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Research Article

Genetic Assignment and Monitoring of Yellow Cardinals

MARISOL DOMINGUEZ,[1] *Laboratorio de Ecología y Comportamiento Animal, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Instituto IEGEBA UBA-CONICET, Universidad de Buenos Aires, Pabellón II, Ciudad Universitaria, C1428EHA, Argentina*

GIMENA PIZZARELLO, *Laboratorio de Ecología y Comportamiento Animal, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Instituto IEGEBA UBA-CONICET, Universidad de Buenos Aires, Pabellón II, Ciudad Universitaria, C1428EHA, Argentina*

MELINA ATENCIO, *Laboratorio de Ecología y Comportamiento Animal, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Instituto IEGEBA UBA-CONICET, Universidad de Buenos Aires, Pabellón II, Ciudad Universitaria, C1428EHA, Argentina*

ROMINA SCARDAMAGLIA, *Laboratorio de Ecología y Comportamiento Animal, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Instituto IEGEBA UBA-CONICET, Universidad de Buenos Aires, Pabellón II, Ciudad Universitaria, C1428EHA, Argentina*

BETTINA MAHLER, *Laboratorio de Ecología y Comportamiento Animal, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Instituto IEGEBA UBA-CONICET, Universidad de Buenos Aires, Pabellón II, Ciudad Universitaria, C1428EHA, Argentina*

ABSTRACT The yellow cardinal (*Gubernatrix cristata*) is a passerine bird endemic to southern South America. At present, the species is threatened with extinction, in part because of the capture of wild birds to supply the illegal caged-bird market. Previous genetic evidence supports the existence of 3 management units for the species in Argentina, where the largest populations are currently found. Our objectives were to guide the reintroduction of these animals to their respective management units by determining the origin of seized specimens from illegal trade using 2 molecular markers, mtDNA and microsatellites, and to monitor the success of the released birds through radio-telemetry. We compared the performance of different molecular markers and assignment approaches to optimize a technique capable of assigning the origin of confiscated yellow cardinals in a reliable way. Five of 10 released radio-tracked individuals were predated shortly after liberation; however, 3 were successful in finding a mate and starting reproductive activities. Individual success was independent of the time spent in captivity, the liberation with a partner, the settlement type (semi-open or closed), and the maximum distance traveled from the point of release. Cardinals that survived had higher individual heterozygosity. Our findings contribute a robust genetic assignment technique to be used in future yellow cardinal seizures and identify factors that might improve subsequent releases. © 2019 The Wildlife Society.

KEY WORDS endangered, *Gubernatrix cristata*, monitoring, releases, yellow cardinal.

Wildlife trafficking is the third most profitable illicit commerce in the world, after drugs and weapons, and is estimated at US$10 billion a year (Haken 2011). Birds are the most commonly trafficked taxa, with 2–5 million wild birds illegally traded every year (Bush et al. 2014). Latin America is one of the most biodiverse regions in the world, yet little is known about the scale, methods, and perpetrators of wildlife trafficking in this region (Reuter and O'Regan 2016). Illegally traded wildlife is confiscated yearly by authorities and because of a lack of existing management strategies, these individuals are kept in rehabilitation centers for the rest of their lives. However, these institutions have a limited capacity that is often outweighed by the volume of seizures, which prompts the need to take strategic measures that deal with these animals.

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[1]*E-mail: soldominguez@ege.fcen.uba.ar*

One option for these individuals is the movement and release into an existing population of conspecifics, termed reinforcement (International Union for Conservation of Nature [IUCN] 2013), which has become an integral part of many endangered species programs (Griffith et al. 1989, Bright and Morris 1994, Fischer and Lindenmayer 2000, Brichieri-Colombi et al. 2018). In cases of highly threatened species, these actions may offer the only chance for survival (Hayward et al. 2007*a*, *b*); however, the success of these programs should be thoroughly monitored during all stages (including pre-release, release, and post-release; Letty et al. 2007, Sutherland et al. 2010, IUCN 2013) to improve their effect on species viability.

The yellow cardinal (*Gubernatrix cristata*) is a South American passerine, and the only representative of the monotypic genus *Gubernatrix* of the family Thraupidae (Barker et al. 2013). Its historical distribution encompasses the southern tip of Brazil (Rio Grande do Sul), Uruguay, and central Argentina (Ridgely and Tudor 2009, BirdLife International 2018), where the largest natural populations

are currently found (Birdlife International 2018). The main causes of decline of yellow cardinals are habitat loss due to wood extraction and the advancement of agricultural activities, and the capture of individuals in wild populations to stock the illegal caged-bird market (Ortiz and Aceñolaza 2008). The species is categorized as endangered because of the accelerated decline in population size and the fragmentation of remnant populations (BirdLife International 2018). A previous study that analyzed the genetic structure of natural populations of yellow cardinals in their current distribution using neutral nuclear and mitochondrial molecular markers supports the existence of 3 genetically distinct management units (MUs; Moritz 1999) in Argentina (Domínguez et al. 2017; Fig. 1). This previous information can be used to genetically assign individuals to a specific MU (Waser and Strobeck 1998).

