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Assessing potential Argentine Ant recruitment to pipping eggs in the Red-tailed Tropicbird on Rapa Nui (Easter Island)

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ABSTRACT

The invasive Argentine Ant, *Linepithema humile*, is known to prey on land bird chicks, but there are few reports of the ants' impacts on breeding seabirds. We conducted a manipulative study using hen eggs to estimate the potential Argentine Ant recruitment to pipping eggs in the Red-tailed Tropicbird, *Phaethon rubricauda*, at Rapa Nui (Easter Island), Chile. Hen eggs were experimentally opened to simulate pipping eggs and control eggs were left unopened. The eggs were monitored for 90 min. The time elapsed until the arrival of the first ant did not differ between experimental and control eggs. None of the control eggs were visited by more than two ants. In contrast, the inferred cumulative number of ants that arrived at experimental eggs varied from 1 to >450. Simulated pipping eggs attracted a high number of ants in a short period of time, and the probability of an egg being attacked by ants after 5 and 60 min of exposure was 75% and 99%, respectively. A long-term monitoring study is needed to determine the actual Argentine Ant recruitment rate on pipping eggs, and the potential effects on the post-fledging survival rate of this native seabird which is threatened by a number of introduced species.

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Introduction

The negative economic and ecological effects of invasive ants (Formicidae) have been well documented (e.g. Holway *et al.* 2002; Cooper *et al.* 2008). The Argentine Ant, *Linepithema humile*, is one of the six most destructive invasive ants (Holway *et al.* 2002; Wetterer *et al.* 2009). Native to South America, Argentine Ants have invaded many continents and islands (Wetterer *et al.* 2009), having negative effects on biodiversity, including mammals and lizards (e.g. Laakkonen *et al.* 2001; Fisher *et al.* 2002). Argentine Ants are known to prey on chicks of some land bird species, and have been observed both killing nestlings of the endangered songbird *Vireo bellii pusillus* (Peterson *et al.* 2004), and causing nest failure in *Junco hyemalis* (Suarez *et al.* 2005). Because Argentine Ants recruit to food items in extremely large numbers they may overwhelm their prey through suffocation, or direct predation by continuous biting, causing chick mortality (Suarez *et al.* 2005).

Despite the worldwide distribution of the Argentine Ant, published studies regarding their impacts on nesting success in seabirds are scarce. It has been suggested that *Sterna antillarum browni* chicks are susceptible to attacks by Argentine Ants (Choe *et al.* 2010), but no significant impact was observed on the nesting success rates of *Pterodroma phaeopygia sandwichensis* (Krushelnycky *et al.* 2001). Recently, we directly observed large numbers of Argentine Ants in a nesting area of the Red-tailed Tropicbird, *Phaethon rubricauda*, at remote Rapa Nui (Easter Island, Chile) in the south-eastern Pacific Ocean. Red-tailed Tropicbirds nest on the bare ground, potentially making them more at risk from Argentine Ants. At Rapa Nui the nests are exposed to a minimum temperature of 18°C, which is above the minimum required for Argentine Ant foraging (11–12°C; Krushelnycky *et al.* 2001). The Argentine Ant is able to displace other ants and increase rapidly in numbers (Holway *et al.* 2002), which may be one of the potential mechanisms for the current widespread distribution of this species on Rapa Nui.

The seabirds of Rapa Nui and other islands in the Pacific Ocean were heavily impacted by anthropogenic disturbance (Steadman 1995). The widespread introduction of alien species had negative effects on native seabirds because they lack defence mechanisms against introduced species (Furness and Monaghan 1987). The

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Red-tailed Tropicbird is the only seabird species found nesting in Rapa Nui itself (not only in the nearby islets), although approximately 30 species bred on the island in prehistoric times (Steadman 1995).

