



Interpopulation Comparisons of Antipredator Defense Behavior of the Thorn-Tailed Rayadito (*Aphrastura spinicauda*)

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Abstract

The high impact of predators on breeding success has favored the evolution of diverse behavioral strategies that enhance both individual survivorship and reproductive success. Self- and nest-defense against predators are inter-related behaviors that vary according to life history traits and the risks involved in defense. In addition, interpopulation differences in the composition of predator guilds, and hence predation pressures, may result in geographic variation in antipredator defense strategies. It is generally accepted that the absence of predators could drive the loss of antipredator behavior. However, it is unclear how species respond if one type of predator (e.g., a specialized nest predator) is absent but others are present. The multipredator hypothesis suggests that the absence of only a few predators is not sufficient to drive a change or loss of antipredator behavior in a species. We examined interpopulation variation in defense behavior in the thorn-tailed rayadito (*Aphrastura spinicauda*), a suboscine bird inhabiting a wide latitudinal range in southern South America. In populations where nest predators were either present or absent, we examined the responses of rayaditos to an adult predator (an owl) and to a nest predator (a marsupial). Owl-defense behavior involved more intense and longer responses than marsupial-defense. This suggests that adult rayaditos may allocate more energy to defend themselves than their young. However, because the presence of adult predators near the nest also prevents parents from feeding the nestlings, owl-defense may simultaneously represent a form of nest-defense. No differences in marsupial-defense were found among the three sampled populations, despite the absence of native mammalian nest predators in one population. The lack of variation in nest-defense behavior provides some support for the multipredator hypothesis, although the high interpopulation gene flow in this species could also contribute to this behavior's maintenance.

Introduction

Nest and adult predation is the most important factor impeding successful breeding in birds (Martin 1995; Lima 2009). To counteract these threats, birds have evolved diverse morphological and behavioral strategies. During the breeding season, antipredator behavior

comprises both self- and nest-defense against predators (Lima 1998; Caro 2005). These behaviors are inter-related and have strong implications for nesting success and survival. For example, nest-defense can reduce the risk of nest predation but also simultaneously increases the probability of injury to the parents (Montgomerie & Weatherhead 1988).

Activities involving parental care, such as nest visitations during nestling feeding, may also provide information to predators on nest location, thus increasing the risk of predation of nests and breeding adults (Martin et al. 2000).

Antipredator behavior may vary in response to many factors. For example, the species or type of predator can influence antipredator displays (Knight & Temple 1988) and vocalizations (Evans et al. 1993). Although variation in alarm calls with predator-type are well documented among mammals (e.g., Zuberbühler 2001; Fichtel et al. 2005), relatively scarce knowledge exists for predator-specific calls in birds. For example, some birds use aerial and ground alarm calls to differentiate predator types (Evans et al. 1993; Seddon et al. 2002), or employ different types of calls according to the current behavior of the predator (i.e., whether the predator is perched, attacking, or scanning) (Griesser 2008). Moreover, the repetition rate of alarm calls can convey information concerning the risk to the nest (Leavesley & Magrath 2005; Welbergen & Davies 2008) or to relatives in the group (Griesser 2009).

Antipredator behavior may also vary geographically (Foster 1999). Predation pressure is typically not constant along species distributional ranges, resulting in variation in antipredator behavior (Berger 1998; Stoks et al. 2003). As defense behavior plays a key role in breeding success, and hence fitness, certain behaviors are predicted to lose intensity and eventually disappear when selection pressures are relaxed. The evolutionary time required for this process will depend on the costs of maintaining the antipredator behavior (Blumstein 2006). However, it remains unclear how antipredator behavior changes when only one type of predator is absent. The absence of only one or some predator species may not be enough to generate a loss or a change in antipredator behavior (Blumstein & Daniel 2002). Known as the 'multipredator hypothesis' (Blumstein et al. 2004; Blumstein 2006), this phenomenon may result from genetic mechanisms, such as pleiotropy or gene linkage (Blumstein 2006).

Antipredator behavior consists of both learnt and genetic components (Curio et al. 1978; Griffin et al. 2001). When a certain predator is absent, there should be no learning of defense behavior against it. Nevertheless, in cases where a predator has disappeared locally, antipredator behavior may still be maintained via dispersal or interpopulational gene flow. Therefore, research targeting geographic variation in different components of antipredator behavior, in relation to the presence of certain predators, can

provide insights into how inter-related behaviors can vary and affect microevolutionary behavioral changes (Foster 1999; Blumstein 2006).