A collaboration of government and non-governmental organizations outlined a management plan that deals with yellow cardinals confiscated from the illegal wildlife traffic. Animals seized by governmental offices were sanitarily rehabilitated by Temaiken Foundation, and liberations were planned using presence data provided by Aves Argentinas. Our objectives were to genetically determined the origin of confiscated individuals to guide their release into their respective MUs and evaluate the success of this conservation action with a post-release monitoring program.

STUDY AREA

We carried out the field study in La Pampa province in Argentina (36° 48' S; 64° 37' W; Fig. 1) during the breeding season of 2017. The study site encompassed a private field of 1,320 ha used for livestock ranching characterized by the presence of thorny shrubland forests dominated by calden mesquite (*Prosopis caldenia*) with variations in vegetation cover.

METHODS

Genetic Procedures to Identify Origin

DNA extraction and amplification.—In 2016 and 2017, we analyzed blood and feather samples from 109 confiscated yellow cardinals. The samples and the permissions to transport them to our laboratory were provided by Secretaría de Ambiente y Desarrollo Sustentable. We extracted DNA using a Qiagen extraction kit (Hilden, Germany). We amplified a 736 base-pair fragment of the mitochondrial DNA control region using primers LCR3 and H1248 (Tarr 1995) following the protocol detailed in Domínguez et al. (2017). We purified amplification products with the ExoSAP method and sequenced them in a genetic analyzer (3130xl; Applied Biosystems, Foster City, CA, USA) at the Instituto Nacional de Tecnología Agropecuaria. We edited and aligned mitochondrial DNA sequences using Bioedit (version 7.0.5.3; Ibis Therapeutics, Carlsbad, CA, USA) and compared them to reference populations (Domínguez et al. 2017) to establish the haplotype for each confiscated individual.

We genotyped 10 microsatellite loci following the protocol detailed in Domínguez et al. (2017). We used the above-mentioned sequencer to size the fluorescently labeled polymerase chain reaction products and software

