

## Contact calls are used for individual mate recognition in free-ranging green-rumped parrotlets, *Forpus passerinus*

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Contact calls function to coordinate movements in a wide variety of social animals. Where population density is high, visibility is low and repeated interactions occur between known social companions, calls are often individually recognizable. Parrots are highly social and make substantial daily movements, which appear to be mediated through contact calling. However, there is little experimental evidence for how free-ranging parrots recognize social companions. We quantified intra- and interindividual variation of contact calls in a banded population of green-rumped parrotlets in Venezuela. Recordings of a sample of males were made on replicate days as they announced their return to incubating mates. Spectrographic structure of these contact calls showed significantly more variation between than within individuals, and calls could be correctly classified to individuals more often than was expected by chance. Males varied across multiple dimensions simultaneously, including duration, frequency and frequency modulation of contact calls. Playback experiments showed that free-ranging female parrotlets responded significantly more often to their mates' calls than to calls of males of other nests. Mate recognition via contact calls may be selectively advantageous during incubation and brooding if this reduces the potential costs associated with confusing contact calls of mates with those of nonmates, including infanticidal conspecifics.

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Contact calls function to coordinate activities between two or more individuals in many social animals (Kondo & Watanabe 2009). Specific functions of contact calls include maintaining the cohesiveness of a group during local or long-distance relocation (dolphins: Janik & Slater 1998; primates: Snowdon & Cleveland 1980; birds: Farnsworth & Lovette 2005), negotiating fission–fusion of multiple groups during foraging and roosting (bats: Willis & Brigham 2004; dolphins: Connor et al. 2000; primates: Symington 1990; parrots: Balsby & Bradbury 2009) and coordinating activities between parents and their dependent offspring (seals: Charrier et al. 2003; manatees: Sousa-Lima et al. 2002; dolphins: McCowan & Reiss 1995; bats: McCracken 1984; swallows: Beecher et al. 1986; penguins: Jouventin & Aubin 2002). While there are probably additional contexts (Marler 2004a), it is clear that contact calls function to coordinate movements

of individuals at multiple levels of social organization and life history stages, across different spatial scales, and in a wide diversity of vertebrate taxa.

Where contact calls are used to reunite social companions, one of the primary functions is to signal the identity of the caller. While the ability to identify individual conspecifics is widespread in vertebrates, there have been fewer studies that demonstrate benefits to either signaller or receiver (Tibbetts & Dale 2007). In highly social species, dense aggregations and adult philopatry may select for networks of social relationships that could favour signalling individual uniqueness via contact calls (Connor et al. 1998; Bradbury 2003; Marler 2004b; Kondo & Watanabe 2009). Individual recognition might be selectively advantageous if this allows participants to penalize cheaters (Reeve 1989), establish a beneficial reputation within foraging and roosting groups (Nowak & Sigmund 1998), or assemble regular partners during foraging and roosting.

Signature information seems particularly important when individuals need to identify each other in crowded, noisy or dangerous reproductive contexts. Classic examples are the reuniting of pairs and offspring in nesting seabird colonies (reviewed in: Beer 1970; Falls 1982) and colonial nesting swallow species (summarized in Medvin et al. 1993). To function as identification tags, variation in an individual's calls must consistently exceed

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some baseline level relative to that of the total variation by which participants might be confused (Beecher 1989; Bradbury & Vehrencamp 1998). Furthermore, the departure from baseline variation has to exceed the acceptance thresholds of auditory mechanisms of receivers in order to be memorized and acted upon in future interactions (Sherman et al. 1997). While auditory acuity is required for recognition, the motivational state of the receiver can be expected to ultimately affect response rates to recognition cues (Searcy & Yasukawa 1996; Stoddard 1996).

We studied vocal recognition between mated pairs in a well-known population of green-rumped parrotlets (*Forpus passerinus*) in Venezuela. Individual recognition based on contact calls has been demonstrated in captive colonies of the congener, spectacled parrotlet, *F. conspicillatus* (Wanker et al. 1998; Wanker & Fischer 2001), and the domesticated budgerigar, *Melopsittacus undulatus* (Brown et al. 1988), but there is limited evidence in free-ranging parrots (Saunders 1983; Vehrencamp et al. 2003; Buhrman-Deever et al. 2008). There are many potential benefits to parrotlets of having individualized calls: they are highly philopatric as adults (Sandercock et al. 2000; Veran & Beissinger 2009), and being the smallest New World parrot (25 g; Juniper & Parr 1998), they occur in such densities that as many as 10 pairs may breed concurrently within earshot of one another (Beissinger 2008; Bonebrake & Beissinger 2010). They are socially monogamous, have pair bonds that last throughout the year and from year to year, and coordinate biparental care (Waltman & Beissinger 1992; Beissinger 2008). During incubation, males leave to forage and announce their return with contact calls, to which female mates respond by ascending to the nest cavity entrance (Waltman & Beissinger 1992). During mate absences, females rarely ascend to the cavity entrance, despite the ubiquity of contact calls given by many other males in the vicinity of nests (K. S. Berg, unpublished data). Because the male–female pair is the principal social unit (Beissinger 2008) and females in their nest cavity cannot use visual cues for individual recognition, it is an ideal context in which to experimentally test for individual mate recognition based on contact calls. We first characterized female responses to male arrivals in free-ranging breeding pairs. We then quantified individual variability in spectrographic parameters of contact calls of males recorded during the same visits to incubating females. Finally, we provide experimental evidence of recognition through playbacks to a subset of unrestrained incubating females.

