

Recruitment Calling: A Novel Form of Extended Parental Care in an Altricial Species

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Experimental Procedures

Observational Data Collection

Breeding occurred between October and March with a maximum of three successful attempts per group. A "breeding stage" was categorized as: "adults only" (when the group contained only adult individuals), "nest building/incubation," "dependent young" (either nestlings or dependent fledglings present), or "independent fledglings." When two stages occurred simultaneously (e.g., a new brood was being incubated while fledglings from the previous brood were being fed), the breeding stage of the oldest brood was recorded (i.e., "dependent young" in the aforementioned case).

Prey items were classified as follows: tiny = barely visible, small = visible in the bill, medium = up to $\frac{1}{2}$ of prey hanging out the side of the bill, and large = $\frac{1}{2}$ - $\frac{3}{4}$ of prey hanging out the side of the bill; items larger than this were scored as multiples of "large." Fifty prey items representative of each size category were weighed, and prey sizes were subsequently converted to biomass values as follows: tiny = 0.02 g, small = 0.11 g, medium = 0.45 g, and large = 0.84 g.

Experimental Data Collection

Using Wavelab, version 2 (Steinberg Media Technologies, Hamburg, Germany), we constructed playback loops by editing original recordings of calls that we had previously made with a Sennheiser MKH416T microphone and a Marantz PMD670 hard-drive sound recorder and which had been digitized (44.1 kHz, 16 bits). No loop was used more than once, thus avoiding pseudoreplication. Trials were conducted in May and June 2005, between 0700 and 1000 hr and between 1500 and 1800 hr, and the order of trial presentation was randomized. Playbacks to the same group were given at the same time of day but were separated by 3–4 days so that habituation to the protocol was minimized. All calls were played back at the same sound intensity from a Sony SRS-A35 speaker placed on the ground.

Molecular Sexing

Birds were caught in walk-in traps with mealworms as bait. On the birds' capture, a blood sample was obtained by brachial venipuncture (under SAFRING licence no. 1263), kept cool in the field, and then stored at 4°C. Genomic DNA was extracted from blood samples with a commercial kit (Qiagen, Dneasy Blood kit, Venlo, The Netherlands) according to the manufacturer's protocol. PCR reactions comprised 1 μ l of DNA (~0.2 μ g/ml), 0.012 mM of each dNTP, 2.5 mM MgCl₂, 10 picamol each of primers P2 (6-FAM-5'-TCTGCATCGCTAAATCCTTT-3') and P8 (5'-CTCCAAGGATGA GRAAYTG-3'), and 0.5 units of Super-Therm DNA polymerase (Southern Cross Biotechnology, Cape Town, South Africa) in a total volume of 10 μ l. Reactions were performed with the following temperature profile: initial denaturation at 94°C for 4 min; 10 cycles of denaturation at 94°C for 30 s, annealing at 46°C for 30 s, and template extension at 72°C for 20 s; 25 cycles of denaturation at 94°C for 30 s, annealing at 44°C for 30 s, template extension at 72°C for 20 s; and final extension at 72°C for 10 min. PCR products were then prepared for a genescan on an ABI3100 sequencer machine, and alleles were detected with the programme Genemapper (Applied Biosystems, Foster City, California). Birds were sexed according to the presence of the PCR products of *CHD-Z* (357 bases) and *CHD-W* (378 bases).

Statistical Analysis

The main analysis assessed the terms that influenced whether an individual gave a purr call during a focal watch (binomial variable: 1 = call and 0 = no call). A generalized linear mixed model (GLMM) was used so that random terms (which allow the analysis to take account of repeated measures of the same individual and group) could be defined in addition to fixed terms. All terms were entered into a GLMM with a binomial error structure and a logit link function and then sequentially dropped until only terms whose elimination

would have significantly reduced the explanatory power of the model remained (the minimal model). All two-way interactions were tested, but only those that were significant are presented in the Results. Adding the eliminated terms individually to the minimal model allowed their significance to be estimated. The GLMM considered only focal watches on adults ($n = 48$ individuals, 2,319 watches) because fledglings do not produce the purr call. The following fixed terms were included in the model: sex, dominance status (dominant or subordinate), month, year, and breeding stage (adults only, nest building/incubation, dependent young, or independent fledglings). We also considered the following continuous terms: foraging group size, body weight, maximum daily temperature, daily rainfall, foraging success rate (biomass per minute), and patch divisibility (number of prey items per minute). Foraging success rate was used as an indicator of food abundance. If a purr call had been given during the focal watch, we used the values for foraging success rate and patch divisibility from the patch at which the call was given. If no purr call had been given, values from the entire focal watch were used.

To investigate potential costs and benefits of recruitment to a foraging patch, we compared the foraging success rate, total time, and vigilance time in patches at which the purr call was given with the mean values from the focal individual's previous patches in that foraging watch. If values for a particular individual were available from more than one focal watch, means were used. Proportions were arcsine-square-root transformed so that the data were normalized prior to parametric testing. All statistical tests were two-tailed and were deemed significant at $p < 0.05$. The GLMM was conducted in GenStat (8th edition, Lawes Agricultural Trust, Rothamsted, UK), and all other statistics were conducted in Minitab (13th edition, Coventry, UK).