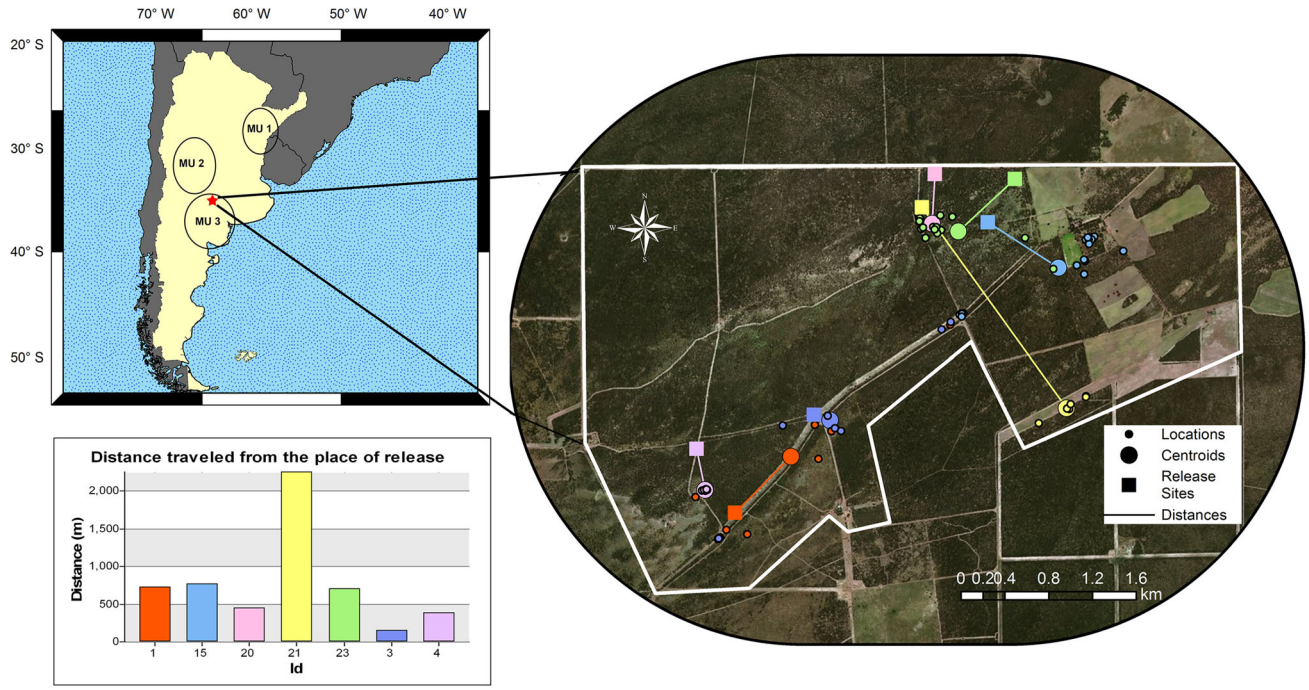

Figure 1. The reference map (upper left) shows the location of the study site and of the 3 management units (MUs) found for the yellow cardinal in Argentina, 2017. Study area (white delimited polygon, upper right) including the site where each radio-tagged yellow cardinal was released (squares), the locations where each bird was observed (small circles), and the centroid of their 95% kernel density estimate territory (big circles). Each color symbolizes a different male. Distances traveled from the place of release to the centroid are shown in the bar graphic and as lines in the map.

Peak Scanner version 1.0 (Applied Biosystems) and GeneMarker version 2.6.7 (Soft Genetics, State College, PA, USA) to determine allele sizes.

Assignment tests.—Assignment tests give the probability of an individual's multilocus genotype of belonging to a certain population within a set of populations (Iyengar 2014). We used the Bayesian clustering approach of Structure version 2.3.4 (Pritchard et al. 2000), applying the same parameters previously determined for the natural populations grouped in 3 clusters ($k = 3$), with a burn-in of 200,000 steps, 1,500,000 Markov chain Monte Carlo iterations, and each simulation repeated 10 times (admixture level found between natural populations = 0.1936; Domínguez et al. 2017). We inferred the ancestry of seized individuals with the Popflag model (Pritchard et al. 2000). We plotted likelihood values using the online program Structure Harvester (Earl and vonHoldt 2012) and averaged the estimated cluster membership coefficient matrices of the multiple runs of Structure using a FullSearch algorithm in CLUMPP software (version 1.1.2, https://rosenberglab.stanford.edu/clumpp.html, accessed 16 Jun 2018). We visualized the output from CLUMPP using the program Distruct (version 1.1, https://rosenberglab.stanford.edu/distruct.html, accessed 16 Jun 2018). We also analyzed the multilocus genotype with Geneclass 2.0 (http://www1.montpellier.inra.fr/CBGP/software/GeneClass/GeneClass2/Setup.htm, accessed 18 Aug 2018) using the Bayesian criterion of Rannala and Mountain (1997), which is based on a frequency distribution that estimates the probability of the multilocus genotype obtained for each individual of belonging to each reference population (Holbrook et al. 2012).

Given that programs Structure and Geneclass2 allot a probability of assignment of each individual to a certain population, we established a threshold value above which assignment was considered reliable. To obtain this threshold value, we used the multilocus genotypes of the individuals sampled in natural populations by Domínguez et al. (2017). For both programs we carried out jackknife resampling, leaving out 1 individual as if its origin was unknown in each run and then obtaining the percentage of assignment of this individual to the 3 previously established MUs. We built confusion matrices for different threshold values, including all individuals that scored above that threshold. From those matrices, we calculated the number of assigned yellow cardinals for each threshold value, the number of animals that were incorrectly assigned, and the percentage of correct assignments calculated as the quotient between the sum of individuals correctly assigned to their MU and the number of assigned individuals (assigned correctly and incorrectly). We established the optimal threshold value for each program considering type I error (an individual not reassigned to its population of origin) and type II error (an individual assigned to the wrong population; Negrini et al. 2009, Kurushima et al. 2013). We considered animals with values below this threshold to be unassigned. For each program we chose the threshold value that best reflected a compromise between maximizing the number of assignments and minimizing the incorrect ones (Figs. S1 and S2, available online in Supporting Information).

To compare the genetic characteristics of yellow cardinals that had survived at the end of the monitoring period versus the ones that did not, we estimated individual heterozygosity for birds with a complete genotype as the proportion of heterozygous loci in an individual. Then, we performed a Wilcoxon non parametric test to compare heterozygosity between both groups. We performed this analysis in Program R version 3.4.3 (www.r-project.org, accessed 8 Feb 2017).