At Rapa Nui a small Red-tailed Tropicbird colony was established on Rano Raraku volcano, being used regularly since 2007 (Flores *et al.* 2017). Although Rano Raraku is under the protection of the Rapa Nui National Park, the colony is threatened by several invasive animals (i.e. cats, dogs, rats, and a bird of prey (*Phalcoboenus chimango*; Jaramillo *et al.* 2008; Flores *et al.* 2017). Flores *et al.* (2017) reported predation by Argentine Ants on one 2-day-old Red-tailed Tropicbird chick, which were invading the rectum, throat and nostrils. Considering the latter and Argentine Ant predation on land bird chicks (see above), we aimed to estimate the potential Argentine Ant recruitment to Red-tailed Tropicbird eggs when they are pipped (as part of the process prior to hatching).

We performed a manipulative experiment using hen eggs as a proxy for Red-tailed Tropicbird eggs. We aimed to determine (a) how fast, and (b) how many Argentine Ants could recruit to eggs, and (c) the probability of an egg being attacked by ants at the Rano Raraku nesting area.

Methods

Experimental design

The experiments were conducted on 25 and 26 November 2016 starting at 19.30h at two different sites (separated by *ca.*30 m) on empty Red-tailed Tropicbird nests at Rano Raraku at the most active breeding sector on the outer slope of the crater (Flores *et al.* 2017).

We used domestic hen eggs with a small hole to simulate pipping eggs, as had been done in a previous study of Argentine Ant recruitment to bird eggs (see Suarez *et al.* 2005). The underlying assumption is that the odours of the pipping eggs act as a cue that the Argentine Ant uses to locate prey (d'Ettorre 2016). The domestic hen eggs used in this study came from free-living hens raised at Rapa Nui. A total of 30 eggs (mean length = 5.4 cm, width = 3.9 cm and weight = 48.8 g) were used in two independent experiments. The hen eggs we used were smaller than the eggs of the Red-tailed Tropicbird (mean length = 5.6 cm, and width ranging from 4.1 to 5.6 cm; Fleet 1974), which we considered unlikely to influence the behaviour of the ants. Fifteen eggs were manipulated to simulate pipping eggs (hereafter experimental eggs) and the other 15 were left unopened as controls. A small hole (0.5–1 cm diameter) was poked into each experimental egg using a narrow wooden stick and most of the albumin was removed 1 h before the experiments. This was done to prevent eggs toppling and leaking into the nests while conducting the experiments. Only the yolk was left inside as an olfactory attractor, as it is likely that any egg component on chicks' bodies at hatching would be an attractor for the Argentine Ant which has a generalist diet (Silverman and Brightwell 2008). Five experimental eggs and five control eggs were used on the first day, and 10 experimental eggs and 10 control eggs were used on the second day. Each experimental egg was placed 10 cm away from a control egg. Each pair of eggs (experimental and control) was spaced randomly by at least 1 m, at one of the two sites. The possible degree of association among the neighbouring spatial units was tested using Moran's index. No significant spatial autocorrelation was found ($I = 0.1129$, $P = 0.3679$), thus indicating the absence of spatial pseudo-replication in the data set. The eggs were monitored by two observers for 90 min to register: (1) the time when the first ant reached each egg, and (2) the number of ants on each egg at intervals of 5 min.

For each pair of experimental and control eggs, the number of ants counted in each interval was added to obtain the inferred cumulative number (ICN) of ants. We cannot ascertain the time an individual ant was preying on the experimental eggs but, based on the foraging behaviour of diverse ants (e.g. Traniello 1989; Pinter-Wollman *et al.* 2013), it is reasonable to assume that during a foraging trip an Argentine Ant remains on the prey for a maximum of 3–4 min. Therefore, for the 5-min intervals, we assume that the ants preying on eggs at any time interval are different from those in the preceding time interval.

Statistical analyses

To analyse the data we used R (R Core Team 2012) and functions in the *lme4* package (Bates *et al.* 2015). In all analyses the eggs were included as a random effect, considering that each experimental egg and its corresponding control egg were deployed at the same time and in the same place at the nesting site.