The thorn-tailed rayadito (*Aphrastura spinicauda*; *Furnariidae*) is a suboscine passerine endemic to the temperate forests of Chile and Argentina (Remsen 2003). It is a small (10–13 g) insectivorous non-migratory bird that nests in tree cavities and forages in single- or mixed-species flocks in winter (Vuilleumier 1967; Grigera 1982; Ippi & Trejo 2003). A common antipredator strategy adopted by rayaditos is mobbing behavior, which involves approaching the predator (e.g., perched owls or walking humans) and emitting loud alarm calls. Mobbing occurs either in pairs during the breeding season or in flocks during the non-breeding season (Ippi & Trejo 2003; Ippi et al. 2011). The species occurs over a wide latitudinal distribution (30–55°S) where a variety of predator species are present in different populations. The rayadito is therefore an ideal species for the study of interpopulational differences in defense behavior against adult and nest predators. This may provide insights into how the species' behavior has changed across populations and how they respond to novel predators. We studied these questions in three populations encompassing a large fraction of the rayadito's entire geographic distribution: Navarino Island (55°S), where rayaditos have no native mammalian nest predators, and Chiloé Island (41°S) and Cerro Manquehue (33°S), where rayaditos live sympatrically with diverse mammalian nest predators, including some species that inhabit both populations.

This study focused on the characteristics of antipredator behavior, as well as geographic variation in antipredator responses. Specifically, by presenting rayaditos with models of two different predator types, we examined: (1) whether their behavioral displays or antipredator vocalizations differ when exposed to an adult predator and a nest predator located close to the nest; and (2) whether interpopulation variation in nest-defense behaviors exists, in relation to the presence of nest predators in those populations. Because no mammalian nest predators exist on Navarino Island, rayaditos should express no or less intense nest-defense behavior in this population. To obtain a more comprehensive overview of defense behavior in rayaditos, we also compared antipredator defense and baseline aggressive behavior among the three populations. As gene flow is high between the populations (González & Wink 2010) and because the adult predator and control species are present in all three populations, we predict that interpopulational differences in these both behaviors will be low or absent.

Methods

Study Area

Field work was conducted during the austral spring and summer (September–January) in three populations: Navarino Island (55°S and 67°W; 2007), Chiloé Island (41°S and 73°W; during 2006 and 2007), and Cerro Manquehue (33°S and 70°W; 2007). Since 2002, 600 nest boxes have been installed in these populations (300 in Chiloé, 200 in Navarino and 100 in Manquehue). For details about the features of the nest boxes see Moreno et al. (2005) and for details of the three study areas see Ippi et al. (2011).

The avian forest assemblage of Navarino Island includes approximately 29 species, of which eight are raptor species and 18 are passerines (Ippi et al. 2009). Additionally, no native mammalian or reptilian predator species occur at this latitude. The forest bird assemblage of Chiloé Island includes 38 species, of which 13 are raptor species and 22 are passerine, excluding aquatic and semi-aquatic species (Rozzi et al. 1996). Furthermore, in Chiloé Island, there is a marsupial species, the monito del monte (*Dromiciops gliroides*), that predated nests (eggs and possibly young chicks) (Jiménez & Rageot 1979), as well as a native cat, a snake, and several rodent species that can also predate nests. No detailed fauna censuses have been conducted in Cerro Manquehue, but some reports indicate a total of 32 species of birds in the area, with 12 birds of prey, and 14 passerines (Chicharro et al. 2007; Armesto et al. 2008). Non-avian nest predators include the elegant fat-tailed mouse opossum (*Thylamys elegans*), two species of snakes and several rodent species (Torres-Mura et al. 2011).

Moreno et al. (2005) reported an overall nest failure rate of 32% in Chiloé, with 24% of nests depredated during the egg stage and 8% depredated and deserted after hatching. Nest predation in our nest boxes (without antipredator protection) is higher in Manquehue than Navarino. Of 29 nest boxes monitored during 2007 in Manquehue, six were depredated (two nests during the incubation stage and four during the nestling stage), four were abandoned in the nestling stage, and two failed for unknown reasons during the egg stage. This resulted in an overall failure rate of 41.4%. In Navarino, of 14 nest boxes monitored during 2007, none were depredated. Although predation rates in nest boxes are not always indicative of predation on natural nests (Brawn 1988), no information on predation rates on nests in natural cavities exist for Navarino and Manquehue.

In Chiloé, Cornelius (2008) found that nests of rayaditos in boxes had lower nest success than nests in natural cavities.

Monitoring Protocol

Nest boxes were monitored following Moreno et al. (2005, 2007). Adult captures (one or both adults at each nest) occurred on the day the first egg was laid. We captured rayaditos with mist nets and playback and banded each animal with a unique combination of colored rings and a numbered metal band. We took a small sample of blood by brachial venipuncture and stored samples on FTA[®] cards for DNA preservation (Gutiérrez-Corcheró et al. 2002). This allowed us to genetically determine the sex of the subjects because sexes are indistinguishable in the field (see Moreno et al. 2007). Rayaditos were released immediately after sampling.

