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Genetic Diversity and Population Structure of Mesoamerican Scarlet Macaws in an Ex Situ Breeding Population in Mexico

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Abstract: Given the interest in the conservation of the Mesoamerican scarlet macaw (*Ara macao cyanoptera)*, the Xcaret Park formed an initial reproductive population about 30 years ago, which has progressively grown to a considerable population in captivity. In this work, we focus on the evaluation of the genetic diversity of the captive population, taking two groups into account: its founding (49) and the current breeding individuals (166). The genetic analysis consisted of genotyping six nuclear microsatellite loci that are characterized by their high variability. Tests for all loci revealed a Hardy–Weinberg equilibrium in four loci of the founders and in no loci of the breeding groups. The results showed that the genetic variation in the Xcaret population was relatively high (founders He = 0.715 SE = 0.074, breeding pairs He = 0.763 SE = 0.050), with an average polymorphism of 7.5 (4–10) alleles per locus in founders and 8.3 (4–14) in breeding pairs. No significant differences in the evaluated genetic diversity indexes were found between both groups. This indicates that the genetic variability in Xcaret has been maintained, probably due to the high number of pairs and the reproductive management strategy. Bayesian analysis revealed five different genetic lineages present in different proportions in the founders and in the breeding pairs, but no population structure was observed between founders and breeding individuals. The analyzed captive individuals showed levels of genetic diversity comparable to reported values from *Ara macao* wild populations. These data indicate that the captive population has maintained a similar genetic diversity as the metapopulation in the Mayan Forest and is an important resource for reintroduction projects, some of which began more than five years ago and are still underway.

Keywords: ex situ conservation; Psittacidae; *Ara macao*; conservation genetics; Xcaret; captive breeding

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1. Introduction

A recommendation made by the Species Survival Committee of the International Union for the Conservation of Nature [1] concerning reintroduction projects mentions the need to include genetic studies, since it is important to try to introduce sufficient genetic variability in the founding individuals of a new population to avoid bottlenecks, greater inbreeding, and possible problems of local adaptation to diseases or environmental changes [2,3]. Genetics is therefore an important aspect in the conservation or recovery program of any species, though by no means the only one [4].

The Mesoamerican scarlet macaw *(Ara macao cyanoptera)* is classified as endangered in Mexico [5] because it has disappeared from most of its original distribution, which used to extend from Tamaulipas through Veracruz, Oaxaca, Tabasco and Chiapas, and as far south as Costa Rica [6–8]. The IUCN received a proposal to consider this subspecies as

endangered [9]. The drastic decline in its populations is caused by the poaching of nestlings for the pet trade and loss of its natural habitat: the high evergreen forest [10]. In Mexico, only small remnants are left of what were once abundant populations. Experts estimate that this subspecies may be lost forever if no preservation action is taken in the next 10 years [11]. At present, we can do more for the conservation of this subspecies by reintroducing it in areas where it can be viable in the mid and long term with the use of captive breeding.

Wiedenfeld [6] found that wild populations of scarlet macaws distributed from Mexico to Central America and to the Amazon River basin may in fact be divided into two subspecies, one distributed from Costa Rica to southern Brazil *(Ara m. macao)* and the other from Mexico to Costa Rica *(Ara m. cyanoptera).* García-Feria [12] analyzed the genetic variation of four contemporary populations of *A. m. cyanoptera* in Chiapas (Mexico), Guatemala, Belize, and Honduras, using two fragments of mitochondrial DNA and a nuclear gene. His conclusions were that there is no genetic break between the studied populations, and that they comprise a cohesive reproductive unit. He observed that 91% of genetic variation was found within populations and only 9% between populations. A more complete phylogeographic study of *Ara macao* using sequences of mitochondrial genes, based on museum specimens, confirmed the existence of two lineages, which must indeed be recognized as subspecies [13]. Their findings in relation to the Mexican and Central American populations indicated that populations from separate sites from the Isthmus of Tehuantepec to the Caribbean slope in Belize and Guatemala did not present any significant substructure at separate sites, forming a single demographic unit or panmictic population, the Mayan Forest metapopulation (Isthmus Tehuantepec-Lancadona-Guatemala-Belize), but found a second demographic unit in the area, the Honduras–Nicaragua–NE Costa Rica metapopulation.