Post-Release Monitoring

Release site.—We monitored a subset of animals that were released in La Pampa province, Argentina (36° 48' S; 4° 37' W) on 27 September 2017 (procedures approved by Dirección de Fauna de la Provincia de La Pampa), according to previous genetic assignment to that MU. The 26 released cardinals (7 females and 19 males) had been confiscated by Argentinean authorities from illegal pet trade in procedures that took place at different dates and in different locations; thus, the birds spending unequal time periods in captivity prior to release. The Temaiken Recovery Center held birds until release and provided rehabilitation and physical training to ensure their flight ability. Medical personnel monitored weight of the birds, took x-rays, and examined birds for the presence of infectious diseases and parasites. Sanitary and physical conditions were optimal at release. Staff of the Temaiken Recovery Center transported yellow cardinals to the release site by truck at night to reduce the negative effects of stress by minimizing exposure to daytime temperatures and avoiding the times of greater bird activity. The trip from the rescue center to the release site lasted 9 hours, including stops every 2 hours to check the animals. The birds were transported in wooden cages, divided into 7 individual compartments of $17 \times 15 \times 20$ cm with a sliding top lid. Individuals were last fed the afternoon prior to release. To determine potential release sites, we used information provided by Aves Argentinas from their annual yellow cardinal census program and identified areas that presented connectivity with other yellow cardinal populations. Local authorities selected the final field site based on the feasibility to implement control actions that minimize the risk of illegal re-capture of the released birds. Within the release site, we identified suitable areas based on vegetation type and density, avoiding overlap with territories of wild yellow cardinals.

We banded each confiscated cardinal with a unique color-ring combination for future individual identification. Additionally, we fitted 12 males with radio-transmitters weighing 1.0 g (model A1055; Advanced Telemetry Systems, Isanti, MN, USA), which corresponds to < 3% of cardinals' mass, with an 88-day lifespan and a range of detection of approximately 800 m considering the characteristics of our study area. We released 6 of the radio-tagged males with a female. We carried out the releases during the morning, immediately after arrival, with optimal weather conditions (no rain, 17°C average temp). We released birds every 500 m, given that the minimum distance between territories in this species is approximately

200 m (Domínguez 2015). For each release, we removed the top lid of the cage so that we opened only 1 compartment at a time. In the cases where we released the animals as couples, we opened 2 compartments. Upon release, we verified the identity of each individual by checking the ring combination and radio-transmitter frequency for those that were radio-tagged. We released the 7 females with the males with which they shared the aviary during rehabilitation, whereas we released the rest of the cardinals individually.

Post-release monitoring.—We carried out radio-tracking on foot for 21 consecutive days in the morning (0700–1200) and in the afternoon (1600–1900). We tracked each radio-tagged bird daily using a Yagi antenna and a hand-held receiver (model Sika; Biotrack, Wareham, United Kingdom). We tracked the cardinals until we achieved visual contact and recorded the location using a global positioning system device (eTrex Legend HCx; Garmin, Olathe, KS, USA). We also described the area where we found the birds as open, semi-open, or closed, according to vegetation density. We designated areas where trees or shrubs were < 2 m apart as closed, areas with trees or shrubs situated > 2 m apart from each other with small open areas between them as semi-open, and savannah-like areas with few scattered trees as open. At the end of the breeding season, we returned to the study site for 8 days (5–13 Nov) to monitor the yellow cardinals and recapture those that were still alive to remove the radio-transmitters. We opportunistically re-sighted ringed untagged birds while conducting radio-telemetry.

Geographical analysis.—We estimated individual ranges through a kernel density estimate (KDE) using ArcMap 10.3 (Environmental Systems Research Institute, Redlands, CA, USA) and Geospatial Modelling Environment version 0.7.4 (Beyer 2010). We obtained data for ≥ 3 days of tracking for 1 ringed and 6 radio-tagged cardinals. We excluded from our analysis 7 birds from which we had geographical information for only 1 or 2 days. We calculated 95% and 50% KDE estimates to determine individual ranges and core areas used during the monitoring period. For each individual, we also analyzed how cumulative area changed as a function of days passed from release to see if and when birds settled in an area or if they continued exploring the environment. We then calculated the distance between the centroid of each cardinal's 95% KDE and the release location.

Survival analysis.—We used generalized linear models (GLMs) with binomial distributions to assess whether survival of the monitored birds (0 = did not survive to the end of the breeding season, 1 = survived to the end of the breeding season) was affected by the time they spent in captivity prior to release, the type of settlement area (open, semi-open, or closed), or the distance between the centroid of each cardinal's 95% KDE and the release site. We used a data set of 10 radio-tagged cardinals that we monitored from their release until they were either successful or died. We considered an individual predated or scavenged when we found feathers or body parts attached to the radio-transmitter or within the area where we last found the bird. No inference on predator type was possible. Time spent in captivity prior to release included 3 categories determined by the date of confiscation: short (< 1 yr), medium (1–2 yr), and long (> 2 yr). We defined type of settlement area by the vegetation density of the place where it was found most frequently (or found last in cases where we found the individual dead). We checked normality of the residuals for all models. We conducted these analyses using the lme4 package (Bates et al. 2015) in Program R.

