. panamica* (Nieh and Roubik 1998). The *unloading phase* begins when a forager enters the nest and ends when she stops unloading her collected food. The *exiting phase* begins when she stops unloading her food and ends when she exits the nest.

At all sucrose concentrations and locations, we measured pulse duration, average pulse duration per performance, interpulse duration, and average interpulse duration per performance. A *pulse* is a continuous burst of sound. An *interpulse* is the period between pulses when there is no sound. We focus on the average pulse per performance and the average interpulse per performance because this parameter appears to have the most biological significance (based upon the results of Hrncir et al. 2000). In the sucrose-concentration experiment, we also measured fundamental frequency.

Statistics

We used JMP IN v4.0.4 statistical software to perform linear regression, ANOVA for parametric data, and the Kruskal-Wallis test with a χ^2 approximation for non-parametric data. *Melipona* recruits may average the results of several performances to obtain distance information (Hrncir et al. 2000). If so, then the standard error (the standard variation of a distribution of means) is a more appropriate measure of the signal variance experienced by receivers (Sokal and Rohlf 1981). In the text and table, we therefore present averages as mean±SE, and give the number of measurements (N_m), performances (N_p), and individuals (N_i).

Results

Behavior of recruiting foragers

Recruiting foragers of both species produced similar motions (Fig. 1) and sounds inside the nest. Recruiters entered the nest, contacted nestmates to unload their food (trophallaxis), and then turned and left the nest. Foragers did not display any pronounced spinning behavior.

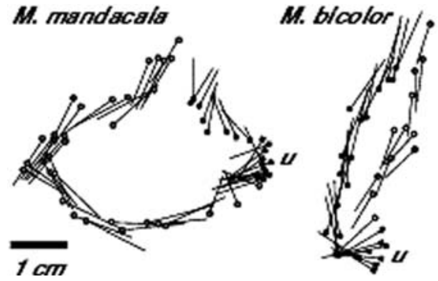

Fig. 1 Typical paths of *Melipona mandacaia* and *M. bicolor* foragers recruiting inside the nest for a 2.5-M sucrose solution 140 m away. *Lines* show the head to abdomen distance. Head indicated by *symbol*. The unloading and exiting phases, respectively, correspond to *filled* and *unfilled symbols*; *filled circles* indicate entry, *filled squares* unloading food (*u*), and *unfilled circles* exiting. Entry and exit positions shown every 0.167 s. Unloading positions shown every 2.5 s because foragers were largely stationary during unloading. Recruitment rates given in Fig. 2 legend

Variation in recruiter paths inside the nest consisted of paths with shorter and greater lengths (±7 cm) and smaller and larger radii (±0.8 cm, measured from the center of the elliptical path). During a recruitment performance, nestmates unloaded the recruiter for extended periods (>2 s), received a food sample (≈1 s), or oriented and held their splayed antennae around the abdominal area of the forager, the zone of highest sound intensity. It has been suggested that honeybees use their antennae to detect airborne sounds (Michelsen et al. 1987; Towne and Kirchner 1989; Michelsen 2003) and stingless bees may do the same. Foragers usually took the shortest route to exit, but could also take a zigzag path, particularly when the nest exit was blocked by other bees. The average performance lasted for 25.0±1.5 s (N_m=N_p=76, N_i=20) in *M. mandacaia* and 21.6±0.5 s (N_m=N_p=118, N_i=20) in *M. bicolor* (ANOVA, $F_{1, 194}$=5.65, P=0.018).

M. mandacaia and *M. bicolor* foragers entered the nest while producing pulsed sounds, with a fundamental frequency of 551±8 Hz (N_m=60, N_p=N_i=20) for *M. mandacaia* and 538±6 Hz (N_m=63, N_p=N_i=20) for *M. bicolor* (NS, ANOVA, $F_{1,121}$=1.9, P=0.168).

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, Fig. 2). Moreover, recruitment behavior for lower sucrose concentrations (0.6–1.5 M) was ineffective at recruiting nestmates to the food source (Fig. 2 legend).

In *M. mandacaia* and *M. bicolor*, the *average pulse* duration per performance decreased significantly with