The Xcaret Ecoarchaeological Park is a private institution whose income comes from tourism, and that has been conducting ex situ reproduction of the species for the past 30 years [14]. It is registered as an UMA (Management Environment Unit) in the Wildlife Federal office in Mexico (Dirección General de Vida Silvestre, DGVS), under permit INE/CITES/DFYFS-ZOO-P-0011-99-Q.ROO. This permit does not allow commercial use of the macaws regarding direct sales, since this activity was banned in 2008, but allows the Park to manage its captive population for exhibition/education, and conservation purposes. Within this restriction, the Park has built many facilities for the macaws depending on the use of the specimens; some facilities are only used for night housing the macaws that are imprinted with humans and fly and return to the aviary and are not of reproductive age. Other facilities are used for macaws that are used for exhibition in close encounters with the public, and other facilities are for macaws that are reproducing.

Breeding at Xcaret is managed in aviaries of different sizes. At the beginning of the breeding program, pairs were artificially made by placing two adults together in the same cage, but later, bigger cages were built to include several adults of both sexes to let them choose their mates by themselves. Once the pair is formed, they are put in a cage of mid-size with a nesting box. Raising is performed both by hand and by their parents until they are three months old. There are climate-controlled facilities to raise nestlings by hand and more open facilities to feed youngsters until they feed themselves. All the macaws born in the population are banded with a closed marked steel ring according to the permit at 1–1.5 months old because, later, it would not be possible to insert the ring anymore. The ring has a unique key number and the Xcaret name. The reproductive output is reported annually to the DGVS with the list of rings placed with the specimens.

In a study of ex situ conservation genetics assessment, Witzenberger and Hochkirch [3] recommended that the number of founders must be a minimum of 15 and the population size at least 100 in order to minimize inbreeding problems in ex situ programs. The macaw population started with 29 pairs obtained from other captive populations in Mexico and from seized illegal specimens given to Xcaret by PROFEPA (Procuraduría Federal de Protección al Ambiente) for an approximate total of 60 individuals of unknown kinship. Breeding in captivity started in 1994 with six nestlings. The captive population grew to the goal of 100 breeding pairs, and more. The maximum number of macaws in the population

was 946 (2012). As a result of different donations for reintroduction programs, the total number today is 596. Given these numbers, the captive population is expected to meet the criteria to avoid inbreeding.

Over the course of the first 20 years of management, decisions were made to maximize reproductive success by avoiding reproductive pairing of close relatives, using the record keeping system. Such decisions showed improvement regarding reproductive output and population growth. Although records were not kept at the beginning of the Park's operation, breeding data were recorded starting in 2007.

The actual breeding capacity now is 200 newborns per year but, due to space limitations, many nests are kept closed. All macaws born in captivity are issued a birth certificate with their parents' IDs and ring numbers. Available pedigree information has been used to avoid inbreeding.

Evaluation of the maintenance or loss of genetic diversity in the current ex situ population is very important, since this population is the foundation for reintroduction programs in Mexico (Palenque 2013–2018, Los Tuxtlas 2014–2021). This information could help optimize strategies to select individuals for reintroduction by maximizing genetic variation, thus avoiding bottlenecks and negative effects of inbreeding in the new populations to be established.

The objectives of the present study were to estimate the genetic diversity of the founders and breeding pairs of the Xcaret population in order to contribute to the conservation of this subspecies and to compile previous genetic studies to compare them with this captive population. To achieve this objective, we genotyped the founding individuals and breeding pairs with six microsatellite loci to therefore compare the diversity of both groups (founders and breeding pairs).