We modeled daily survival rate (DSR) of the 10 birds using package RMark (Laake 2013) in R to explore the influence of the 3 covariates. We assessed 3 single-variable models, 3 2-variable models, and a null model without covariates. We used Akaike's Information Criterion corrected for small sample sizes (AIC_c) and the associated Akaike weights (w_i) to evaluate support for competing models within the set of candidate models (Burnham and Anderson 2002). We considered that models with AIC_c differing by ≤ 2 units and $w_i \leq 0.90$ were equally supported by the data (Burnham and Anderson 2002).

RESULTS

Genetic Analysis

We successfully identified the haplotypes belonging to 104 of the 109 confiscated yellow cardinals and found 1 new haplotype (not sampled in the natural populations). For 78 of the confiscated animals, we obtained their complete multilocus genotype, whereas for 23 birds we could amplify 9 out of 10 microsatellite loci, for 7 birds 8 of the 10, and for 1 bird only 7 of the 10 loci.

We determined threshold values upon which an assignment was reliable by establishing the cut-off values where correct assignment did not improve in relation to miss-assignment and the curve of this proportion reached a plateau (Tables S1 and S2). It corresponded to 65% for Structure and 70% for Geneclass.

We assigned most of the confiscated individuals to 1 of the 3 MUs (95/109). For 13 individuals, multilocus assignment values were below the threshold and therefore only a western origin (Domínguez et al. 2017) could be established based on the mtDNA haplotype identity. For the individuals with assignment values above the threshold, haplotypes were concordant with the assigned MU. One individual was left unassigned because of an unidentified haplotype and contradictory information given by the assignment programs. Assignment was independent of incomplete genotyping. Of the 13 unassigned individuals (with below-threshold values), 10 were completely genotyped and 3 missed ≥ 1 loci. All of the seized cardinals except for 1 belonged to the western region of the species' distribution (5 were assigned to MU2 and 89 to MU3; Fig. 2).

Individual heterozygosity ranged from 0.2 to 0.9 and was marginally non-significant between cardinals that survived and the ones that did not survive ($W = 11.5$, $P = 0.074$). The average heterozygosity of those who survived was twice the heterozygosity of those who died (Fig. 3).

Release and Monitoring

By the end of the second post-release monitoring period, 2 of the 12 radio-tagged individuals were never found, 5 were predated, and 1 was found dead with no signs of predation. Of the 4 cardinals that survived, 3 paired up with females (1 with a released female and 2 with wild females) and 2 of these pairs built nests (Table 1).

Regarding the 14 yellow cardinals that were released but not fitted with radio-transmitters, we observed only 2 females and 1 male during monitoring events. One of the females (identification number [ID] 20) paired up with a radio-tagged cardinal (ID 23), different from the one at release (ID19; Table 1). We saw the remaining male and female separately only in 2 of the days.

Mean size of individual range was 105.88 ha (range = 0.21–341.90 ha). Core areas also varied greatly between birds, with an average of 26.91 ha (range = 0.05–92.70 ha). Only 3 cardinals established a home range at the end of the monitoring period (Fig. 4), considering that the cumulative area reached an asymptote and did not change in at least the last 3 observations. These corresponded to 1 pair (female: ID 20; male: ID 23) and 1 male (ID 15) that built nests. The distance traveled from the release site to the centroid of each cardinal's 95% KDE varied from 157 m to 2,258 m (Fig. 1, Table 1, Video 1, available online in Supporting Information). Cardinals released in pairs separated shortly after they were released.

The GLM analysis did not indicate that probability of survival was related to any of the explanatory variables (time in captivity: $P = 0.24$, type of settlement area: $P = 0.74$, maximum distance traveled: $P = 0.51$). Additionally, DSR analysis indicated that all the models tested were equally supported by the data (Table S1, available online in Supporting Information).

