Breeding biology of the endangered Yellow Cardinal

Gubernatrix cristata in Brazil

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ABSTRACT: The Yellow Cardinal, Gubernatrix cristata, has a small geographic range in Brazil, Uruguay, and Argentina. We studied the natural history of the only known Brazilian population of this “Endangered” species (~50 individuals), which is socially monogamous and may breed cooperatively. During two breeding seasons (October to February 2013–2015), we monitored nests and described the main breeding traits. The breeding season started from the first week of October, with a peak of active nests in mid-November and lasted until mid-February. We found 32 nests, of which 30 were monitored. Female built the open-cup nests in six days (n = 1). All nests were built on Prosopis trees, on average at 2.4 m from the ground. Modal clutch size was three eggs (n = 19), and female incubated for 13 days on average (n = 7). Hatching rate was 76% and nestlings fledged after 16 days (n = 6). Nestling survival rate was 67% with mean productivity of 1.6 fledglings/successful nest. Shiny Cowbird, Molothrus bonariensis, parasitized nests of the Yellow Cardinal, with a frequency of 67% and intensity of 1.9 egg per parasitized nest (1–4 eggs; n = 13). Parasitism in nestlings by botfly larvae (Philornis sp.) occurred in 33% of nests with nestlings. Nest predation was the main cause of nest losses (73%) and we recorded a Geoffroy’s Cat, Leopardus geoffroyi, preying on a nest with eggs. The probability of success using the Program MARK was 13%. Fledgling survival rate during the first month outside the nest was 62% (8/13 fledglings). We found a longer breeding season, occurrence of second broods, and higher rates but fewer nest losses due to brood parasitism in comparison to the Argentinean population. We highlight the importance of natural history studies for the conservation of different populations of the same species.

KEY-WORDS: brood parasitism, Molothrus bonariensis, nest predation, nesting success.

INTRODUCTION

The Yellow Cardinal, Gubernatrix cristata, is a passerine that was more abundant in the past and now have a very fragmented distribution through northeast Argentina, Uruguay and southern Brazil (Ridgely & Tudor 2009). Records in Brazil were few and it was even thought that the species was possibly extinct in the country (BirdLife International 2018, Jaramillo 2019). Historical records are concentrated in southern and western Rio Grande do Sul state (Bencke et al. 2003). Nowadays, there is at least one established resident population in the Rio Grande do Sul with less than 50 individuals (Martins-Ferreira et al. 2013, Beier et al. 2017). Bird trapping and habitat loss led to a drastic population decline of the Yellow Cardinal in all its range (Dias 2008, Ridgely & Tudor 2009, Azpiroz et al. 2012, Martins-Ferreira et al. 2013).

Studies of breeding biology and life-history comparisons between populations enable early identification of threats, even before any evident population decline occur (Martin & Geupel 1993). Despite the decreasing trend of populations advised for the Yellow Cardinal (BirdLife International 2018), until recently its natural history was poorly known. Previous information about its breeding biology was limited to a few old nesting records (Castellanos 1932, Höy 1969), a nest description (de la Peña 1981), and the breeding status of collected specimens (Belton 1994). Domínguez et al. (2015) published the first study of the breeding biology of a Yellow Cardinal population in Corrientes province, Argentina. Some potential threats to breeding cardinals are nest predation, brood parasitism, botfly parasitism (Domínguez et al. 2015), hybridization (Bertonatti & López-Guerra 1997), and endogamy (Beier et al. 2017).

Shiny Cowbird, Molothrus bonariensis (hereafter, cowbirds), is a generalist brood parasite and its eggs were found in nests of 270 bird species (Lowsrth 2018), including Yellow Cardinal (Domínguez et al. 2015). The main impact of cowbirds that parasitize hosts with similar or larger body masses is the egg puncturing, which increases the probability of nest abandonment (Massoni & Reboreda 2002, Reboreda et al. 2003, Domínguez et al. 2015). Also, botfly larvae, Philornis sp. (Diptera), may infest nestlings of the Yellow Cardinal (Domínguez et al. 2015). Botflies put their eggs on nests of several bird species and their larvae infest the nestlings, feeding
mainly on blood cells for four to eight days until they drop out to pupate (Dudaniec & Kleindorfer 2006). Depending on the infestation intensity, i.e. the number of larvae infesting a nestling, it may have negative effects on nestling survival and increase nest abandonment after all nestlings died (Dudaniec & Kleindorfer 2006, Rabuffetti & Reboreda 2007).