Antipredator Experiments

To document antipredator behavior, we conducted experiments with three stuffed mounts. The first was of an adult predator, the Austral pygmy owl (*Glaucidium nanum*), a common diurnal predator of juvenile and adult birds and mice (no records exist of this species depredating a cavity nest). This species is an important threat to small passerines (Jiménez & Jaksic 1989). The second mount was a nocturnal and crepuscular nest predator, the monito del monte, a predator of insects, eggs and probably nestlings, although they also consume fruits (Jiménez & Rageot 1979; Amico & Aizen 2000). Last, a mount of a male of rufous-collared sparrow (*Zonotrichia capensis*) served as a control. This species is a mainly granivorous passerine (López-Calleja 1995) and, to our knowledge, does not represent a threat nor strong competition to rayaditos. We therefore considered the reaction of the focal rayadito to this stimulus as a control baseline response (following other studies that have used non-interacting species as control stimuli (see e.g., Stenhouse et al. 2005; Duckworth 2006). The owl and the sparrow are present in all three study populations, whereas the monito del monte is only found at the Chiloé Island site. However, in Cerro Manquehue, another nest predating marsupial is present, the elegant fat-tailed mouse opossum, which is similar in size, morphology, diet, and behavior to the monito (Palma 1997). Assuming an integrated predator recognition system (see Curio 1973 and Blumstein 2006), birds should respond similarly to the two marsupial species.

Trials commenced when nestlings were 4 d old. Mounts were presented on successive days (weather permitting), and the order of the treatments was chosen randomly. All trials were conducted between 0650 and 1300 h. Models were mounted on top of the nest box of the focal pair at Navarino and Manquehue. As nest boxes in Chiloé experienced a very high predation rate (probably by the *monito del monte*), we installed a metal bell-like structure above the nest boxes to impede access by mammalian predators. Therefore, for this population, we placed the decoy on a telescopic pole at a standardized distance from the nest box (i.e., approximately 0.2 m from the entrance). All experiments were recorded using video cameras (Panasonic NV-GS 320) coupled with personal observations of activities recorded with a digital audio recorder (Olympus VN-960PC). Vocalizations were recorded with a digital recorder (Sony PCM-M1) and a Sennheiser ME 66 microphone, with a sampling rate of 48 kHz. The microphone was mounted 0.5 m above the ground level on a tripod at a distance of 2–4 m from the nest. To prevent damage to the models, all experiments were stopped when physical aggression by focal animals toward the model exceeded five pecks. Behavioral observations were conducted by one observer positioned close to the nest, but hidden from view (between 8 and 20 m away).

As rayaditos visit their nests very frequently when nestlings are 4–6 d old (Moreno et al. 2007), it is difficult to install the predator models and video camera without disturbing their normal behavior. Therefore, although latency (i.e., time taken before the first response by the focal bird) is a commonly used variable to measure caution in antipredator experiments (see e.g., Dunn et al. 2004; Stenhouse et al. 2005), we excluded this variable from the analyses and waited 2 min after installation of the stimuli before starting behavioral recordings. After the initial 2-min period, we conducted behavioral observations for 10 min and also recorded all vocalizations during this time (no playbacks were used).

Data Analyses

We used the software JWATCHER 1.0 (Blumstein et al. 2000) to extract the data and produce the ethograms from the recorded behavior. A few trials involving the sparrow were excluded from analyses because of high aggression by the focal rayaditos, which pecked the sparrow model more than five times before the two-first minutes had passed (two cases in Navarino and three in Manquehue). This non-random elimination of some trials could potentially bias the results,

via the exclusion of more aggressive individuals. However, the proportion of eliminated trials was very low (3.6%, $n = 138$ trials). As some trials were stopped before 10 min had passed, we converted several variables to proportions. The dependent variables measured were: proportion of time out of sight, average distance to the mount, minimum distance to the mount, proportion of time spent within 2 m of the mount, proportion of time performing alarm calls, movements rate (number of movements including flights, jumps, and flutters relative to the duration of the trials), proportion of time spent inside the nest box, rate of wing spreading (number of times that the bird opens its wings relative to the duration of the trial), pecking rate (number of times the model was pecked relative to the duration of the trial), and proportion of time spent searching for food or eating. We included all trials in the analyses, including those where one or both parents were absent. To analyze the effects of populations on presence/absence of both parents, we fitted generalized linear models (GLM) to our data with a binomial error distribution and a logit link function. In cases where individuals were absent during the trial, average and minimum distances were fixed at 20 m and other variables, such as proportion of time feeding or alarming was fixed at zero.