## METHODS

### Study Site

Research was conducted at Hato Masaguaral (8°31'N, 67°35'W), Guarico, Venezuela, a research station in the Llanos. Breeding and demography of parrotlets have been studied here since 1988–1989 using 106 Polyvinyl Chloride (PVC) nestboxes separated by 10–25 m and situated along 3 km of disjoint fence lines that traverse seasonally flooded savannas and gallery forest (Beissinger & Waltman 1991; Bonebrake & Beissinger 2010). Parrotlets were captured with mist nets and fitted with unique, permanent, aluminium colour-band combinations that were readable with a spotting scope. Breeding extends from late May to early December. Only females incubate, beginning with the first egg and continuing for 18–28 days (median clutch size = 7 eggs; Beissinger & Waltman 1991).

### Pair Behaviour during Incubation

We surveyed pair behaviour during incubation on multiple days at 25 active nest attempts comprising 50 colour-banded individuals. Nests were distributed amongst three populations and took place in one of three breeding seasons: 1 June–15 July 2006,

1 June–30 November 2007, and 2 June–6 November 2008 (Supplementary Material, Appendices S1, S2). We quantified the percentage of times that females ascended and or vocalized after the male arrived to compare to results of playback experiments. Because copulations take place outside the nest and feedings take place inside the nest, we noted whether the pair was eventually reunited inside or outside the nest.

### Recording and Analysing Contact Calls

From this sample of nests, contact calls of 18 individual males were recorded during incubation on multiple days at active nests in the two populations and from the 3 years. All recordings analysed were taken from each male during a narrow temporal window (mean = 8.6 days, range 23 days,  $N = 18$  males) and behavioural context (i.e. arrival prior to incubation feeding). Recordings of individuals were made with a directional microphone (MKH816 and MKH70, Sennheiser, Wedemark, Germany) and digital recorder (PMD670 Marantz, Longford, Middlesex, U.K.) with 16 bit/44.1 kHz sampling rate and recordings saved as wave files. The microphone was enclosed in a blimp wind-screen with the entire structure mounted on top of the spotting scope to identify colour-band combinations of vocalizing individuals from a distance of 25–30 m from the nest.

Wave files were saved to a computer (Satellite A105, Toshiba, China) and working copies stored on DVD for spectrographic analysis and use in playback experiments. Spectrograms were produced with Syrinx v2.5 ([www.syrinxpc.com](http://www.syrinxpc.com)) using a Hann window and 256 fast Fourier transform (FFT) sample size. Pilot recordings indicated that the contact calls used by familiar individuals when they are visually separated (e.g. when a parent searches for fledglings or when adults are separated during banding) are typically loud and have one or two elements to each call. Based on these observations, we restricted analyses to one- and two-element contact calls, which facilitated a balanced design in playback experiments because these contact call types were the most common (78%,  $N = 1012$  calls by 18 males). Green-rumped parrotlets have large, albeit poorly known, vocal repertoires and could have individual information encoded in additional call types. A more exhaustive treatment of call repertoires is given elsewhere (K. S. Berg, unpublished data).

We measured spectrograms of calls using two methods. First, latent measures were extracted using spectrographic cross-correlation and principal coordinate analysis (SPCC–PCO; SoundXT v2.0) routines in Matlab (for details and examples see Cortopassi & Bradbury 2000, 2006). SPCC has the advantage of comparing all attributes of spectrographic structure (Clark et al. 1987). From the square correlation matrix, SPCC–PCO generates an orthogonal set of PCO axes, which become latent variables describing the distribution and satisfying independence assumptions inherent in multivariate statistical comparisons. However, by itself, the method cannot identify which specific spectral attributes vary most across individuals; thus, we compared PCO axes to spectral attributes of call structure from the same set of calls with a series of energy distribution measurements (EDM) and batch-extracted the calls with code developed by Kathryn Cortopassi (<http://www.birds.cornell.edu/brp/research/algorithm>) using the platform XBAT (<http://xbat.org>) in Matlab (see Supplementary Material, Appendix S3, Fig. S1; also see Ellis 2008). EDM estimates signal parameters based on ordered frequency or temporal bins that together contain 95% of the aggregate energy and are thus robust to extreme values in both frequency and temporal domains. Elements were measured separately for two-element calls. In both methods, spectrograms were made with a Hann window and a 256 FFT, with 50% overlap. Files were band-pass filtered between 3.8 and 7.5 kHz, which encapsulated the fundamental frequency while avoiding other

animal sounds above and below that window. We conducted these analyses on all calls from 18 males for which we had at least five calls on each of 2 days (mean number of calls per date = 19, range 45). Individuals were pooled across years and populations, but because the within-year and the within-population samples may better reflect the discrimination task confronted by typical females, we also report results of statistical tests of discriminability of calls of 10 males from the same population recorded during the same 6-week period in 2006. The proximity of nestboxes and frequent interactions among breeding and nonbreeding birds (Beissinger 2008) provided females with opportunities to become familiar with many of the breeding males included in this sample.