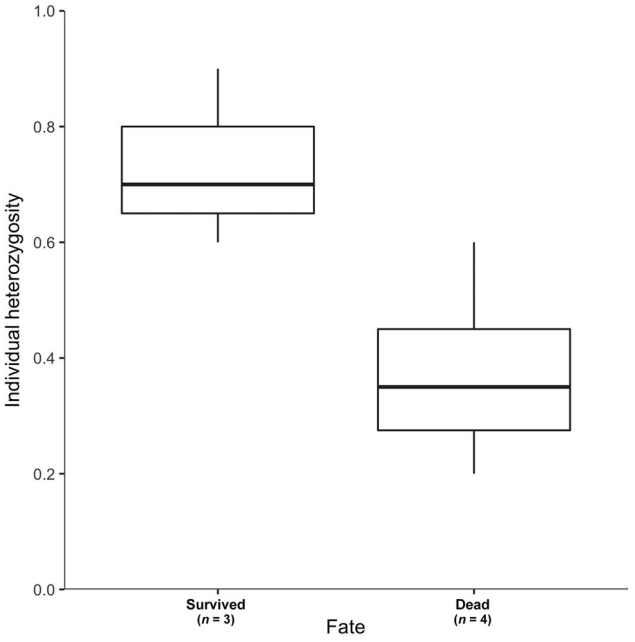

Figure 3. Boxplots of the average heterozygosity levels of the group of yellow cardinals that survived versus the group that died at the end of the monitoring period in Argentina, 2017. Box plots show the 25th to 75th percentiles (boxes), medians (thick lines within boxes), and the maximums and minimums (vertical lines).

DISCUSSION

Genetic Assignment

The genetic assignment method proposed in this study can greatly improve the success of establishment after liberation for many animals. Chances of survival and reproductive success increase when individuals are released in the environment where they were captured (Wanjtal and Silveira 2000). This is partly explained by the familiarity of an individual to the environment, predators, and food sources. Also, local adaptations might be present in different areas of the distribution (Slatkin 1987). Particularly in birds, song dialects are critical in attracting females, and male success is related to song recognition (Podos 2007). In this sense, it has been shown that the yellow cardinal's genetic MUs also exhibit different dialects (Domínguez et al. 2016). Thus, incorporating genetic analysis into a management plan could help assigning the unknown origin of many confiscated cardinals kept in captivity and increasing their chances of survival.

We determined reliable thresholds for both genetic assignment methods. These can be used in a complementary way because the approaches of both programs differ. Geneclass 2.0 is not specifically designed to identify individuals whose genomes possess a mixed ancestry (admixture); thus, cases of low assignment percentage can

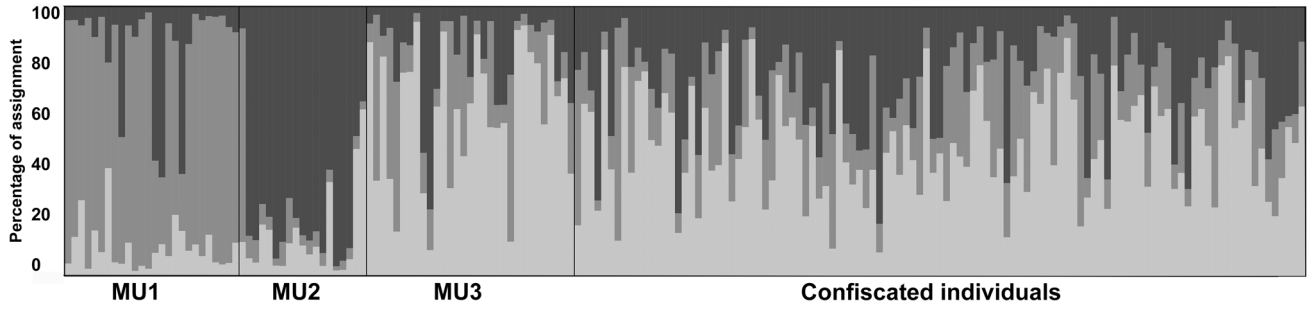

Figure 2. Percentage of assignment of confiscated individuals (each one is represented by a vertical bar) to the 3 clusters (management units MU1–MU3) identified by Structure in the natural populations of yellow cardinals, Argentina, 2017. Bars above MU1, MU2, and MU3 correspond to the assignment of origin of individuals from the natural populations studied in Dominguez et al. (2017). Bars above confiscated individuals represent the confiscated poached yellow cardinals analyzed in this study.

Table 1. Fate and characteristics for monitored yellow cardinals (10 males, 1 female: identification [ID] number 20) in La Pampa, Argentina, 2017. Captivity refers to the time spent in captivity prior to release and includes 3 categories determined by the date of confiscation: short (<1 yr), medium (1–2 yr), and long (>2 yr).