To reduce the number of variables used in the analyses, principal component analysis (PCA) was performed on the behavioral variables. Four principal components (PCs) were extracted. PC scores did not follow a normal distribution (Kolmogorov–Smirnov; $p < 0.0001$, for all four components), included negative values and PC1 and PC2 were left-skewed. We therefore transformed the data to approximate a gamma distribution. However, interpretation and figures presented here are based on the original untransformed PC scores. We analyzed the four PCs as dependent variables using generalized linear mixed models (GLMMs; Bolker et al. 2009) including focal bird identity, nest box, and year as random effects. This accounted for multiple measures from the same individual, variation of the pair of a same nest and trials conducted in different years. Population, predator model, sex, and their two-way interactions were included as fixed effects. When we found significant global differences among populations, we conducted new GLMMs for each mount separately. When we found significant global differences in GLMM among predator mounts or populations, we applied pairwise *a posteriori* contrasts adjusting for multiple comparisons with sequential Bonferroni correction.

Vocalizations recorded during experiments included alarm calls and loud trills (see Ippi et al. 2011 for

details). Spectrogram analyses of vocalizations were conducted with the software RAVEN 1.2 (Cornell Bioacoustics Laboratory, Cornell, USA). During the experiments, alarm calls of both parents overlapped. We therefore selected and analyzed three consecutive non-overlapping notes for each individual, with a note being defined as any continuous trace on the spectrogram (Baptista 1977). These notes were selected based on their quality. The bioacoustic variables extracted from the spectrograms were minimum frequency (kHz), maximum frequency (kHz), bandwidth (difference between minimum and maximum frequency; kHz), peak frequency (frequency in the call with the most energy; kHz), and notes rate (notes/S). We analyzed differences between alarm calls emitted when faced with different types of predator with a GLMM, using nest box and year as random effect. Individual identity was not included as random effect because it was not always possible to identify which individual was alarming in the spectrogram. Bandwidth and peak frequency followed normal distributions (Kolmogorov–Smirnov; $p = 0.200$) and were analyzed with an identity link. Notes rate was transformed to approximate a gamma distribution, and the remainder variables followed gamma distributions (and analyzed with a log link). All statistical analyses were conducted with IBM SPSS Statistics 20.0 for Windows and were considered significant at $p < 0.05$.

Results

Antipredator experiments were conducted at 12 nests in Navarino, at 26 nests in Chiloé, and at 13 nests at Manquehue. Generally, both parents were present during the experiments in all three populations, and no differences were found among populations in the presence/absence of parents during the trials (GLM; Owl: $\chi^2 = 2.610$, $df = 2$; $p = 0.271$; Monito: $\chi^2 = 3.061$,

$df = 2$, $p = 0.216$; Sparrow: $\chi^2 = 1.544$, $df = 2$, $p = 0.462$) (Table 1).

Defense Behavior During Nestling Stage

PCA was performed on ten behavioral variables, and we obtained four PCs that explained 80% of the total variance (Table 2). Positive scores for PC1 corresponded to less distant individuals, with more time spent near the model and more alarm responses. We therefore interpret PC1 as approaching behavior. The second PC was negatively correlated with the time spent inside the nest box and positively with time spent alarming and spreading wings rate. We thus interpret PC2 as alarm activity behavior. The third PC correlated with pecking rate, and finally, the fourth PC was correlated with the time spent foraging during the experiments. All the following analyses were conducted with these four component variables.

We found no differences between males and females in all principal component variables (GLMM; PC1: $F_{1, 262} = 0.605$; $p = 0.437$; PC2: $F_{1, 262} = 2.068$; $p = 0.152$; PC4: $F_{1, 262} = 0.722$; $p = 0.396$), except that males pecked the models more than females (PC3: $F_{1, 262} = 6.417$; $p = 0.012$) (Fig. 1). Females only ever pecked the sparrow ($n = 14$), while males pecked all three models ($n = 19$). No interaction between sex and population, or sex and predator occurred for any PC (data not shown).

Behavioral responses to the different types of stimuli were significantly different, when including all three stimuli (PC1: $F_{2, 262} = 8.328$; $p < 0.001$; PC2: $F_{2, 262} = 29.141$; $p < 0.001$; PC3: $F_{2, 262} = 27.107$; $p < 0.001$; PC4: $F_{2, 262} = 6.597$; $p = 0.002$) (Fig. 2). However, in pairwise post hoc comparisons, we failed to detect any difference between the monito del monte and the sparrow in all PCs (PC1: $t = 0.494$; $df = 262$; $p = 0.621$; PC2: $t = -1.753$; $df = 262$; $p = 0.081$; PC4: $t = -0.392$; $df = 262$; $p = 0.695$),

Table 1: Presence/absence of males, females or both thorn-tailed rayadito parents during defense behavior experiments

Population	Experiment (model)	Parents present (%)	Female absent (%)	Male absent (%)	Parents absent (%)	Total nests
Navarino	Austral pygmy owl	90.9	9.1	0.0	0.0	11
	Monito del monte	81.8	0.0	18.2	0.0	11
	Rufous-collared sparrow	100	0.0	9.1	0.0	8
Chiloé	Austral pygmy owl	88.5	3.8	3.8	3.8	26
	Monito del monte	96.2	0.0	3.8	0.0	26
	Rufous-collared sparrow	92.0	8.0	0.0	0.0	25
Manquehue	Austral pygmy owl	90.9	9.1	0.0	0.0	11
	Monito del monte	81.8	9.1	9.1	0.0	11
	Rufous-collared sparrow	77.8	8.3	16.7	8.3	9