We studied the breeding biology of the only known population of the Yellow Cardinal in Brazil. The main breeding traits such as breeding season, nest, eggs, clutch size, incubation, nestlings, brood parasitism, and nest survival were described.

### METHODS

#### Study area

We conducted this study in the municipality of Barra do Quaraí, state of Rio Grande do Sul, Brazil. Three study areas were in the Espinilho State Park (ESP), and one at São Marcos Ranch (SMR), adjacent to the ESP. The climate is classified as humid subtropical with hot summer (Cfa) in Köppen’s climate classification, with 1300 mm accumulated annual rainfall and 19°C mean annual temperature (Alvares et al. 2013; maximum 46°C was taken by a camera trap in December 2013). The vegetation is characterized by an area of insertion of Espinal Province (Cabrera & Willink 1973), which consists of grasslands with scattered thorny trees and shrubs, dominated by *Prosopis affinis* and *Vachellia caven* (Fabaceae). This is one of the last and the largest reminiscent of this vegetation in Brazil (Marchiori & Alves 2011). Three areas were grazed by cattle (Fig. 1).

We studied the breeding biology of the Yellow Cardinal from October through February (2013–2015), since nesting activity has not been recorded before October in a previous pilot study. Our dataset is the same used by Beier et al. (2017). We searched for adults at the beginning of each breeding season. Adults found were captured using mist nets and marked with a numbered aluminum ring (standard CEMAVE/ICMBio, the Brazilian Banding Agency) and a unique combination of colored plastic rings. We banded nestlings at 10 days of age or captured soon after fledging.

#### Nest search

We searched for nests only in areas with savanna vegetation mainly observing the mating pair, following the female and/or any individual carrying materials for nest building or feeding the nestlings. Each nest found was georeferenced and monitored every two or three days (rarely four or five days), when we recorded the nest status (active and inactive) and contents (number of eggs and/or nestlings), from the day that nest was found until it became inactive. When it was not possible to infer the exact day that a nest event occurred (laying, start of...

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Figure 1. Espinilho State Park with the localization of the four study sites in Barra do Quaraí, Rio Grande do Sul state, at the frontier of Brazil, Argentina, and Uruguay. Study sites B, C, and D were cattle grazed.
incubation, hatching, fledging or nest loss), we considered that it occurred halfway between nest observations.

Breeding season

We considered the breeding season length as the period of days between the beginning of the first nest construction and the day the last nest ended, for both breeding seasons together. To estimate the start of the first nest construction in the breeding season, we used the mean duration for each nest period (construction, laying, incubation, and nestling). We estimated nest building initiation date of the first nest based on the mean duration of nesting stages and plumage development of fledglings, for a mating pair found with two fledglings in November 2014. Last day of breeding period was estimated using the approximated date when the last active nest became inactive.

Description of nests and eggs

We described nest format and support type (as recommended by Simon & Pacheco 2005), supporting plant species, and materials of which it was composed. The nest construction period was considered from the placing of first materials on nest site until the first egg laying. Nests and eggs were measured only after they were abandoned, or when the egg failed to hatch, to avoid possible negative effects of nest manipulation. Nests were measured using a ruler (1.0 mm precision) and nest height was measured using a retractable ruler (1.0 cm precision). Egg measurements were taken using a caliper (0.05 mm) and weighed using a digital scale (0.01 g).