#### *Playback Call Selection and Trials*

Playbacks of male calls to female mates were conducted in a partially overlapping set of 18 breeding pairs during 2006–2008. Playbacks were conducted on average 6.4 days after recordings of focal males were made ( $N = 18$  nests). We first selected one high-quality common example of one- and two-element call types from each individual for use in the playback experiment. Calls were later identified in PCO plots and compared to their centroids. A Mantel test comparing Mahalanobis distances between calls used in playback and all calls for that male indicated that the two were positively correlated ( $r = 0.17$ ) and that calls used in playback were thus representative of each male's larger sample. Digital copies of calls were band-pass filtered using Syrinx between 1.8 and 20 kHz, and extraneous sounds that did not overlap the parrotlet calls were removed with the filter cursor tools. Filtered copies' amplitudes were then standardized ('Maximize' command in Syrinx), which avoids clipping by amplifying the signal until the loudest part is just at 100% of the power permitted by the 16-bit depth. To simulate the natural delivery of contact calls when males arrive, we created two templates based on typical bouts of calling. We used this template to create two audio files for playback at each nest. Each file contained seven calls (one- and two-element originals and five copies), which lasted on average  $6.4 \pm 1.2$  s and were separated by 5 s of silence (i.e. A: 1211121; B: 2221121; A: 1211121). Because the two templates differed in their distribution of one- and two-element call types, we alternated whether the sequence started with A or B templates at each nest, but we had no a priori reason to suspect either call was more or less implicated in providing individual recognition cues. Thus a maximum of 21 calls, with at least one of the templates repeated (see below), were broadcast at each trial, which lasted on average  $35 \pm 3.2$  s ( $N = 18$  nests).

To control for female motivational levels, experiments were conducted once the female had ceased laying and was thus not fertile. The end of the fertile period was determined by two consecutive nest checks at 3-day intervals without discovering a new egg (Beissinger & Waltman 1991). Sound files were replayed from a laptop computer (Satellite A105, Toshiba, China), using Syrinx and a Harman/Kardon CA212 (Stamford, CT, U.S.A.) power amplifier positioned 30–40 m from the nest. Calls were broadcast from a camouflaged directional, Control 1Xtreme speaker (JBL, Stamford, CT) positioned at the typical height (mean = 1.5 m), direction and distance (mean = 4.4 m) of the arriving male. Sample broadcast calls were re-recorded along with live birds and wave forms compared; amplitudes were adjusted to natural amplitudes when the volume on the laptop was set to approximately 75% of maximum, which was then used as the standard in playback experiments.

Once the equipment was in place we monitored the nest until the male arrived and entered the box or perched with the female nearby. Because males typically visit the nest once per hour to feed their mates (Waltman & Beissinger 1992), we scheduled playback trials 30–40 min after the male had left to simulate the male's