ID	Fate	Nest	Captivity	Type of settlement area	Max. distance from released site (m)	Survival (days)
1	Survived	No	Long	Semi-open	725	>45
15	Survived	Yes	Medium	Semi-open	766	>45
20[a]	Survived	Yes	Medium	Semi-open	452	>45
21[b]	Survived	No	Medium	Closed	2,258	21
23	Survived	Yes	Long	Semi-open	704	>45
3	Predated		Medium	Semi-open	157	10.5
4	Died		Short	Closed	384	12.5
9	Predated		Long	Closed	709	3
11	Predated		Short	Semi-open	106	2
12	Predated		Medium	Semi-open	1,893	11
19	Predated		Long	Closed	1,095	1.5

[a] Denotes the only bird that was not radio-tagged but was still monitored.
[b] The bird was relocated at the end of the first monitoring period, but was not found during the second monitoring period.

be discerned with Structure. In addition, Structure assumes that all populations of origin have been sampled, whereas Geneclass 2.0 does not. Similar to Negrini et al. (2009), we found that the percentage of assignments increases when both programs are used. Establishing a threshold for assignment based on the data of natural populations provided robust results. One way of further improving the method would be to expand the sampling area of yellow cardinals in the wild because the inability to assign some individuals to their MUs (13%) could derive from an incomplete sampling of natural populations (Holbrook et al. 2012). Incomplete genotyping, on the contrary, did not reduce probabilities of assignment.

The use of molecular analyses in forensic investigations has become a very common practice, and crimes such as poaching and the illegal trade of protected species are increasingly being investigated all around the world through the use of DNA-based evidence (Iyengar 2014). Discerning the origin of confiscated individuals can be useful to guide actions against illegal wildlife trade by identifying possible routes used by traffickers and the natural populations that are being harvested (Fernandes and Caparroz 2013, Presti et al. 2015). This study shows that the majority of the seized yellow cardinals came from MU3, in the southern part of the distribution (Fig. 1). This correlates with bibliographic data suggesting that, historically, this region has had more stable and numerous populations (Pessino and Tittarelli 2006).

The association between individual genetic diversity and fitness-related traits, known as heterozygosity-fitness correlation, has been intensively studied in the last decades (Chapman et al. 2009) and a positive correlation has been found in many organisms (Lesbarreres et al. 2005, Da Silva et al. 2006, Marr et al. 2006). Microsatellite neutral markers can be used as proxy of genome-wide levels of heterozygosity (Hansson and Westerberg 2002) and we found that mean individual heterozygosity of polymorphic loci of the group of yellow cardinals that survived was double that of the group that did not (0.70 vs. 0.35 respectively; Fig. 3). Although the difference was only marginally significant, this could be an interesting trend to further investigate on a bigger sample size in future releases.

Post-Release Monitoring

Monitoring of individuals after they have been released is critical to assess the success of this management action (Sutherland et al. 2010, IUCN 2013). Radio-tracking of the released cardinals showed that predation during the first days after liberation was high. However, we did not find any explanatory variable for survival probability because individuals were predated independently of their time in captivity, distance they moved from the release site, and the phytologic characteristics of the area they used. Other factors, like personality, could be playing an important role in the individual's success after being released. Individual responses to novel environments can be arranged along a shy or bold axis (Wilson et al. 1994). Shy individuals react to novelty by retreating, reducing activity levels, and becoming more vigilant, whereas bold individuals are more likely to approach novel objects and increase activity levels and exploratory behavior (Garamszegi et al. 2008, Cole and Quinn 2014).

Future studies could evaluate exploratory and risk-taking behavior to decide if a soft-release (individuals maintained in an enclosed area at the release site for a period of time before liberation) is necessary. We found that cardinals released in pairs did separate shortly after they were released, indicating that pairs formed in captivity do not hold.

Although 3 of the 10 released individuals that could be tracked in this study were successful in establishing a territory and attempted breeding, some improvements can be planned for future releases to maximize their success. We detected a high predation rate of released yellow cardinals. Sosa and Lopez de Casenave (2017) reported an edge effect related to predation rates in a study that analyzed nest predation in this area, probably because of higher abundance of predators associated with the agricultural matrix surrounding patches of forest. We released yellow cardinals in a continuous fragment of thorny deciduous shrubland forest and we did not find an association with vegetation density. Prior to future releases, we recommend a preliminary study of predation rates in the study site. Knowledge of predators in the area could help train yellow