Clutch size, incubation, and nestlings

Clutch size was noted at all nests, excluding those with evidence of partial losses during egg laying. The clutch was considered completed after two consecutive visits without an increase in the number of eggs, and only from nests found during building, laying, or up to four days after incubation started (Lopes & Marini 2005). Incubation period starts after laying of the penultimate to the last egg until hatching, according to our observations and literature (Domínguez et al. 2015). Hatching rate was calculated as the ratio between the number of hatchlings and eggshells. Nests were considered as abandoned if nestslings too young to fledge vanished, including nests with eggshells. Nests were considered as abandoned if their contents remained with no sign of parental care. Other potential causes of nest losses were nestling death and brood parasite success. We installed camera-traps (Bushnell Trophy Cam HD) at eight nests to identify nest predators, during incubation and nestling periods, 5–10 m far from the nests, and recorded a total of 12.5 h of video. A nest was considered successful when at least one cowbird egg from the total of nests that completed laying. For calculating brood parasitism intensity, we used the mean number of cowbird eggs per parasitized nests, considering only nests without partial clutch losses. Prevalence of botfly parasitism was calculated as the ratio between the number of parasitized nests and the number of nests where nestlings were at least 5 days old.

Nest survival

We considered the nest as predated when eggs or nestlings too young to fledge vanished, including nests with eggshells. Nests were considered as abandoned if their contents remained with no sign of parental care. Other potential causes of nest losses were nestling death and brood parasite success. We installed camera-traps (Bushnell Trophy Cam HD) at eight nests to identify nest predators, during incubation and nestling periods, 5–10 m far from the nests, and recorded a total of 12.5 h of video. A nest was considered successful when at least one cowbird egg from the total of nests that completed laying. For calculating brood parasitism intensity, we used the mean number of cowbird eggs per parasitized nests, considering only nests without partial clutch losses. Prevalence of botfly parasitism was calculated as the ratio between the number of parasitized nests and the number of nests where nestlings were at least 5 days old.
Statistical analysis

To assess differences on mean time interval and distance between successful and unsuccessful nesting attempts we used non-parametric Mann-Whitney U-test (or Wilcoxon rank sum test). We also used Z-test to assess differences of DSR and DSR’ between incubation and nestling stages and parasitized and unparasitized nests. We used R software (R Core Team 2015) to run the statistical tests. Values are presented as mean ± SD and considered statistically significant when $P < 0.05$.

RESULTS

Nest search and breeding season

We found 32 nests, 14 in 2013–2014 of nine breeding pairs and 18 in 2014–2015 belonging to 12 pairs. Two nests found during construction were apparently abandoned (never seen with eggs). Of the 30 remaining nests, six were found during nest-building, eight during egg laying, 13 during incubation, and three nests with nestlings. Both individuals of the pair were marked in 13 nests, only the male was marked in 13 nests and in four nests only the female was marked. We estimated that the beginning of nesting season was 03 October and the last nest became inactive on 12 February, totalizing 131 days. The peak of active nests was in late November (Fig. 2).

Description of nests and eggs

Nest building was performed by females but closely followed by males. The nest is a high cup/fork, with an external layer of twigs (*Prosopis* spp.) and an inner layer of thinner sticks, grass, and other plants, lined with filamentous plants and horse/cattle hair. The nest was built in six days ($n = 1$), considering the period from the first twigs until laying of the first egg. Mean nest height was $2.43 ± 0.67$ m (1.07–4.43 m, $n = 30$). Nests were built mostly in *Prosopis affinis* trees (97%), and one nest was built in *P. nigra*. Eggs were ovoid with a light bluish-green background color and black spots (sometimes it could have a few streaks) distributed over the entire surface or concentrated at the blunt pole (Figs. 3A & C). Mean egg mass was $3.84 ± 0.15$ g and measured $24.7 ± 1.1$ mm in length and $17.7 ± 0.6$ mm in width (seven eggs at different ages from three nests).