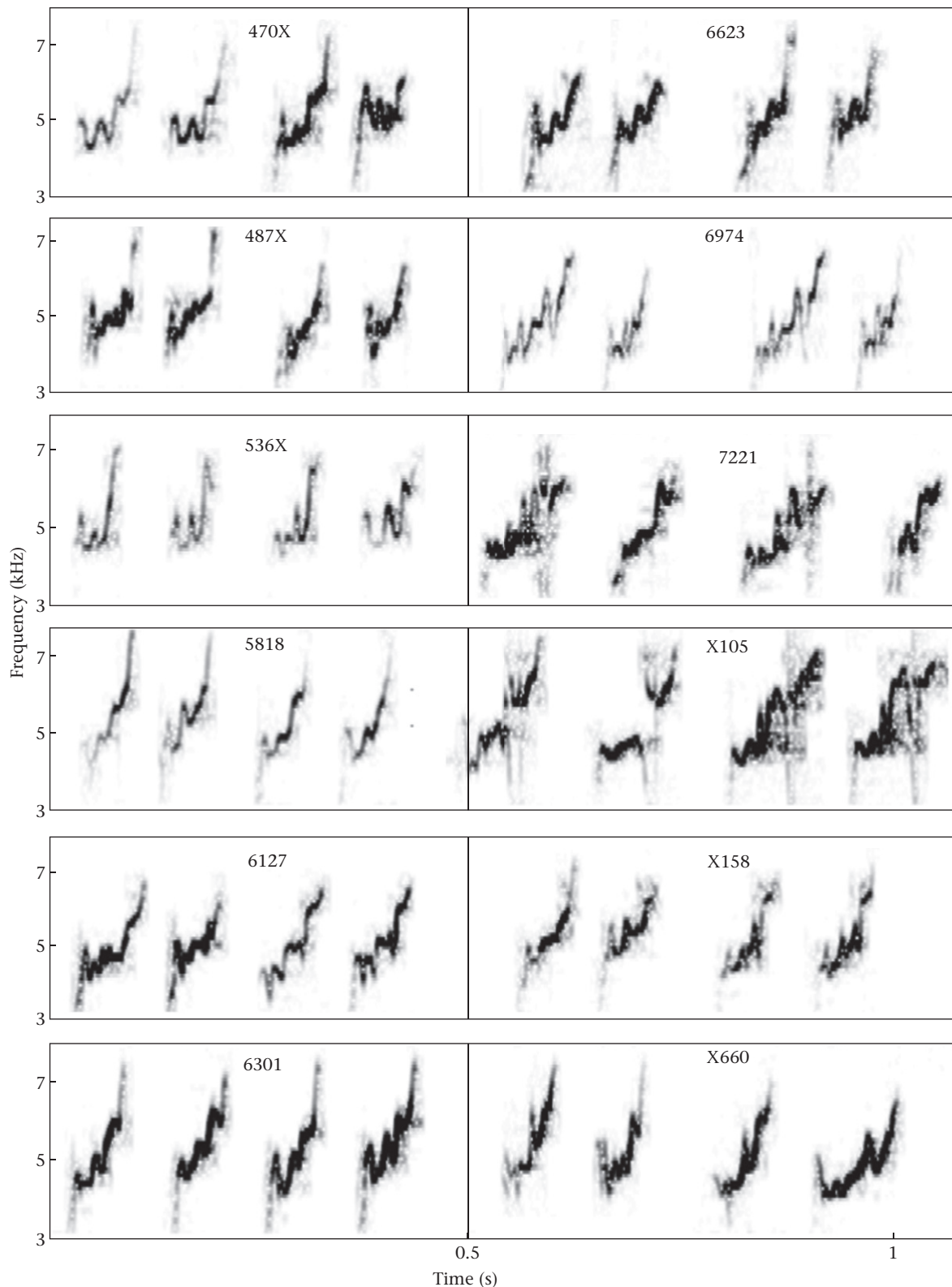
absence and to again approximate the female's normal motivational state, while avoiding the male's arrival during playback. We postponed playback if the male arrived earlier or if other individuals were in the vicinity. The directional microphone and digital recorder (see [Recording and Analysing Contact Calls](#)) helped monitor the quality of playback calls and female vocal activity for 5 min beginning with the first calls broadcasted. We visually observed the nest for an additional 5 min to determine any post-playback response from the female. Positive responses were based on whether the female ascended to the cavity entrance, vocalized, or both. A negative response was determined by lack of visual and/or acoustic evidence of a response.

We conducted one playback trial to each incubating female using their mate's calls and one control to each female, using the same file used to simulate actual mates at other nests. Distances between focal nests and the nests of males used in controls, were on average, out of earshot of incubating females ( $248 \pm 122$  m, range 432 m). Sample calls of each male were used in both control and experimental trials only once. Position, direction and distance to the speaker were the same for controls and experiments at each nest. Playback trials consisted of playing three call-sequence bouts. Playback was discontinued if a response was noted. Experimental and control trials at each nest were separated by an average of 1.4 days (maximum = 5 days), and we alternated the order of control and experimental playback trials. All control playbacks were from recordings of males made in that same year and population as the focal nest attempt (Supplementary Material, [Appendix S1](#)).

#### *Statistical Analysis*

All statistical analyses were conducted with SAS v9.1.3 (SAS, Cary, NC, U.S.A.). To test for associations between rates of female responsiveness to male arrivals and whether the male subsequently entered the nest, we used generalized linear mixed models (GLMM, Glimmix Procedure) with female response (yes or no) as fixed effects and the nesting pair included as a random effect. Response variables were categorical with two levels (male enters nest = yes or no) so we modelled the data using a binary distribution linked to a logit function.

Analysis was conducted on high-quality recordings of calls from 18 males for which we had recordings on replicate dates. Cross-correlation coefficients ranged between 7% and 88%. Eigenvalues of the first 11 PCO axes were all greater than one and together explained 94% of the variation in cross-correlation values. All PCOs and spectral measurements were normally distributed with the exception of the average absolute derivative, the cumulative absolute derivative and the number of inflexions, all based on the centre frequency contour (hereafter CFC). Both derivative estimates were normalized by log transformation. Mean  $PCO_1$  of individuals was significantly and positively correlated with centre frequency ( $R = 0.63$ ,  $P < 0.001$ ). Mean  $PCO_4$  was positively correlated with the average derivative of the CFC ( $R = 0.24$ ,  $P < 0.001$ ), and with inter-percentile range (IPR) duration ( $R = 0.53$ ,  $P < 0.001$ ); the rest of the PCOs and spectral measurements were weakly or nonsignificantly correlated. We used a forward selection process (Stepdisc Procedure) to estimate initial suitability of nine measurements (Supplementary Material, [Appendix S3](#)) and 11 PCO axes for inclusion in the initial models below. All nine measurements were entered at  $\alpha = 0.001$ . However, second percentile frequency, first percentile frequency and IPR bandwidth added little to the model and were highly correlated with each other or with centre frequency (all  $R > 0.80$ ,  $P < 0.001$ ). To avoid biased parameter estimates we removed second percentile frequency, first percentile frequency and IPR bandwidth from the final model. Log (average absolute derivative of the CFC) was also correlated with log(cumulative absolute