The first analysis was a comparison between control and experimental eggs of the time elapsed until the arrival of the first ant. The second analysis was a comparison of the ICN of ants throughout the experimental period for both egg categories. For both analyses we applied a linear mixed-effects model with egg categories as a fixed effect and the random effect as describe above. The best model was obtained by likelihood ratio tests of the full model with the effect in question against the model without the effect in question. Means estimated by the models are reported with standard error (SE). We also analysed the ICN of ants recruiting to the experimental and control eggs over time. We performed a generalised linear mixed-effects model (GLMM) using the *glmer* function and assuming a Poisson error structure. As random effects we used intercepts for subjects (eggs), and by time random slopes to reduce residual variance by accounting for the observed between-subject variation in slopes that reduce the Type I error rate (Schielzeth and Forstmeier 2009). Finally, we calculated the predicted probability of ants recruiting to an experimental egg from the start of the experiment. Odds ratios were obtained for each pair of experimental and control eggs. Univariate logistic regression was performed using *glmer* assuming a binomial error structure. As above, intercepts and slopes were allowed to vary randomly across model estimation. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. Figures were made using the *ggplot2* package (Wickham 2009) within R.

Results

The time until the arrival of the first ant varied between 1 and 85 ($\bar{X}$ = 21.9 ± 5.58) min in control eggs and between 1 and 40 ($\bar{X}$ = 11.3 ± 6.92) min in experimental eggs. The time elapsed until the arrival of the first ant did not differ between control and experimental eggs ($X^2_{(1)} = 2.6046$, $P = 0.1066$). None of the control eggs were visited by more than two ants; in contrast, the ICN of ants that arrived at experimental eggs varied from 1 to >450 ants within 90 min. Therefore, our simulated pipping eggs attracted the ants (Figure 1(a)), which after 90 min reached a mean ICN of 207.9 ± 38.1 ants, significantly higher than those found on closed control eggs ($X^2_{(1)} = 19.756$, $P < 0.0001$).

The ICN of ants on experimental eggs was highly variable compared to the control eggs, although significant differences were detected between groups (Figure 1(a); $P < 0.001$; Table 1). Moreover, there was a significant increment in the ICN of ants recruiting to the experimental eggs over time ($P < 0.001$). This result is consistent with that obtained by logistic regression, which showed that simulated pipping eggs attracted a high number of ants in a short period of time, where the probability of being recruited by ants was 75% and 99%, after 5 and 60 min of exposure, respectively (Figure 1(b)). Thus, we conclude that the proportion of experimental eggs that are recruited by ants increases significantly with increasing time of exposure at the nesting sites of the Red-tailed Tropicbird at Rapa Nui.

Discussion

Our results show that the Argentine Ant is present at Red-tailed Tropicbird nests even when these are not occupied by breeding birds. This was clearly evident considering that the time of arrival of the first ant was as early as 1 min in one experimental egg and between 1 and 10 min in 53% of the experimental eggs. In the case of control eggs, 57% of the eggs were visited by the first ant within the first 10 min. However, none of the control eggs were visited by more than two ants and the ants abandoned the eggs within the duration of the experiments in most cases. This totally differed from the results of the experimental eggs, where the