Clutch size, incubation, nestlings and fledglings

Mean clutch size was $2.95 ± 0.52$ eggs (2–4 eggs; mode = 3 eggs; $n = 19$), laying one egg/day. The incubation was initiated on the penultimate egg laid, lasted 12.9 ± 0.9 days (12–14 days; $n = 7$) and only the female incubated the eggs. Hatching rate was $0.76 ± 0.25$ ($n = 14$ nests) and there was no difference between unparasitized and parasitized nests (unparasitized: $0.80 ± 0.16, n = 6$; parasitized: $0.73 ± 0.31, n = 8$; $U = 23, P = 0.95$).

Hatchlings (Fig. 3B) were orange skinned, with light grey down feathers on the head and back, red-carmine mouth, yellowish gape, and opened their eyes around seven days old. The nestlings (Fig. 3D) remained in the nest for 16.0 ± 1.3 days (15–18 days; $n = 6$). Nestling survival rate of successful nests was $0.67 ± 0.28$ ($n = 6$). Mean productivity was $1.6 ± 0.7$ (1–3) fledglings per successful nests ($n = 8$) and $2.6 ± 1.8$ (1–5) fledglings per female ($n = 5$). Fledglings had bare nape and belly, with remaining grey down feathers on the crown; greyish and streaked breast, yellowish margins in wing feathers; flight and tail feathers not fully developed; yellowish gape. Fledglings at the age of 35–40 days from hatching were muddy forms of the adult female (Figs. 3E & F). The fledgling survival rate was 62% (8/13 fledglings).

![Figure 2. Number of estimated nests of Yellow Cardinal by nesting stage (laying/incubation: white bars; nestling stage: grey bars), total active nests (black bars), and nests parasitized by cowbirds (dots) by two-week intervals during two breeding seasons (2013–2015) in Barra do Quaraí, Rio Grande do Sul state, Brazil. Data from both breeding seasons were combined.](image-url)
We observed up to three nesting attempts performed by the same female (two females). From all nesting attempts, 40% was renesting, 35.7% (5 of 14) in 2013–2014 and 43.8% (7 of 16) in 2014–2015 breeding seasons, eight pairs did one renesting attempt and two pairs did two renesting attempts. We observed renesting after a successful attempt (n = 2) in two breeding pairs with helpers. Other two renesting occurred after successful attempts, but the fledglings of the previous nests did not survive the first month after fledging, then we did not consider them as second broods. Mean interval between renesting attempts was 15.6 ± 10.1 days (6–36 days, n = 11), and the interval was significantly longer after successful (n = 4) than after unsuccessful (n = 7) attempts (25.2 ± 10.3 days vs. 10.1 ± 4.3 days; U = 1.5, P = 0.02). Mean distance between renesting attempts was 220.75 ± 86.36 m (99–330 m, n = 12), and there was no difference between successful (n = 4) and unsuccessful (n = 8) attempts (174.75 ± 74.58 m vs. 243.75 ± 86.74 m; U = 7, P = 0.15).

**Brood parasitism**

The frequency of brood parasitism was 67% (20/30). The intensity of parasitism was 1.9 ± 1.3 eggs per parasitized nest (1–4 eggs, n = 13). Three cowbird eggs measured 25.1 ± 0.6 mm in length, 20.4 ± 0.1 mm in width and weighed 5.23 ± 0.12 g. Parasite eggs were white or creamy, completely covered with brown spots, except for one egg...
that was white with a single brown spot (Figs. 3B & C). Cowbird eggs were never rejected. We found punctured host eggs in 15% (3/20) of parasitized nests, and then the owners abandoned the nest. Punctured eggs were often consumed by ants. In four parasitized nests (20%, 4/20) cardinals were successful (unparasitized successful nests: 40%, 4/10). Even on nests where cowbirds hatched \((n = 8)\), in three (37.5%), the cardinals were successful and in only one (12.5%) the two cowbirds fledged (without cardinal fledglings). In three cases, the cowbird nestling hatched about three or four days after cardinals, and was seen in only one nest revision, then disappeared. In one nest we found the corpse of the cowbird nestling at the bottom of the nest after the cardinals fledged.