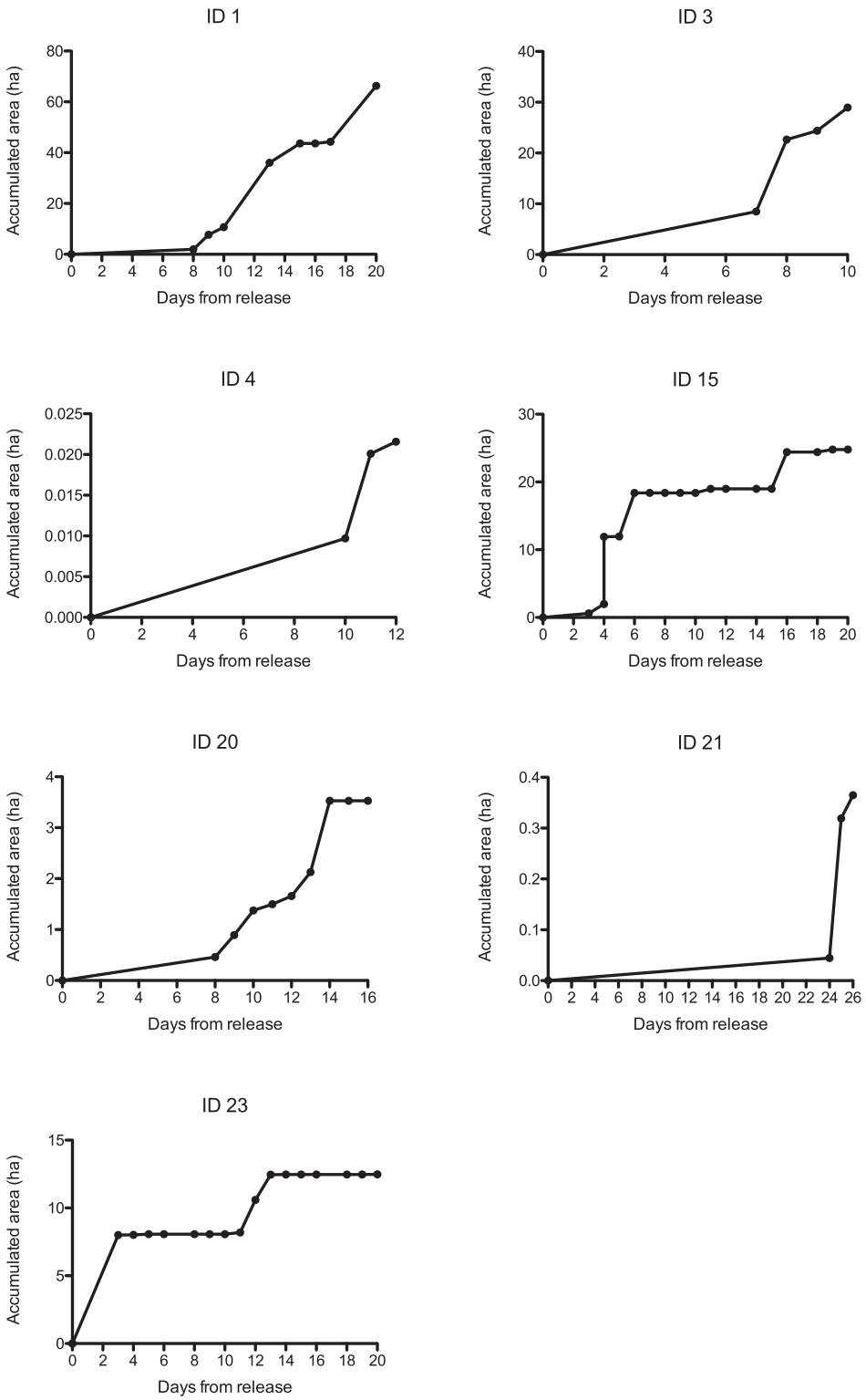

Figure 4. Accumulated area (ha) as a function of successive days from release for radio-tagged yellow cardinals (with identification [ID] number) that were re-located in 3 or more days in La Pampa, Argentina in 2017.

cardinals in predator awareness during the recovery phase and might increase survival, as might a progressive adaptation of individuals to their release site using a soft release method. Post-release monitoring of yellow cardinals during future liberations of individuals recovered from the illegal wildlife trade will provide more information to optimize this management action and aid in the conservation of this endangered bird species.

MANAGEMENT IMPLICATIONS

A combination of Structure and Geneclass methods effectively assigned confiscated poached yellow cardinals to their area of

origin. We recommend the continued collection of genetic samples from the field to improve genetic assignment to a population of origin to determine release sites for healthy birds that have been confiscated. Cardinals with higher individual heterozygosity tended to have a greater probability of survival after release; additional information to confirm this trend will help managers determine if heterozygosity should be considered when selecting birds for release. Monitoring of released birds revealed that their success was independent of the time spent in captivity, the liberation with a partner, the vegetation density of the settlement area, and the maximum distance traveled from the point of release. Because canopy cover did not have a relationship with survival, releasing yellow cardinals in areas characterized by the presence of thorny shrubland forests dominated by calden mesquite (*Prosopis caldenia*) was an effective strategy. Cardinals were able to disperse long distances (up to 2,258 m) with no apparent effects on survival and locate suitable breeding sites (2 of 10 radio-tracked males built a nest with a mate). Release of confiscated yellow cardinals effectively added breeding individuals to the wild population and we recommend continued monitoring of released yellow cardinals to identify any factors that could improve survival and breeding propensity of released birds.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.