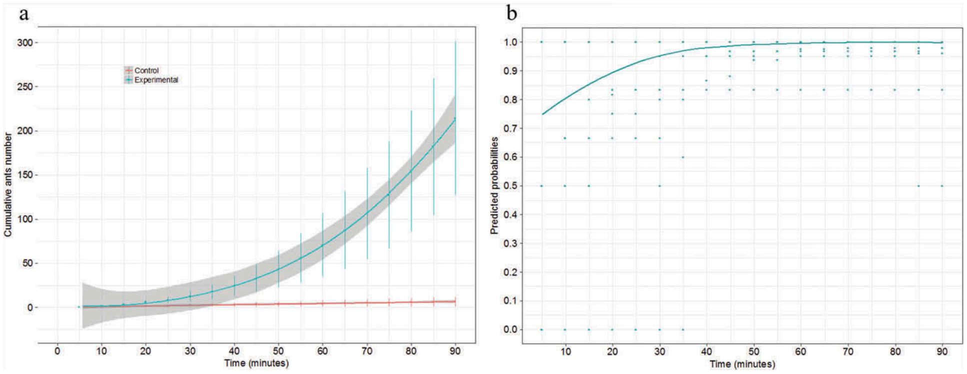

Figure 1. (a) Inferred cumulative number (ICN) of Argentine Ants recruiting to control and experimental eggs deployed at nesting sites of the Red-tailed Tropicbird at Rapa Nui. Solid lines are the fitted responses with 95% confidence interval for the relation between time and ICN. The dots are the means with 95% confidence intervals for each time interval. (b) Predicted probability of the proportion of eggs that are invaded by Argentine Ants over time at nesting sites of the Red-tailed Tropicbird on Rapa Nui. The solid line is the fitted line.

Table 1. Summary statistics of a generalised linear mixed-effects model evaluating the inferred cumulative number of Argentine Ants recruiting to experimental and control eggs over time.

Coefficients	Estimate	SE	*Z* value	*P*
Intercept	−0.2991	0.3208	−0.9320	0.351
Experimental eggs (A)	1.2266	0.1335	9.1900	<0.001
Time (B)	0.0297	0.0033	8.9380	<0.001
A × B	0.0160	0.0019	8.2410	<0.001

recruitment of ants increased over time. Therefore, we infer that the smell of the opened eggs and not the presence of eggs attract a high number of ants in a short period of time (within 90 min). Furthermore, the probability of an opened egg being recruited by ants increases with the exposure time, with recruitment almost 100% after 1 h of exposure. This is in line with reports that the Argentine Ant is able to recruit resources in large numbers within short periods of time (Suarez *et al.* 2005; Brightwell and Silverman 2007). As a consequence, the potential for Argentine Ant recruitment to Red-tailed Tropicbird eggs may be high, as the pipping process can take 19 h (Whittow and Grant 1985). However, is important to underline that our results reflect the potential for Argentine Ant recruitment, but not the actual failure of Red-tailed Tropicbird nesting success in the colony at Rano Raraku due to Argentine Ant predation. For example, Suarez *et al.* (2005) found that Argentine Ants recruited to more nests and in higher numbers than the native ants they displace. However, after monitoring real nests of *Junco hyemalis* over 3 years they found that only 4 of 247 nests that had nestlings failed as a result of Argentine Ant predation. In another study, Peterson *et al.* (2004) noted that Argentine Ants were present in nests of the songbird *Vireo bellii pusillus* in small numbers prior to hatching, but increased greatly in numbers during the process of hatching. Nestlings were bitten repeatedly until they succumbed and the nestlings of one nest were effectively predated by ants. These studies show that the Argentine Ant is capable of causing chick mortality, although in low proportions. In the specific case of the Red-tailed Tropicbird, Flores *et al.* (2017) recorded the death of a single 2-day-old chick due to ant predation in a study that included just two breeding seasons. A long-term monitoring study is necessary to determine the rate of nest failure due to Argentine Ant predation in the Red-tailed Tropicbird colony at Rano Raraku.

Other invasive ant species are known to have negative effects on growth and/or survival of seabirds (Drees 1994; Plentovich *et al.* 2009, 2011; DeFisher and Bonter 2013). Therefore, it is possible that the Argentine Ant is having negative consequences for the Red-tailed Tropicbird colony at Rano Raraku. Future research on this topic can provide valuable information to local authorities to evaluate the need to control Argentine Ants to help preserve this native seabird at Rapa Nui.

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