**Botfly parasitism**

Prevalence of botfly parasitism was 33% of nests \((6/18)\). In one nest, nestlings were only cowbirds and they were predated. The earliest nest with botflies was recorded on 07 November and the latest on 18 December. Four botfly-parasitized nests \((80%, n = 5)\) were successful and one nest was lost with the nestling death, for which we were not able to determine the cause. The intensity of botfly parasitism was in average \(12.7 \pm 5.5\) larvae/nestling were not able to determine the cause. The intensity of botfly parasitism was in average \(12.7 \pm 5.5\) larvae/nestling were not able to determine the cause. The intensity of botfly parasitism was in average \(12.7 \pm 5.5\) larvae/nestling were not able to determine the cause.

Nest survival

Only eight nests (26.7%, \(n = 30\)) were successful, four (28.6%, \(n = 14\)) in 2013–2014 and four (25.0%, \(n = 16\)) in the 2014–2015 breeding seasons. The main cause of nest loss was predation (73%), followed by egg puncture (14%), nestling death (9%) and nest parasite success (4%) \((n = 22)\). One Geoffroy’s Cat \((Leopardus geoffroyi)\) was recorded by the camera trap preying on a nest with eggs. The nest was about 1.8 m above ground, in a fork of the tree trunk. We did not record any other predation attempt or identified other predators.

The DSR was 0.938 ± 0.018 during incubation and 0.948 ± 0.016 during the nestling stage. The DSR during incubation was 0.431 ± 0.105 \((n = 193\) nest-days, 12 nest losses) and during the nestling stage was 0.425 ± 0.117 \((n = 193\) nest-days, 10 nest losses). The Mayfield Nesting Success was 18.3% (23.65% in 2013–2014 and 12.32% in 2014–2015). There was no difference between the unparasitized and parasitized nests during incubation \((0.492 \pm 0.178, n = 76\) nest-days, 4 nest losses vs. 0.395 ± 0.132, 117 nest-days, 8 nest losses; \(Z = 0.44, P = 0.66\)) and nestling stage \((0.485 \pm 0.179, n = 91\) nest-days, 4 nest losses vs. 0.391 ± 0.154, 106 nest-days, 6 nest losses; \(Z = 0.40, P = 0.69\)). For *Philornis* parasitism, the DSR during the nestling stage was 0.279 ± 0.129 \((n = 105\) nest-days, 8 nest losses) for unparasitized and 0.691 ± 0.183 \((n = 88\) nest-days, 2 nest losses) for parasitized nests, and there was no significant difference between them \((Z = 1.84, P = 0.07)\). We found a DSR of 0.937 ± 0.013 (SE) and a DSR of 0.134, using Program MARK.

**DISCUSSION**

The natural history of the Yellow Cardinal was poorly known until recently when a study was conducted in Argentina (Domínguez *et al.* 2015), relatively close to our study site (at two sites, about 130 and 180 km northwest, respectively). Despite the geographic proximity, we found some differences: a longer breeding season, occurrence of second broods, and higher frequency and intensity but fewer nest losses due to brood parasitism. Domínguez *et al.* (2015) conducted their study prior (2011–2012) to ours (2013–2015), so we cannot determine whether the differences found were related to temporal or spatial factors. Additionally, we have already reported cooperative breeding in the Brazilian population (Beier *et al.* 2017), which was not reported in other populations (Domínguez *et al.* 2015, Segura *et al.* 2019).

We found higher frequency (67%) and intensity \((2\) eggs/parasitized nest) of brood parasitism by cowbirds than in Argentina (33%, 1 egg/parasitized nest; Domínguez *et al.* 2015). Despite that, only 18% of nest losses were due to cowbird parasitism in our study. While in Argentina nest abandonment due to egg puncturing by female cowbirds (54% of parasitized nests) represents a threat of concern (Domínguez *et al.* 2015), in our study, it seems to have lesser importance (15% of parasitized nests). Shiny Cowbird has an incubation period of 11–12 days (Fraga 2011), very similar to the Yellow Cardinal (13 days), and cowbird nestlings disappeared from nests where cardinals hatched first. Cowbirds hatchlings may not get enough food competing with cardinal nestlings three or four days older, or the host parents rejected the parasite, for which we do not have other evidence. Brown-headed Cowbird, *Molothrus ater*, nestlings are more successful in nests of hosts with similar or intermediate body size...
(Kilner 2003), which is the case for Yellow Cardinal (47 g; Beier et al. 2017) and Shiny Cowbirds (45–51 g; Fraga 2011), but we did not find nests where both, host and parasite, were successful.