2. Materials and Methods

2.1. Sample Collection

Blood samples were obtained from 49 macaws identified as founders that are still alive, though they are no longer breeders. We also sampled the 166 scarlet macaws comprising the 83 breeding pairs of 2015, descendants of these founders. A drop of blood was collected from each individual and placed on labeled FTA cards (Whatman™, Florham, NJ, USA). All samples were collected at the Xcaret aviary and then sent to the laboratory for analysis and storage at room temperature. All samples were confirmed as *Ara macao cyanoptera* based on mitochondrial DNA (unpublished data).

2.2. DNA Extraction

DNA was extracted from blood samples using the proteinase K digestion technique with the kit and DNeasy Blood & Tissue® (Qiagen Valencia, Santa Clarita, CA, USA). Two snips of blood were used for lysis, 60 µL of PBS 1X, and 7 µL of proteinase K (1 mg/mL), adjusted at 220 µL of lysis buffer.

The extracted DNA was then quantified in a nucleic acid spectrophotometer (Nanodrop® Thermo, Wilmington, DE, USA) and visualized on 2% agarose gel. All samples had a final DNA concentration between 20 and 58 ng/µL at a purity of 1.5–1.9 (260/280 absorbance). Such quantity and purity are suitable to amplify microsatellite loci [15,16].

2.3. Amplification and Genotyping

Primers for six microsatellite loci designed for different species of *Ara* [17] were previously standardized in our laboratory (Table 1) and identified as variable and informative for *Ara macao cyanoptera.* Amplification of the loci was performed using the forward primer labeled with a fluorescent dye (VIC, FAM, PET, and NED, (Applied Biosystems® Foster City, CA, USA). PCR amplification was carried out with the Platinum® Taq DNA Polymerase kit (ThermoFisher, Waltham, MA, USA) with 1X Buffer, 2 mM $MgCl_2$, 0.8 µM of each primer, 0.2 µM dNTPs, 0.25 U taq polymerase, 1–2 µL of DNA (5–25 ng/µL), and finally bidistilled water to adjust a reaction volume of 12.5 µL. The reaction was performed with

an initial denaturation of 95 °C for 30 s followed by 14 touchdown cycles and an annealing temperature of 60 °C for 30 s (with 0.5 °C per cycle decreases to 51 °C), 1 min at 72 °C, 30 cycles of 95 °C for 30 s, 51 °C for 30 s, and 72° C for 1 min, and a final extension of 7 min at 72 °C.

Table 1. Estimates of population genetic parameters for founders and breeding pairs from the Xcaret scarlet macaw population, including number of individuals (N), number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), private alleles (P), Nei's gene diversity (D), allelic richness (A_R), and inbreeding coefficient (F). * Significant ($p < 0.05$). ** Significant ($p < 0.05$) after correction using Bonferroni procedure.

Founders											
Loci	N	Range	Na	Ne	Ho	He	P	D	A_R	F	HWE
AgGT17	48	115–137	10	5.592	0.875	0.821	-	0.832	9.997	−0.066	*/-
AgGT19	48	181–189	4	2.386	0.458	0.581	-	0.595	3.939	0.211	-
AgGT21	48	169–189	8	5.224	0.729	0.809	-	0.819	7.939	0.098	-
AgGT42	45	243–271	12	6.784	0.956	0.853	1	0.863	12	−0.121	-
UnaCT21	48	166–172	4	3.022	0.646	0.669	-	0.676	4	0.035	-
UnaCT74	48	150–168	7	2.808	0.625	0.644	2	0.661	6.936	0.029	*/**
Mean			7.5	4.303	0.717	0.729			7.466	0.031	
(SE)			1.31	0.735	0.074	0.046			1.316	0.048	
Breeding Pairs											
AgGT17	159	115–137	12	6.11	0.881	0.836	2	0.839	10.508	−0.053	*/-
AgGT19	166	181–189	4	2.16	0.614	0.536	-	0.537	3.605	−0.147	*/-
AgGT21	155	169–191	9	4.87	0.858	0.795	1	0.797	8.093	−0.08	*/-
AgGT42	165	243–275	14	6.87	0.873	0.854	3	0.857	11.369	−0.022	*/**
UnaCT21	165	150–174	6	2.98	0.715	0.664	2	0.666	5.679	−0.077	*/**
UnaCT74	155	152–168	5	2.53	0.639	0.604	-	0.606	4.995	−0.058	-
Mean			8.3	4.250	0.763	0.715			7.375	−0.073	
(SE)			1.6	0.810	0.050	0.054			1.279	0.017	