**Figure 1.** Two examples of two-element contact calls from each of 12 male green-rumped parrotlets. Calls were given when males arrived to feed incubating females. Individual IDs are given above each set of calls. Males 470X and 536X bred repeatedly within 100 m of each other over several years; males 5818, 6127 and 6301 bred at least once within 100 m of each other; males 7221 and X105 belonged to different populations from each other and from the other males. Spectrograms were made with a Hann window and 256 sample size.

derivative of the CFC) ( $R = 0.50$ ,  $P < 0.001$ ), but these parameters were conceptually closely related and the average explained less variation in individuals and was also omitted from the final model. To make the two models more comparable, the five PCO axes (3, 2, 6,

7 and 8) with the most predictive power were entered into the model at  $\alpha = 0.001$ .

Testing for individual repeatability in vocal signals is usually conducted using MANOVAs with individual as a predictor of

multiple spectrographic attributes that vary continuously (e.g. duration, bandwidth, etc.). In addition, the mathematically similar discriminant function analysis allows for calculation of the percentage of calls or songs correctly classified to individuals, based on linear combinations of the same continuous variables used in the MANOVA (reviewed in: Falls 1982; Beecher 1989). Such methods for assessing individual variation in vocal signals may not include sufficient replication across dates (Ellis 2008; Wilson & Mennill 2010). However, if replicate recording sessions are used, pseudoreplication may result (Mundry & Sommer 2007). To address both of these concerns we tested for individual differences in call structure by running two separate GLMMs. Both models included individual as a multinomial response variable with one level per individual and linked to a cumulative logit function. In this case, SAS calculates an ordered multinomial test, but reversing the order produced identical results. One model included structural measurements as predictor variables while the other model included PCO axes as predictor variables. Because our main objective was to estimate plausible levels of individual uniqueness while accounting for short-term temporal variation, we modelled the GLMM with spectrographic variables as main (fixed) effects and included recording session as a random effect. Where an overall model was significant, we used MANOVA to identify which variables differed most among individuals and we used discriminant function analysis (DFA), with cross-validation, to determine the extent to which calls could be correctly classified to individuals.

The portion of females that responded positively to playback of mate's versus mates from other nests was analysed with a one-tailed Fisher's exact test with one degree of freedom. Speaker height, distance and period of day, and order of playbacks conducted (mates versus nonmates) were also compared to response rates with a Fisher's exact test. Means are provided  $\pm 1$  SD and statistical significance was accepted at  $\alpha = 0.05$ .

## RESULTS

### Contact Call Behaviour

We observed 25 males visiting incubating mates on multiple dates during the same nest attempt. Males made on average one trip per hour to the nest ( $\pm 0.05$  trips,  $N = 55$  trips by 16 males) and gave contact calls on 61.0% of visits ( $N = 157$  visits, 25 males). Females responded to male contact calls by either ascending to the box opening, vocalizing, or both on 60.2% of mates' visits ( $N = 153$  visits, 24 males). Controlling for individual pairs, males were 86% more likely to enter the nest cavity upon arrival if the female did not ascend to the box opening (GLMM,  $F_{1,124} = 55.91$ ,  $P < 0.001$ ). On 63.4% of the visits, males flew off again (either with or without the female), while on 36.6% of the cases males joined the female inside the box after arrival ( $N = 157$  visits, 25 males).

### Structural Basis of Contact Calls and Individual Spectrographic Variability

Contact calls were made up of short (mean =  $77 \pm 12$  ms), frequency-modulated (FM) elements with most energy concentrated between 4.3 and 6.2 kHz, and the centre frequency at 5.0 kHz (Fig. 1, Table 1). All individual elements increased in frequency along a bandwidth averaging  $1.9 \pm 0.45$  kHz, but they also contained from zero to nine finer scale frequency inflections along the course of each element (Table 1). At lower frequencies these inflections were coupled with amplitude spikes (AM), whereas the pattern was reversed at higher frequencies (results not shown), a pattern also found in songbirds (Bradbury & Vehrencamp 1998). In most cases, the second harmonic was concentrated near 10 kHz.

**Table 1**

Summary of energy distribution measurements of contact calls in 18 male green-rumped parrotlets ( $N = 696$  call elements)

Variable	Mean	SD	Minimum	Maximum
Absolute duration (s)	0.077	0.012	0.054	0.103
IPR duration (s)	0.055	0.009	0.038	0.075
P1 frequency (kHz)	4.312	0.199	3.900	4.742
P2 frequency (kHz)	6.218	0.444	5.379	7.077
Centre frequency (kHz)	4.966	0.258	4.479	5.536
IPR bandwidth (kHz)	1.906	0.453	1.096	2.838
CFC average derivative (kHz/s)	24.078	11.572	-2.075	51.016
CFC cumulative absolute derivative (kHz/s)	7074	3356	2566	16 054
CFC inflections (count)	1.48	1.35	0.06	5.06

Estimates are based on means of each individual.