Table 2: Factor loadings of the first four principal components for ten variables of defense behavior experiments. Values of factor loadings correspond to the Varimax with Kaiser normalization rotation method

	PC1	PC2	PC3	PC4
Eigenvalues	4.42	1.42	1.10	1.07
% of variance	44.16	14.16	11.04	10.74
Cumulative% of variance	44.16	58.32	69.36	80.10
Factor loadings				
Proportion of time out of sight	-0.895	-0.187	-0.036	-0.053
Average distance	-0.930	-0.118	-0.035	-0.019
Minimum distance	-0.759	0.104	-0.024	-0.127
Proportion of time spent around 2 m	0.857	0.156	0.024	-0.240
Proportion of time alarming	0.595	0.643	-0.198	-0.189
Movements rate	0.559	0.558	0.280	-0.095
Proportion of time inside the nest	0.232	-0.845	-0.096	-0.130
Rate of wing spreading	0.447	0.618	-0.175	-0.042
Pecking rate	0.062	-0.005	0.974	-0.020
Proportion of time searching food/eat	0.023	0.022	-0.023	0.977

The factor loadings that we used to name the PC are noted in bold.

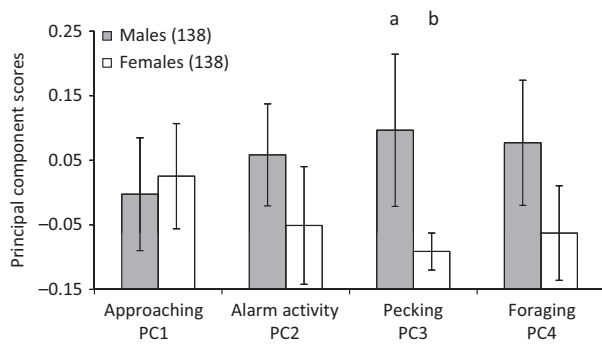


Fig. 1: Principal component scores for defense behavior of rayadito males and females. Different letters above bars indicate significant differences. Data represent mean ± SE and the number in parenthesis represents the sample size.

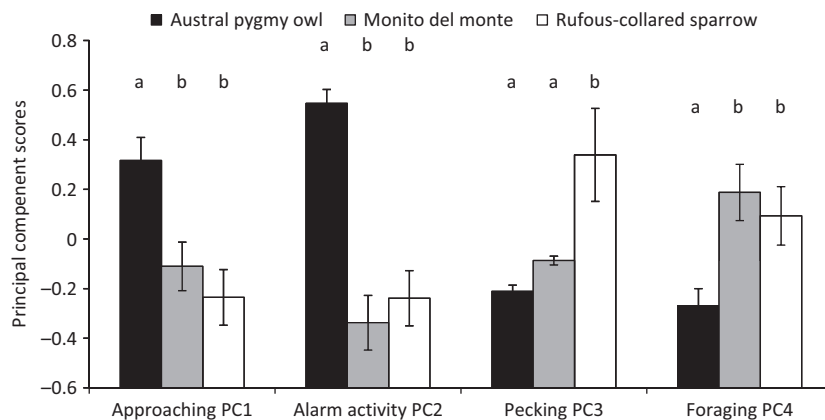


Fig. 2: Principal component scores for owl-defense, marsupial nest-defense and baseline aggressive behaviors of rayaditos. Different letters above bars indicate significant differences with pairwise *a posteriori* contrasts, corrected via the sequential Bonferroni procedure. Data represent mean ± SE.

except for pecking PC3 ($t = 5.231$; $df = 262$; $p < 0.001$). For PC3, the sparrow was pecked more often than monito (Fig. 2). In contrast, for all principal components, defense behavior elicited by the owl was significantly different from the response elicited by the control (PC1: $t = 3.580$, $df = 262$; $p = 0.001$; PC2: $t = 5.037$; $df = 262$; $p < 0.001$; PC3: $t = 6.889$; $df = 262$; $p < 0.001$; PC4: $t = 2.700$; $df = 262$; $p = 0.015$). Similarly, the response to the owl differed from the response to the monito (PC1: $t = -3.333$, $df = 262$; $p = 0.002$; PC2: $t = 7.279$; $df = 262$; $p < 0.001$; PC4: $t = 3.314$; $df = 262$; $p = 0.003$). Only pecking PC3 was marginally non-significant between the owl and the monito ($t = -1.910$; $df = 262$; $p = 0.057$) (Fig. 2).