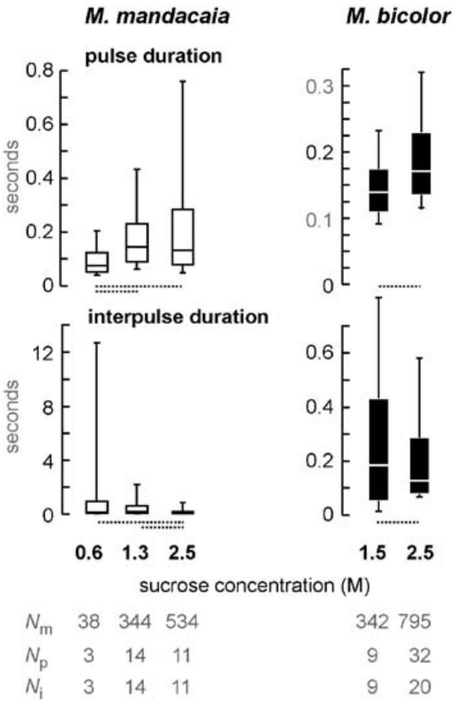

Fig. 2 Effect of sucrose concentration on recruitment sounds in *M. mandacaia* (*unfilled bars*) and *M. bicolor* (*filled bars*). The feeder was 140 m west of each colony. Each box plot shows the 10th, 25th, 50th, 75th, and 90th percentiles of the distribution plotted. *Dashed lines* connect significantly different distributions (Kruskal-Wallis tests, χ^2 approximation, $\chi^2 \geq 16.95$, 1 *df*, $P \leq 0.0001$). Average recruitment rates at 2.5 M were 2 recruits/h (*M. mandacaia*) and 18 recruits/h (*M. bicolor*). In both species, the recruitment rate was zero at all other sucrose concentrations used

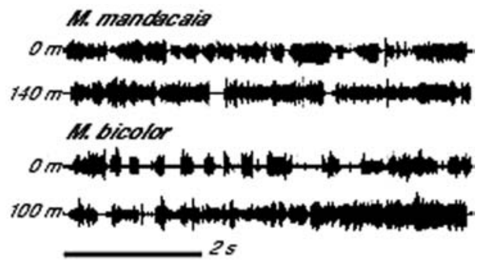

Fig. 3 Oscillograms of recruitment sounds produced by the same individuals recruiting for food sources at different distances from the nest. We show a typical 6-s segment recorded while each recruiter unloaded food. See Fig. 4 for the average recruitment rates at these distances

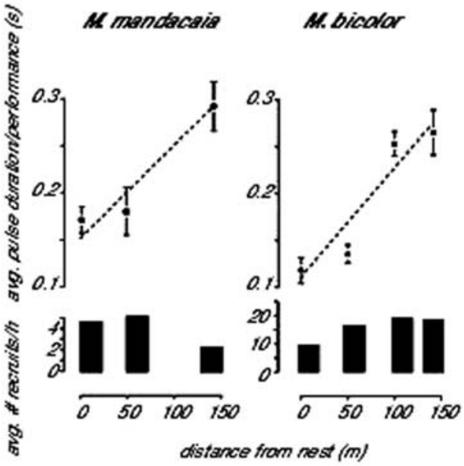

Fig. 4 Relationship between distance to the resource and average pulse duration per performance. Means and SE shown. Linear regression lines shown for the means of the average pulse duration/performance. Sample sizes given in Table 1. The average recruitment rate at each distance is shown

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).

There are significant differences between species (Fig. 2). At 2.5 M and a distance of 140 m from the nest, the *average pulse* duration per performance (ANOVA, $F_{1,42}$=47.1, P<0.0001) and the average interpulse duration (ANOVA, $F_{1,42}$=20.4, P<0.0001) were significantly higher in *M. mandacaia* than in *M. bicolor*.

Effect of distance

Entire performance

In both species, *all pulse* durations during an entire performance significantly increased with increasing distance to the food source (Fig. 3, *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 (Table 1). Foragers successfully recruited nestmates at all tested distances (Fig. 4).

Between species (Table 1), 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 (Table 1). However, there were significant differences between the species: the average interpulse duration per performance for all distances was 396±32 ms (N_m=N_p=59, N_i=20) for *M. mandacaia* and 286±18 ms (N_m=N_p=109, N_i=20) for *M. bicolor* (Kruskal-Wallis test, χ^2=11.5, 1 *df*, P=0.0007).

Table 1 Relationship between recruitment sounds and distance to a 2.5 M food source (SE values are based upon N_p)

	0 m				50 m				100 m				140 m				Regression	
	Mean±SE	N_p	N_m	N_i	Mean±SE	N_p	N_m	N_i	Mean±SE	N_p	N_m	N_i	Mean±SE	N_p	N_m	N_i	R^2	P
M. mandacaia																		
Avg. pulse duration/performance (s)	0.17±0.06	18	1015	18	0.18±0.12	20	1477	19					0.30±0.12	21	840	20	0.29	<0.0001
Avg. interpulse duration/performance (s)	0.39±0.04	18	998	18	0.39±0.06	20	1452	19					0.41±0.06	21	817	20	0.002	0.707
M. bicolor																		
Avg. pulse duration/performance (s)	0.12±0.01	12	526	12	0.14±0.01	38	1996	20	0.25±0.01	27	654	20	0.26±0.02	32	812	20	0.29	<0.0001
Avg. interpulse duration/performance (s)	0.36±0.07	12	513	12	0.18±0.01	38	1950	20	0.38±0.04	27	638	20	0.3±0.03	32	795	20	0.01	0.209

Unloading and exiting phases

The average unloading and exiting *pulse* durations per performance significantly increased in duration with increasing distance to the food source in *M. mandacaia* (unloading pulses: ANOVA, $F_{2,55}$=4.5, P=0.015; exiting pulses: ANOVA, $F_{2,55}$=5.3, P=0.008) and in *M. bicolor* (unloading pulses: ANOVA, $F_{3,32}$=10.7, P<0.0001; exiting pulses: ANOVA, $F_{3,11}$=4.4, P=0.029). There was no significant effect of distance on the average unloading or exiting *interpulse* duration per performance in *M. mandacaia* (unloading pulses: ANOVA, $F_{2,55}$=0.8, P=0.44; exiting pulses: ANOVA, $F_{2,46}$=0.5, P=0.62) or in *M. bicolor* (unloading pulses: ANOVA, $F_{3,32}$=0.8, P=0.49; exiting pulses: ANOVA, $F_{3,11}$=2.8, P=0.09).