Using the mean of each male, IPR bandwidth was correlated with the second percentile frequency (hereafter P2 frequency;  $R = 0.95$ ,  $P < 0.001$ ). The average derivative of the CFC was correlated with the first percentile frequency (P1 frequency;  $R = 0.48$ ,  $P < 0.001$ ) and with centre frequency ( $R = 0.53$ ,  $P < 0.02$ ). IPR duration varied directly with log(cumulative absolute derivative of the CFC) ( $R = 0.62$ ,  $P < 0.005$ ).

There was a significant main effect for five PCO variables predicting calls to individuals in 18 males (GLMM: all  $P_s < 0.01$ ) and the random effect of date was small and not significant (mean  $\pm$  SE =  $0.05 \pm 0.08$ ). Similarly, the main effects of IPR duration, centre frequency, the average derivative of the CFC and the log (cumulative absolute derivative of CFC) were all significant predictors of individuals (GLMM: all  $P_s < 0.01$ ); the random effect of date was again not significant (mean  $\pm$  SE =  $0.02 \pm 0.05$ ). Based on these results we pooled observations across dates for use in MANOVA and discriminant function analysis. Individual identity was a significant predictor of five SPCC-PCO axes (MANOVA: Wilks'  $\lambda = 0.12$ ,  $F_{85,3263} = 15.1$ ,  $P < 0.001$ ; Table 2, Fig. 2). Individuals also differed in four spectral measurements (MANOVA: Wilks'  $\lambda = 0.19$ ,  $F_{68,2651} = 20.9$ ,  $P < 0.001$ ; Table 2). Discriminant function analysis, with cross-validation, used PCO values to correctly classify individuals 39% of the time, and measurements did so 34% of the time, both of which were higher than expected by chance (6%). When we restricted the analysis to 10 males all recorded in the same population in 2006, DFA, with cross-validation, correctly classified PCOs to individuals 55% of the time (Wilks'  $\lambda = 0.10$ ,  $F_{45,1908} = 28.08$ ,  $P < 0.001$ ) compared to 10% random correct classification; measurements were classified correctly 29% of the time (Wilks'  $\lambda = 0.40$ ,  $F_{36,1602} = 12.27$ ,  $P < 0.001$ ).

### Playback Experiments

We conducted playback experiments to 18 incubating females. In trials using the mates' calls, seven females ascended to the entrance and an eighth female responded vocally in rapid, interactive fashion to 8 of 14 of her mate's pre-recorded calls, but she did not ascend to the box entrance. Females responded on two occasions to controls by ascending to the entrance; no vocal responses to controls were recorded. Pooling response types, females called or ascended more often to calls of mates (44.4%) than to calls of mated males from other nests (11.1%) and the difference was significant (Fisher's exact test:  $P < 0.03$ ; Fig. 3). The side of nest on which the speaker was positioned, distance, height and time of playback were unrelated to females' tendency to respond (Fisher's exact test: all  $P_s > 0.21$ ). Females were more likely to respond to their mate's calls if the mate's calls were before the control calls (Fisher's exact test:  $P < 0.002$ ). Females ascended 57% of the time when their mate's call was broadcast first, but they did not ascend to any of the controls that were played first.

**Table 2**

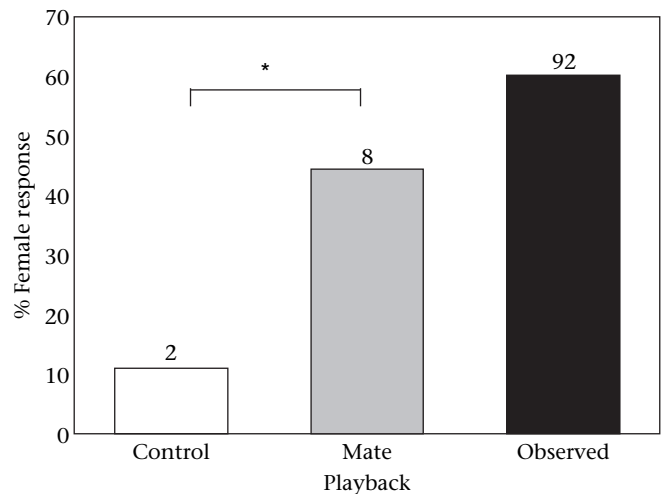
MANOVAs of energy distribution measurements and spectrographic cross-correlation and principal coordinates used to predict individual differences in contact calls of 18 male green-rumped parrotlets ( $N = 696$  elements)

	<i>df</i>	Wilks' $\lambda$	Partial $r^2$	<i>F</i>	<i>P</i>
<b>Measurements</b>					
<i>Model</i>	68	0.19		20.9	0.001
Log (cumulative absolute derivative)			0.60	60.7	0.001
IPR duration			0.35	21.8	0.001
Centre frequency			0.23	11.8	0.001
Average derivative			0.06	2.5	0.001
<b>PCOs</b>					
<i>Model</i>	85	0.12		15.1	0.001
PCO <sub>3</sub>			0.48	36.3	0.001
PCO <sub>2</sub>			0.45	32.5	0.001
PCO <sub>6</sub>			0.25	12.8	0.001
PCO <sub>8</sub>			0.27	14.9	0.001
PCO <sub>5</sub>			0.21	11.0	0.001

In each case the global model is presented first followed by partial regression coefficients for each variable used in the main models.