Geographic Variation in Defense Behavior

Defense behavior of rayaditos was different among the three populations for approaching PC1 ($F_{2, 262} = 3.354$; $p = 0.036$), alarm activity PC2 ($F_{2, 262} = 6.079$; $p = 0.003$), pecking PC3 ($F_{2, 262} = 11.528$; $p < 0.001$), and foraging PC4 ($F_{2, 262} = 3.793$; $p = 0.024$) (Fig. 3). However, no interaction between populations and predator models was found (PC1: $F_{4, 262} = 1.152$; $p = 0.332$; PC2: $F_{4, 262} = 1.467$; $p = 0.212$; PC4: $F_{4, 262} = 0.314$; $p = 0.869$), except for PC3 ($F_{4, 262} = 7.383$; $p < 0.001$). In this case, the sparrow was pecked more often than the predator models and was pecked more in Navarino compared with the other two populations (Fig. 3-c). In a *post hoc* analysis, marsupial nest-defense behavior showed no variation among the populations for any PC (PC1: $F_{2, 93} = 0.153$; $p = 0.858$; PC2: $F_{2, 93} = 0.422$; $p = 0.657$; PC3: $F_{2, 93} = 1.655$; $p = 0.197$; PC4: $F_{2, 93} = 2.024$; $p = 0.138$). Responses to the sparrow were more variable, with approaching behavior PC1 ($F_{2, 81} = 3.529$; $p = 0.034$) and pecking PC3 ($F_{2, 81} = 7.083$; $p = 0.001$) being highest in Navarino, and alarm activity PC2 ($F_{2, 81} =$

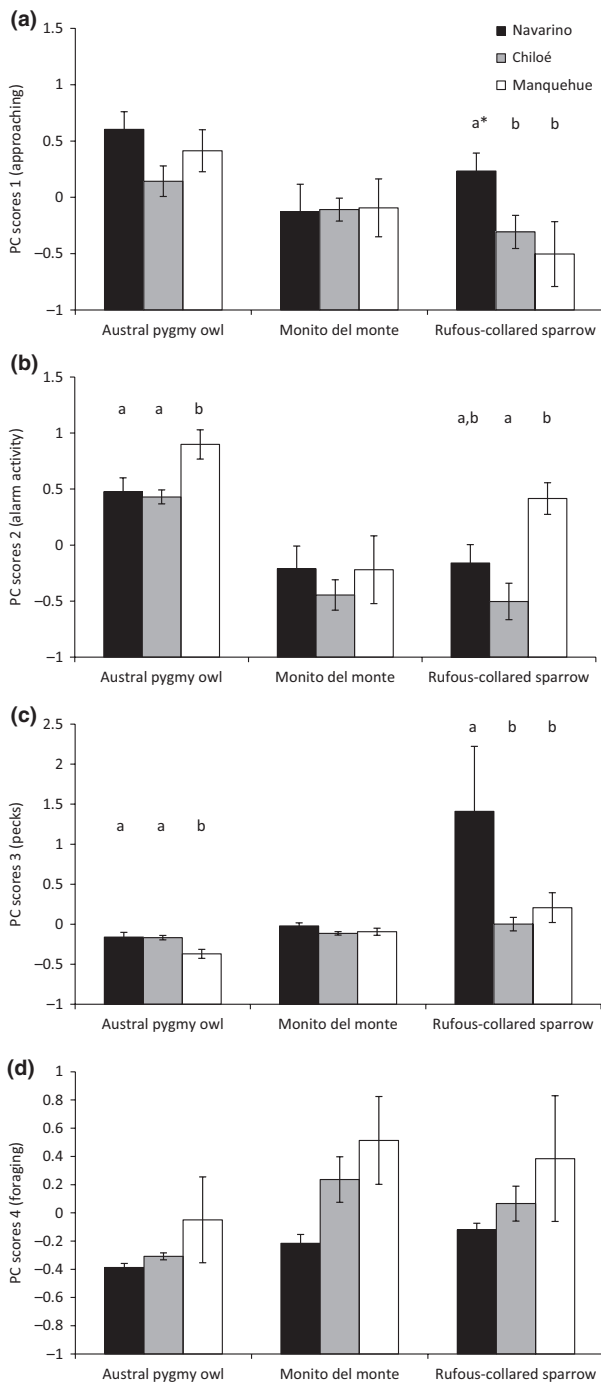


Fig. 3: Interpopulational variation of principal component (PC) scores (a = PC1; b = PC2, c = PC3, d = PC4) for owl-defense, marsupial nest-defense and baseline aggressive behaviors of rayaditos from Navarino, Chiloé and Manquehue. Different letters indicate significant differences obtained with generalized linear mixed model conducted on each model separately and *a posteriori* contrast, corrected via the sequential Bonferroni procedure. Data represent mean \pm SE and * means $p = 0.05$.