The DNA amplicons obtained were sent to the Biodiversity and Health Genomic Sequencing Laboratory of the Institute of Biology of the Universidad Nacional Autónoma de México for genotyping. Each sample was prepared with 500 LIZ® Size Standard (Applied Biosystems) according to the manufacturer, and 1 µL of DNA amplicon per individual per loci, and then subsequently analyzed on an Applied Biosystems™ 3500xL laser sequencer (Life Technologies™, Carlsbad, CA, USA). Allele scoring was performed with GeneMapper v. 4.1 (Applied Biosystems™). All the fragments were analyzed twice to confirm the obtained results. Tandem v. 1.09 [18] was used to correct mobility problems and possible artifacts, which also confirmed the allelic assignment according to the repetition units of each locus. To detect null alleles, large allele dropout, and scoring errors, all loci were analyzed with MICRO-CHECKER v2.2.3 [19]. In GENEPOP Web [20], deviations from the Hardy–Weinberg equilibrium (HWE) caused by an excess or deficit of heterozygotes were analyzed using Fisher's exact test [21]. In GENALEX v. 6.5 [22], the linkage disequilibrium of all loci was assessed using the exact probabilities test. We estimated the significance level values of HWE and linkage disequilibrium by applying a Bonferroni correction ($\alpha = 0.01$). The following diversity indices were also obtained: average number of alleles per locus (Na), effective number of alleles (Ne), observed (Ho) and expected (He) heterozygosity, number of private alleles (P), and Nei's genetic diversity (D). Allelic richness (A_R), standardized with the smallest sample size (46), was obtained in FSTAT v. 2.9.4 [23]. Each index was calculated per locus and per scarlet macaw group studied (founders and breeding pairs).

2.4. Population Structure

The population structure was examined through a principal coordinates analysis (PCoA) based on Nei's genetic distances matrix between individuals using GENALEX ver 6.5. In addition, population structure was examined using STRUCTURE v.2.3 software [24] to perform a Bayesian clustering method in order to infer the number of clusters and structure. An admixture model with the LOCPRIOR option was used. The number of tested populations (K) ranged from 1 to 10 using 20 independent Markov Chain Monte Carlo (MCMC), by sampling 200,000 iterations, and a 200,000 burn-in period. The most likely number of clusters was inferred using STRUCTURE HARVESTER [25]. To infer the optimal K-value, both ln Pr (K) and the ΔK statistic [26] were calculated. To further search for genetic clusters without assuming an underlying population genetic model such as HW equilibrium or linkage disequilibrium, we applied the discriminant analysis of principal components (DAPC, [27]), performed with the package ADEGENET v 2.2.1 [28] of RStudio v 1.4.1717 (©2009–2021 RStudio, PBC) in R v. 4.1.1 [29]. We explored the data with K = 10, K = 20 and K = 30. The lowest value of the Bayesian information criterion (BIC) was used as the number of clusters that best reflect the population structure of the data.

To estimate the degree of kinship (r) between individuals and populations (founders and breeding pairs), we evaluated different "relatedness r" estimators available in the GENALEX software. To compare the informativeness and the power of relatedness detection of available estimators, we used the reciprocal of the mean squared deviations (RMSD) of estimators provided in the KinInfor program v.2 [30]. Our objectives were to detect full sibs (Δ1 = 0.5, Δ2 = 0.5), paternal halfsibs (Δ1 = 1, Δ2 = 0), maternal half sibs (Δ1 = 0.5, Δ2 = 0), and unrelated (Δ1 = 0, Δ2 = 0) individuals. We ran simulated pairs of genotypes and set the confidence level at 0.05. For each estimator, the mean and standard deviation were calculated. The COANCESTRY program [31] was used to compute the R values for each pair of individuals (dyads) and evaluate the statistical errors associated with these estimates using bootstrapping (1000 replicates).