Discussion

We studied the effect of food location on recruitment sounds in two species of stingless bees, *M. mandacaia* and *M. panamica*, 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 low quality (0.6–1.5 M) sufficient 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). We discuss the evolutionary significance of these findings.

Recruitment rates

It is relevant to consider the feeder recruitment rates with respect to the size of the available workforce. Recruitment rates varied in both species (maximum=20 recruits/h, minimum=2 recruits/h, Fig. 4). Widely varying recruitment rates are reported in stingless bees (Biesmeijer et al. 1998) and honeybees (von Frisch 1967) because bees prefer natural food sources over artificial feeders and because colonies vary in their motivation to forage depending upon weather and nest conditions (von Frisch 1967; Roubik 1989). In addition, *Melipona* colonies are relatively small (*M. mandacaia*: 300–400 workers; *M. bicolor*: 800–1,100 workers), not all workers are foragers, and not all foragers are interested in collecting sugar solution because pollen is also a vital resource (Roubik

1989). Natural food sources were seasonally limited, but remained available during our field seasons. Thus recruitment to our feeder represented an allocation from a limited labor pool that was also exploiting preferred natural food sources. Moreover, we excluded all bees that had ever visited any feeder at any time from the pool of potential recruits. Such a rigorous definition is necessary because experienced foragers may search for feeders on their own, instead of acting on communicated information (Biesmeijer and de Vries 2001). Over time, our experiments therefore used up a sizeable fraction of potential recruits (32–45% of *M. mandacaia* and 16–22% of *M. bicolor* workers; Nieh et al. in press). Thus our observed recruitment rates correspond to the mobilization of a substantial fraction of available foragers within each colony.

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* (Hrncir 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, 1987, 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 (Fig. 4, Table 1). Thus pulse duration may reliably encode food distance if potential recruits distinguish between performances for high- and poor-quality food sources (as shown in the recruitment rates, Fig. 2 legend).

In both species, foragers recruited nestmates to all tested distances (Fig. 4) 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, Table 1). 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 (Fig. 2).

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. in press), interspecific differences in the ability to encode and communicate distance may exist. Nonetheless, it is useful to consider whether methodological differences may have led to different results.

First, it is important to determine whether the measured recruitment sounds were associated with successful recruitment. Hrncir et al. (2000) studied correlations between the behavior of potential recruits inside the nest and their eventual success at reaching the feeder, but it is unclear if they conducted these experiments on the same days as their study on the relationship between food distance and recruitment sounds. The motivation to recruit for artificial food sources can change dramatically from day to day depending upon the availability of natural food sources (Jarau et al. 2000; Nieh et al. in press). Thus it would be good to clarify whether *M. scutellaris* and *M. quadrifasciata* recruited newcomers while nest sounds were being recorded. Second, the smaller sample sizes used by Hrncir et al. (2000) may be insufficient to detect the effect of distance (averages for all distances: *M. scutellaris*, $N_{\bar{p}}$=3, $N_{\bar{m}}$=81; *M. quadrifasciata*, $N_{\bar{p}}$=7, $N_{\bar{m}}$=273). In contrast, larger average sample sizes were used by Esch (*M. quadrifasciata*, $N_{\bar{p}}$=16, $N_{\bar{m}}$=1313; *M. merillae* $N_{\bar{p}}$=12, $N_{\bar{m}}$=446, Esch 1967), Nieh and Roubik (*M. panamica*, $N_{\bar{p}}$=28, $N_{\bar{m}}$=1107, Nieh and Roubik 1998), and our study (*M. mandacaia*, $N_{\bar{p}}$=20, $N_{\bar{m}}$=1111; *M. bicolor*, $N_{\bar{p}}$=27, $N_{\bar{m}}$=997).

What is a biologically significant sample size? Hrncir et al. (2000) reported that successfully recruited foragers made their first contact with a recruiter approximately

60 min before appearing at the food source, with the most intense contact in the last 20 min before appearing at the food source. Their study does not give the rate of forager performances inside the nest, but at similar distances, we observed one recruitment performance inside the nest each 2.2 min for *M. bicolor* and 3.2 min for *M. mandacaia* (Nieh et al. in press). Using one performance each 5 min as a conservative estimate and calculating for the three foragers used in their study, each recruit could have experienced up to 36 recruitment performances in the hour and 12 performances in the final 20 min before successfully finding the feeder.

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 *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, 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, 2001; Dornhaus and Cameron 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.

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