7.388; $p = 0.001$) being highest in Manquehue. Foraging PC4 ($F_{2, 81} = 0.985$; $p = 0.378$) showed no significant differences among the populations (Fig. 3-d). Owl-defense behavior showed no interpopulational differences for approaching PC1 ($F_{2, 93} = 1.757$; $p = 0.178$) and foraging PC4 ($F_{2, 93} = 1.746$; $p = 0.180$), but alarm activity PC2 ($F_{2, 93} = 5.916$; $p = 0.004$) was highest in Manquehue and pecking PC3 ($F_{2, 93} = 5.335$; $p = 0.006$) was minimum in Manquehue.

Vocal Responses to Different Predators

When faced with the different mounts, rayaditos mainly used alarm calls and, less frequently, loud trills. We found no differences in acoustic variables in trials using either of the three mounts. Nevertheless, the emission rate of notes was lower in Chiloé (Table 3; Fig. 4).

Discussion

Defense Behaviors During Nestling Stage

Defense behavior of thorn-tailed rayaditos elicited by both the owl and the marsupial, consisted of similar displays, dominated by mobbing behavior, which involve high repetition of alarm calls, wing spreading and constant movements among perches at very close distances from the predator. However, owl-defense behavior involved more intense displays than marsupial nest-defense and baseline aggression. In the vast majority of cases, rayaditos did not enter in the nest when the adult predator was present, which was also found for a congeneric species (*Aphrastura masafuerae*) (Hahn et al. 2004). Although the adult predator elicited a longer vocal response, the mobbing alarm calls were acoustically similar toward the adult and nest predators, as well as the non-threatening passerine bird. This suggests that rayaditos do not have specific alarm calls for different types of predators.

Defense behavior was more intense in response to the owl than to the marsupial and the sparrow. This result suggests that rayaditos are willing to invest more energy in their own defense than in the defense of their nestlings. This situation is predicted for birds with high longevity and small clutch sizes (Ghalambor & Martin 2001). However, although the rayadito has a small clutch size (4.1 eggs in Chiloé; Moreno et al. 2005), it is also a relatively short-lived bird, with an average age of 0.8 yr in Navarino (Rozzi & Jimenez

2013). The higher aggression toward the owl may therefore have arisen due to other factors. For example, mobbing is a common antipredator strategy in birds (Caro 2005) and despite the risks involved, individuals may obtain several non-mutually exclusive advantages. First, predators should leave the area sooner (Flasskamp 1994) and be less likely to return (Caro 2005) when mobbing is more intense. Once the predator has left and does not return, the parents would be able to continue feeding the nestlings with a regular supply of food. The continued absence of the predator should therefore benefit both the parents and their offspring when they leave the nest. Second, when parents emit alarm calls, begging vocalizations of nestlings stop immediately (Ippi S pers. obs.), rendering it more difficult for the predator to locate the nest (Leech & Leonard 1997). Mobbing therefore constitutes a form of parental investment (Barash 1975; Andersson et al. 1980; Caro 2005). However, the fact that rayaditos also mob owls during the non-breeding season and when they are far from nest, suggest that owl mobbing is not exclusively used for nest-defense. Mobbing may instead serve as both an antipredator self-defense and nest-defense strategy. This dual function of mobbing may therefore explain the more intense owl-defense compared with marsupial nest-defense observed in this study.

Unexpectedly, we not only failed to find differences between marsupial-defense behavior and control behavior, but the control model received more pecks than the nest predator. This may occur because the models were placed directly on top of the nest boxes, which may represent a direct challenge to the parents.

Table 3: Bioacoustic characteristics of rayadito alarm calls during the defense behavior experiments in Navarino and Chiloé

Fixed effect	Dependent variable	F	df	p
Population	Minimum frequency	0.583	1, 119	0.447
	Maximum frequency	0.000	1, 119	0.998
	Bandwidth	0.138	1, 119	0.711
	Peak frequency	1.397	1, 119	0.240
	Notes rate	13.163	1, 119	0.001
Predator model	Minimum frequency	2.448	2, 118	0.091
	Maximum frequency	1.418	2, 118	0.246
	Bandwidth	2.414	2, 118	0.094
	Peak frequency	1.099	2, 118	0.365
	Notes rate	0.794	2, 118	0.454
Population x predator model	Minimum frequency	1.127	5, 115	0.350
	Maximum frequency	0.877	5, 115	0.499
	Bandwidth	1.269	5, 115	0.282
	Peak frequency	1.099	5, 115	0.365
	Notes rate	3.643	5, 115	0.004

Significant effects are noted in bold.

Sparrows do not represent a real threat for rayaditos (which is also suggested by our finding that foraging activity is high in the presence of the sparrow, Fig. 2), but they still appear to be treated as intruders in the rayaditos' territories, especially by males. The higher proportion of pecks toward the sparrow is probably due to the lack of perceived threat by the sparrows and suggests interspecific territorial behavior in rayaditos during the breeding season (S. Ippi, unpub. data). The lack of difference between nest-defense behavior and control behavior may occur for at least three different reasons. First, it is possible that rayaditos did not identify the monito del monte as a predator, given that this species has crepuscular and nocturnal behavior (Redford & Eisenberg 1992) or because the model was inert during the trials (Knight & Temple 1986). Second, rayaditos could use another strategy to counteract predation by marsupials, such as cavity selection in trunks with low epiphyte cover (Cornelius 2008). Third, less aggressive defense behavior against the mammalian nest predators could be related to the ability of parents to chase birds away more easily than mammals (Knight & Temple 1988).