3. Results

Genotypes were obtained for six microsatellite loci from a total of 49 founders and 166 breeding individuals of Mesoamerican scarlet macaws. No evidence of null alleles or allelic dropout was found. Linkage disequilibrium (LD) was observed between 11 of 30 loci comparisons and was observed only in the breeding population (Supplementary Materials Table S1). Deviations from the Hardy–Weinberg equilibrium were detected only in AgGT17 and UnaCT74 in the founder population, but in all loci, except UnaCT74 in the breeding pairs group, after Bonferroni correction (Table 1 and Table S2). Based on the overall test, the founder population showed a deficit of heterozygotes (p = 0.0250), while the breeding pairs showed an excess of heterozygotes (p = 0.0164).

In terms of genetic diversity, the six loci were polymorphic for both populations. The number of private alleles was greater in the breeding population. (8 vs. 3). The diversity (D) and allelic richness (A_R) differed very slightly (Table 1). The comparison of allelic richness was not significantly different between founders and breeding populations (A_R= 7.466 vs. A_R = 7.375, p = 0.96), neither in observed heterozygosity (Ho = 0.717 vs. 0.763, p = 0.8325) nor gene diversity (D = 0.731 vs. D = 0.717, p = 0.496).

The pattern of genetic structure determined by PCoA displays the overlap between founder and breeding pair groups (Figure 1, Supplementary Materials Figure S1). The Bayesian analysis of population structure revealed that the maximum value of ln P (K) obtained was K = 5 (Figure 2a), which is concordant with the maximum Delta K (Figure 2b) (Supplementary Materials Table S3). The individuals of the founder group are formed by different proportions of the five genetic lineages observed (Figure 2c); however, there is a higher prevalence of lineages 1 (red) and 2 (green), both in the founders and in the breeders (Figure S1).

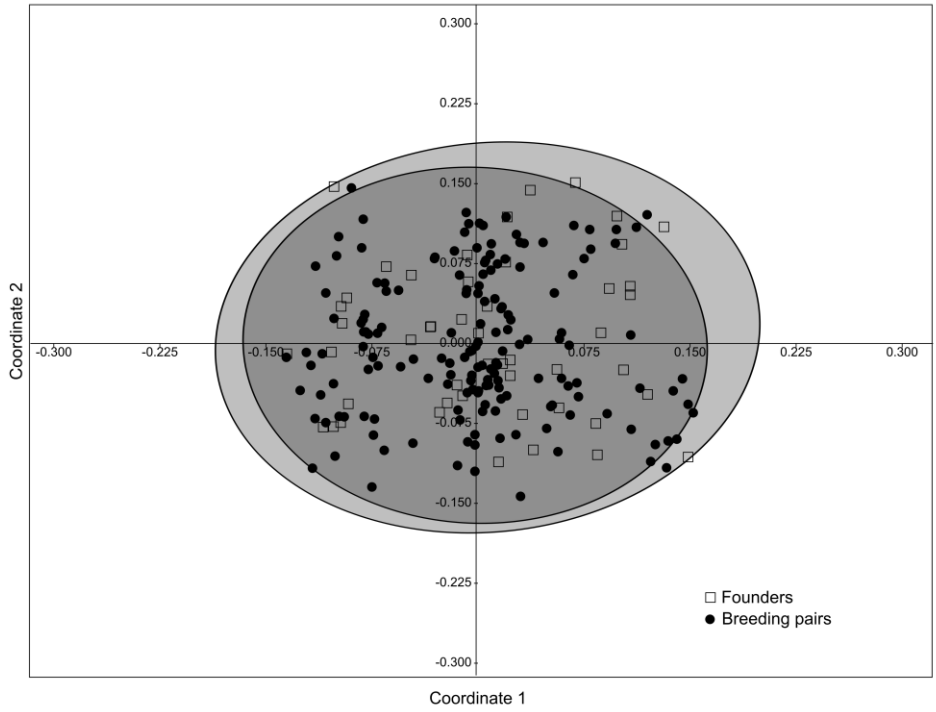

Figure 1. PCoA analysis of 215 individuals of founders (n = 49) and breeding pairs (n = 116) in the Xcaret captive population of Mesoamerican scarlet macaw (*Ara macao cyanoptera*) based on genotyping results of six microsatellites markers.