## DISCUSSION

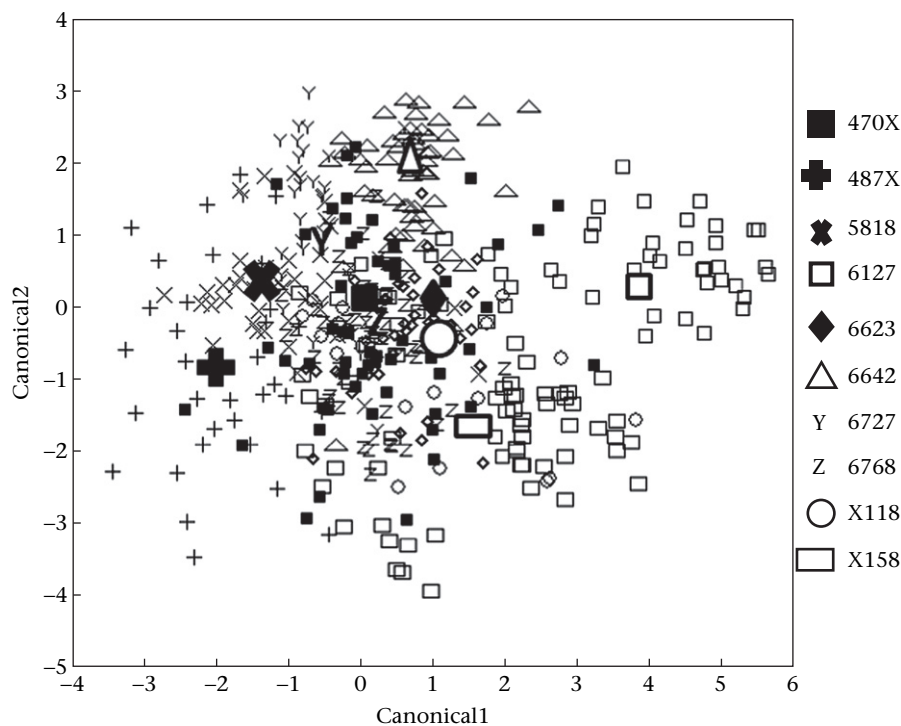
We found systematic differences in acoustic features of two contact calls commonly used by free-ranging male green-rumped parrotlets when visually separated from their mates and that incubating females responded preferentially towards playbacks of their mate's contact calls versus contact calls of nonmates in the same context and in the absence of visual cues. This is the first field study to demonstrate both requirements for individual mate recognition in free-ranging parrots with statistically significant samples. A similar approach was used by Saunders (1983) on nesting cockatoos, but on a small number of individuals. Buhman-Deever et al. (2008) also found evidence for individual mate recognition in brown-throated conures, *Aratinga pertinax*, captured



**Figure 3.** Percentage of positive responses by 18 female green-rumped parrotlets to control playback ( $N = 18$  trials) and to mate's playback ( $N = 18$  trials). Observed female response to mate contact calls in nature is also provided ( $N = 153$  visits by 24 males). \* $P < 0.03$  (Fisher's exact test).

in the wild and tested during short periods in captivity. Vehrencamp et al. (2003) showed that individual orange-fronted parakeets, *A. canicularis*, in wild foraging flocks responded primarily to playbacks of contact calls recorded from nearby conspecifics. This study adds to the list of empirical evidence for individual mate recognition in a marked population of one of the world's smallest parrots.

Green-rumped parrotlets produce high-frequency contact calls of short duration when they are visually separated from their mates. Avian hearing is usually significantly impaired above 12 kHz (Dooling et al. 2000), so it is likely that only the fundamental



**Figure 2.** Canonical variates 1 and 2 from discriminant function analysis of five principal coordinate axes from spectrographic cross-correlation of call elements ( $N = 440$  elements) from 10 male green-rumped parrotlets recorded in the same population in 2006. Each symbol represents one element with different individuals distinguished by unique symbols; larger font symbols indicate centroids of individuals in canonical PCO space.

frequency (4.3–6.2 kHz) and to a lesser extent the second harmonic (8.6–12.4 kHz) of these contact calls are detectable at relevant distances. Calls of similarly sized spectacled parrotlets also have the most energy concentrated near 5 kHz ( $5.1 \pm 0.3$  kHz; Wanker & Fischer 2001; versus  $5.0 \pm 0.3$  kHz for green-rumped parrotlets). However, contact calls of green-rumped parrotlets in our study were of shorter duration on average than those of spectacled parrotlets ( $77 \pm 12$  ms versus  $97 \pm 9$  ms).