Geographic Variation of Defense Behavior

The unexpected lack of interpopulation differences in nest-defense suggests that the hypothesized relaxed selection occurring in Navarino due to the absence of mammalian nest predators and lower nest failure by predation is not enough for a change or loss of this behavior. Unlike Chiloé Island, where refuges existed during the last ice age (Heusser 2002), *Nothofagus* forests appeared in the Fuegian–Patagonian region more recently around 8000 BP (Rabassa et al. 2000). Therefore, the temperate forest avian assemblages probably arrived in Navarino with the *Nothofagus* forests,

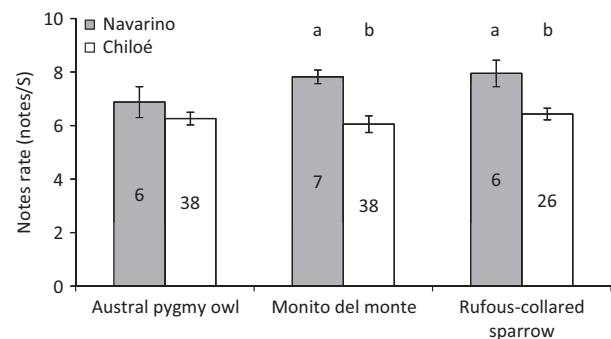


Fig. 4: Emission rate of rayadito alarm call notes in Navarino and Chiloé islands. Different letters indicate significant differences with pairwise comparisons *a posteriori* with generalized linear mixed model. Data represent mean ± SE and numbers in columns are sample sizes.

evolved in the absence of native carnivorous mammals since then. Although a long time is required for a change in innate antipredator behavior (Hollén & Manser 2007), mobbing behavior, which is the main component of antipredator behavior in rayaditos, also involves a learning process (Curio et al. 1978). If there are no mammalian nest predators in Navarino, we predicted that there would be no learning of nest-defense behavior. However, marsupial nest-defense was similar in all populations. This finding supports the 'multipredator hypothesis', which predicts that the presence of a single predator may be sufficient to explain evolutionary persistence of antipredator behavior (Blumstein et al. 2004; Blumstein 2006). Thus, rayaditos inhabiting Navarino Island could maintain the mammalian nest-defense behavior, because aerial predators persist. However, the persistence of this behavior could also be related to the high gene flow between rayadito populations on the island and more northerly populations (see González & Wink 2010), where rayaditos coexist with mammalian nest predators. This is also suggested by the low degree of geographic differentiation in rayadito vocalizations from these populations (Ippi et al. 2011). Finally, our results need to be interpreted with some caution as we failed to find differences between marsupial nest-defense and control behaviors.

In spite of the likely high gene flow between rayadito populations, owl-defense and baseline defense behaviors displayed interpopulation variation. Owl-defense behavior was more intense in Manquehue, where they were more active and emitted alarm calls for more time than in Navarino or Chiloé. Rayaditos also expressed unexpected geographic variation and high aggressiveness in their response to the control model, approaching more and being more physically aggressive (PC1 and PC3) toward the sparrow in Navarino, and more vocal and active (PC2) in Manquehue (Fig. 3). Because the owl and the sparrow are present in all populations, interpopulation variation may arise due to stochastic processes (Armbruster & Schwaegerle 1996). Alternatively, they may reflect plastic behavioral responses to different ecological pressures (see e.g., Lima 2009; Martin & Briskie 2009), such as differences in adult predation pressures (Sandoval & Wilson 2012) or interspecific territoriality enhanced by the low availability of suitable territories.

In conclusion, the key component of the rayadito's antipredator behavior along its entire distributional range is mobbing behavior. We found that owl-defense is more intense and time consuming than marsupial-defense, possibly due to a dual function of owl-defense in both self- and nest-defense. We failed

to find any vocal differences in response to the different types of threats, except that the adult predator elicited a longer vocal response. In addition, rayaditos showed no differences in nest-defense behavior among the three populations, although they expressed more alarm activity during owl-defense and toward the control in Manquehue, and pecked the sparrow more often in Navarino. The lack of variation of nest-defense behavior in populations isolated from mammalian nest predators, gives some support to the multipredator hypothesis proposed by Blumstein (2006). However, this may also have arisen due to the high gene flow among populations (González & Wink 2010). Future studies, which include more isolated populations (in both time and space) and different types of nest predators, could further reveal the processes that shape nest-defense behavior in rayaditos.

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