In contrast to STRUCTURE results, eight gene clusters were obtained with DAPC (Figure 3) for K = 10, K = 20, and K = 30 analysis (Supplementary Materials, Figure S1). However, the separation of the clusters is not entirely clear (Supplementary Materials, Figure S1); clusters 1, 2, 4, 5, and 7 overlap extensively, but 3, 8, and specially 6 are more clearly defined.

Comparisons among the informativeness of different relatedness estimators yielded Ritland [32] as the best estimator (Tables S4–S6). Ranking of the loci according to informativeness was: AgGT42, AgGT17, AgGT21, UnaCT21, UnaCT74, and AgGT19. Mean and variance of the different relatedness estimators are provided in Supplementary Materials Tables S4 and S5. The averaged relatedness values are no greater than −0.004 in founders and −0.002 in breeding pairs (Figure 4). The relatedness values in both groups are within the expected estimated confidence intervals (Figure 4). Mean relatedness was lower in the founders but slightly increased in the breeding pairs, perhaps due to the presence of siblings in the group (although R is close to zero, indicating most individuals are unrelated).

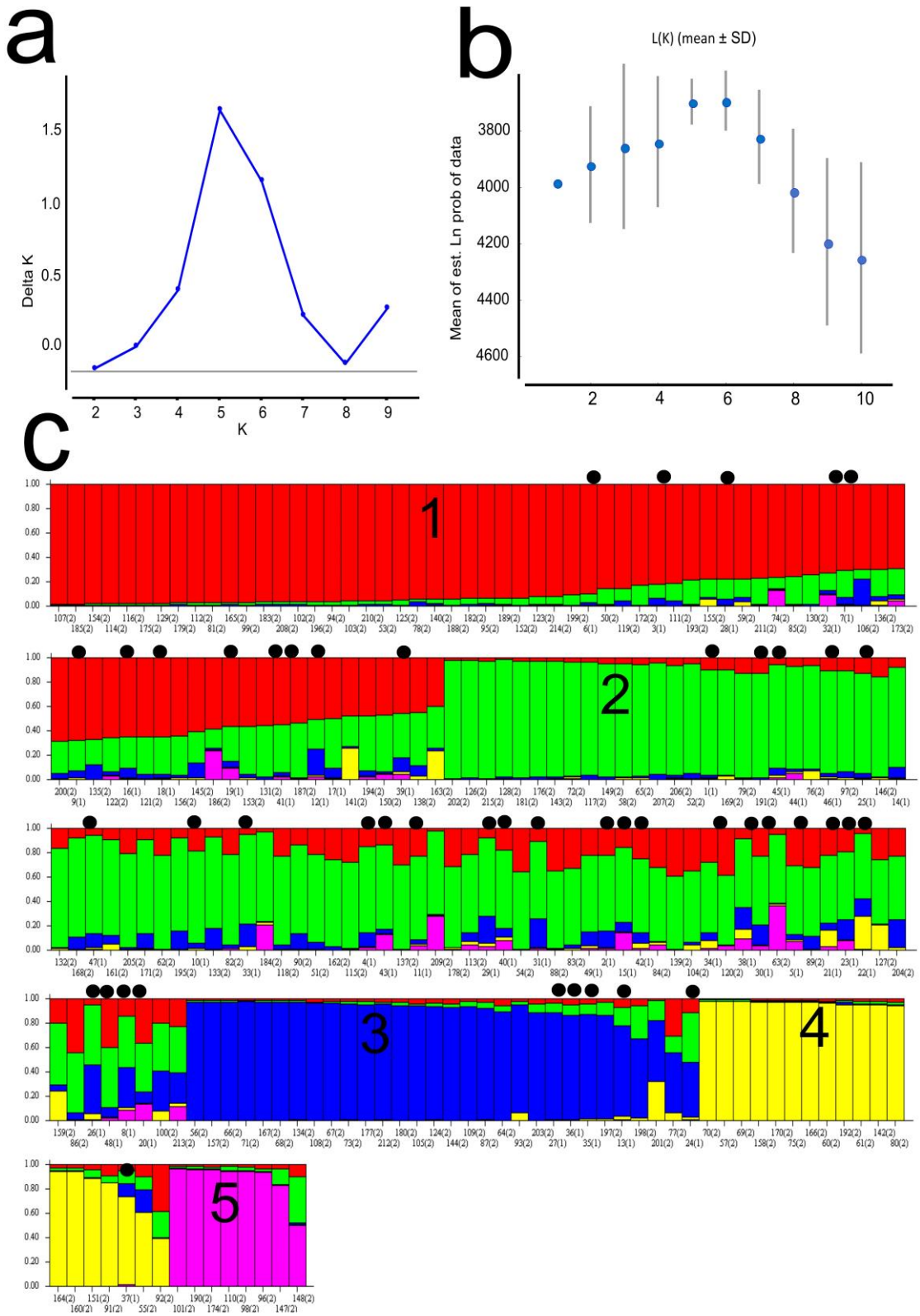

Figure 2. (**a**) Mean ln P (K) graph, (**b**) Evannos ΔK graph, (**c**) Q-membership proportion of K = 5 genetic clusters of founders (black dots), and breeding individuals of the Xcaret Mesoamerican scarlet macaw (*Ara macao cyanoptera*) based in six microsatellites markers. The length of the colored segment indicates the proportion of the individual's composition in specific clusters showing admixture in the population.