Prevalence of bothy parasitism was greater in Brazil (33%) than in Argentina (22%), occurred during a longer period (~40 days vs. 14 days), and apparent success was higher on infested nests (80%, n = 5 vs. 50%, n = 4; Domínguez et al. 2015). Domínguez et al. (2015) found no significant difference in chick survival between parasitized (25%) and unparasitized nests (78%). In our study, some fledglings disappeared a few days after they fledged, which we assumed that they died, based on their developmental stage. Ectoparasites may delay the development of the nestling, and even when they do not affect the nestling success, they could decrease the post-fledgling survival (Stréby et al. 2009).

The breeding season was around 45 days longer in Brazil than in Argentina (Domínguez et al. 2015). A longer breeding season could mean more nesting attempts (Ricklefs & Bloom 1977) and may be a strategy to compensate high rates of nest predation (Slagsvold 1984, Martin 1996, 2014, Di Giacomo et al. 2011), which in our study accounted for 73% of nest losses. Unless it is too late in the season, females will always renest after nest predation and the number of nesting attempts will be determined by the number of days lost in the breeding season for each nest lost (Schmidt & Whelan 1999). We observed more renesting attempts (40% of all nesting attempts) and longer mean interval between attempts (16 days), but less attempts per pair during a breeding season (up to three attempts) than in Argentina (30%, 12 days, and up to four attempts per pair; Domínguez et al. 2015).

The Yellow Cardinal have biparental care, and some nests (n = 7) were also attended by helpers, which contributed to brood provisioning and territorial defense (Beier et al. 2017). Cooperative breeding in this population may be a response to high rates of nest predation and brood parasitism, and/or habitat saturation (e.g., Manica & Marini 2012, Beier et al. 2017). Only mating pairs with nest helpers had second broods, probably due to the extra food and vigilance provided by them. Cooperative breeding may also allow reducing female investment on egg yolk (Russell et al. 2007, Paquet et al. 2013), for which we do not have information for the Yellow Cardinal.

In a pilot study in 2012, we found two nests (n = 8) on cactus Cereus hildmannianus (C.B., M.S. Pereira & M.S. Borba pers. obs.), but all nests of the present study were found on Prosopis trees. Domínguez et al. (2015) also found more nests on Prosopis affinis (76%), followed by 15% on Vachellia caven. Although V. caven occurs in our study area, we did not find any nest on that tree species. The preference to nest on a supporting plant species may be related to nest concealment (Martin & Roper 1988, Martin 1993) or to the most common potential nest site, reducing predation probability (Martin 1993, Liebezeit & George 2002).

The Yellow Cardinal and other threatened bird species are also associated with short grass, which is maintained mostly by cattle grazing in our study area (Pereira 2015). The removal of cattle may lead to the development of taller grass and shrub encroachment, and consequently, ground feeding birds could be evicted from this area. Conservation schemes must consider vegetation management to prevent potential impacts on populations of ground-foraging birds.

The natural history of many bird species is still poorly known, if not completely unknown, especially in the Neotropics (Stutchbury & Morton 2001, Xiao et al. 2017). Various of these species are threatened and the knowledge on their natural histories is of utmost importance to their conservation. Even across distinct populations of a single species, there are remarkable differences, such that each of these populations should be considered as a single unit for the conservation of genetic, ecological and cultural variability, as it is for the Yellow Cardinal (Domínguez et al. 2016, 2017). Our study highlights the importance of autecology studies in different populations of a single species, to allow a better understanding of variations in spatial-temporal patterns and processes and their implications for species and ecosystems conservation.

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