Female green-rumped parrotlets discriminated between playbacks of their own mate's calls and those of other mated males recorded in the same context. Budgerigars can discriminate between the contact calls of up to 30 conspecifics (Dooling 1986). Similarly sized green-rumped parrotlets may also have resolution enabling individual vocal recognition based on fine-scale variations in contact calls. The response rate to playbacks (44%) was similar to that seen in playbacks to other wild parrots (Vehrencamp et al. 2003; Balsby & Scarl 2008; Balsby & Bradbury 2009). However, in this case we have a comparison value since females responded to actual calling by their mates 60% of the time. Similarly, Saunders (1983) reported a 70% response by female cockatoos to playbacks of their mates' calls, but a 100% response to live males' calls. The somewhat higher rates of cockatoos' response in both contexts are not surprising: these birds are about 30 times larger (766 g) than a parrotlet (25 g), calls are of a longer duration with lower frequencies and, as a result, probably transmit individual information at greater distances. Males of this species also take about six times as long as green-rumped parrotlets to return to feed incubating females (Saunders 1983; Waltman & Beissinger 1992; this study), thus female's motivation to respond may also be greater in cockatoos. Lower return rates by male cockatoos may also signify lower costs to females if this decreases conspicuousness to predators (see Martin & Ghilambor 1999 for an example in passerine birds). Despite the quantitative differences in response rates, both studies suggest that factors other than those manipulated influence female call discrimination. One possibility is that call amplitude changes as the male approaches, or, at least in green-rumped parrotlets, the order or diversity of call types varies with individuals, either of which could provide additional clues to the female that her mate has arrived. Since we used standardized amplitudes and standardized combinatorial sequences in playback, these potential sources of individual variation were eliminated.

Recognition systems rarely need to be perfect (Beecher 1989; Sherman et al. 1997). The low response rates of female parrotlets to playbacks (44%) and to their mate's natural calling (60%) might reflect a considerable amount of error in this system. In addition to being short in duration and high in frequency, calls are given in noisy, hot, turbulent environments, all of which may make the fine structure of calls transmit less effectively. Male green-rumped parrotlets disperse less than females, and more than 40% of nestling males recruit locally (Sandercock et al. 2000; Veran & Beissinger 2009). Thus, our sample of playback calls included males hatched in the same population (Supplementary Material, Appendix S1), so individuals' calls might show convergence through genetic and cultural mechanisms. Discriminant function analysis classified calls correctly to individuals 55% of the time (based on SPCC–PCOs from 2006), which is close to female responses under natural conditions (60%). Infanticide is not uncommon in this species, and green-rumped parrotlets must, in addition, elude a suite of larger predators (Stoleson & Beissinger 2001; Bonebrake & Beissinger 2010). Because of their small size and their preference for deep cavity nest sites, incubating females are often unable to escape predation events. It may thus pay for females to err more often by not responding to mate's calls (false negative) than to respond to the wrong individual (false positive) and reveal the location of an active nest to nearby predators.

We tested for individual mate recognition, but calls are noted for having multiple functions (Marler 2004b), which might help explain the somewhat low female response levels to live males and to playback experiments. When females ascended to their nestbox entrance upon their mates' arrival, males were less likely to subsequently enter the box. Because copulations take place outside the cavity (Waltman & Beissinger 1992), males may produce contact calls, at least during the fertile period, to solicit the female to leave the nest cavity, in order to facilitate copulations. Thus, the low response rates in nature (60%) and high true-negative rates to playback (56%) may suggest that no response is necessary if the female is unwilling to copulate or wants to be fed. Lack of a response by the female usually resulted in the male entering the nest cavity, where feedings during incubation typically take place (Curlee & Beissinger 1995). To control for female receptivity to extrapair copulations, we purposefully waited until the fertile period had ended to conduct playbacks, which may have contributed to females ascending less often overall. We cannot discount the fact that females responded more often to mate's calls if they were played back first. However, females never responded to controls that were played first, so the females' reduced response to mate's calls that were played second might alternatively reflect habituation to false arrivals.

Female reluctance to emerge when mates call may minimize predation risk. However, loud announcements by arriving males could easily undermine any benefits. The calls given by males in this context may also minimize localizability. High frequencies and short durations are two structural attributes known to complicate localization of prey by avian predators (Klump et al. 1986; Marler 2004a). Our results suggest that one potential strategy for parrotlets to incorporate individual information, without increasing call duration, is to inflect the fundamental frequency during the course of elements, thereby providing identifiable points in each element onto which frequency modulations can be mapped by the receiver. This is evidenced by the fact that the number and amplitudes of fine-scale frequency inflections along the carrier frequency (as estimated by the cumulative absolute derivative of the centre frequency contour) was the most robust predictor of individuals. The first derivative of the centre frequency contour, an estimate of the carrier frequency, was significantly positively related to centre frequency, so frequency inflection results in overall energy being shifted to higher frequencies. Thus, high-frequency calls may be one consequence of the need to keep durations short while incorporating sufficient individual information. However, the specific cues that females use to discriminate between mates and familiar nonmates remain conjectural: determining this will require experimentally manipulating other attributes of contact call behaviour (Slabbekoorn & ten Cate 1998; Vicario et al. 2001), a focus of future work.

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## Supplementary Material

Supplementary material for this article is available in the online version, at [doi:10.1016/j.anbehav.2010.10.012](https://doi.org/10.1016/j.anbehav.2010.10.012).

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