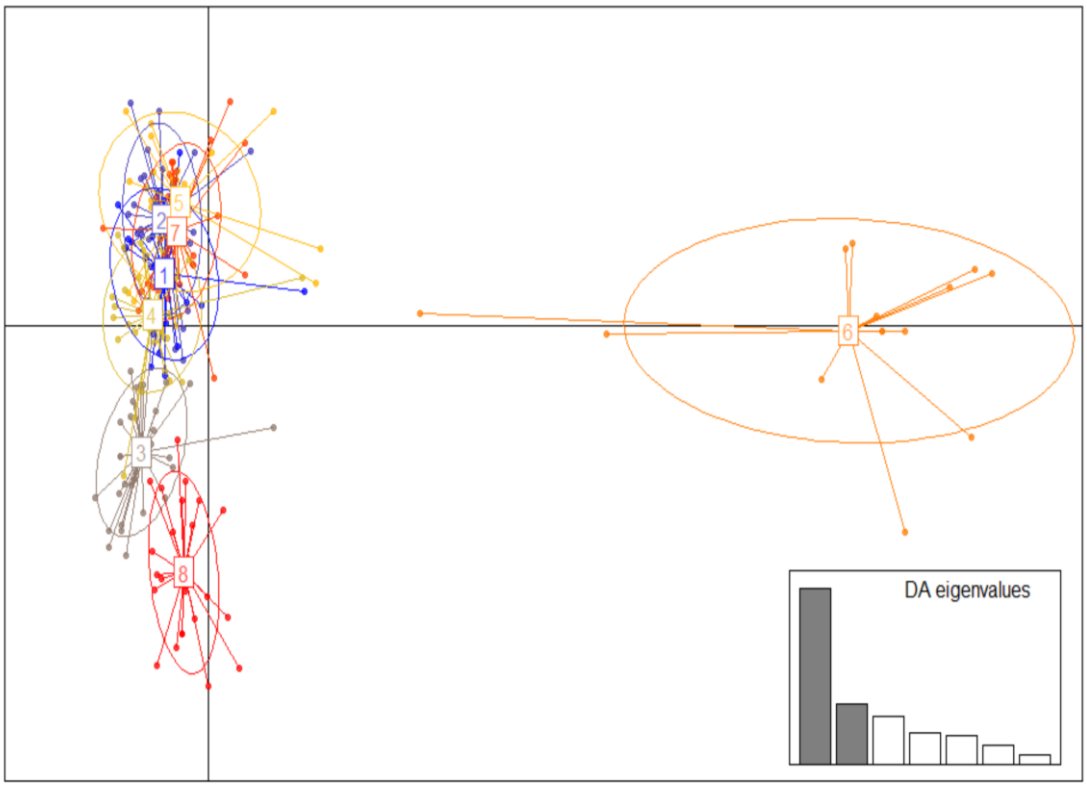

Figure 3. Gene clusters found with the DAPC analysis showing some structure in the captive population of scarlet macaws of Xcaret.

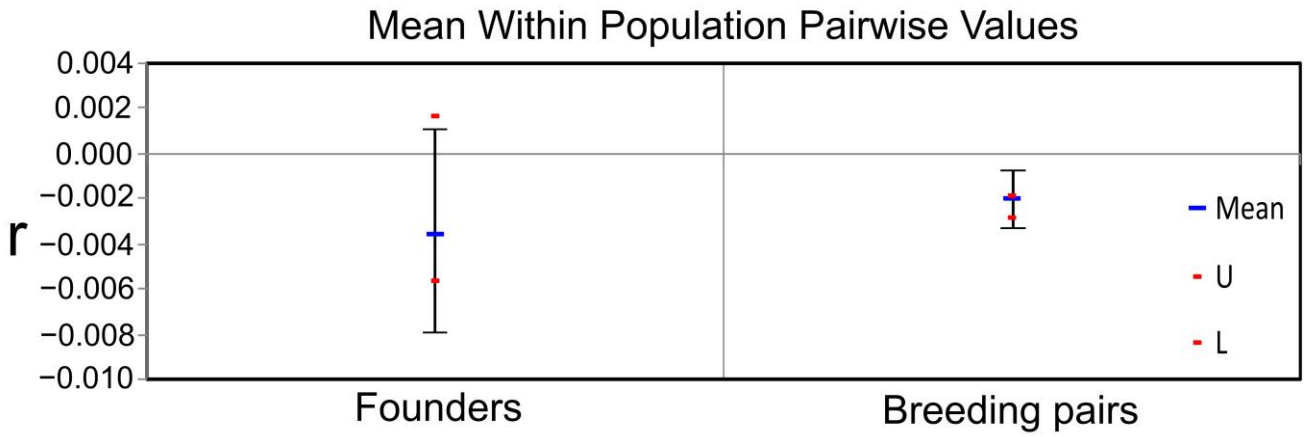

Figure 4. Ritland's relatedness (r) index of the Xcaret Mesoamerican scarlet macaw (*Ara macao cyanoptera*) in founders and in breeding pairs groups.

4. Discussion

The estimates of the population genetic parameters for founders and breeding pairs from the Xcaret scarlet macaw population were very similar. The slightly larger number of alleles in breeding pairs probably reflects the fact that we were not able to obtain samples from all of the founder individuals, since some had died before our study was carried out. Reproduction in the species extends until they are 35 years old, with a longevity record of up to 64–65 years [33]; thus, the number of generations in the population is small. Reproduction begins when individuals are approximately four years old (Xcaret, pers. com.) and generations overlap; this feature has helped preserve the original genetic diversity of the population up to the present time. Populations in captivity have often been observed to

depart from the HW equilibrium [34], and in this ex situ population, significant departures were noticeable in the breeding pairs in comparison with the founders. These results are perhaps accounted for by nonrandom mating management in captivity in this population.

The genetic diversity of some wild populations of *A. macao* have already been studied using the same microsatellites. In addition to trying microsatellites for different species of *Ara* and *Amazona*, Gebhardt and Waits [17] tested *Ara m. macao* from Peru. From a wild population of *A. m. macao*, scarlet macaws were sampled in Pará (Brazil) with 10 microsatellites [35]. From a population of the Mayan Forest in Guatemala, 11 microsatellites were tested [36]. Additionally, two populations from Costa Rica *(A. m. macao)* were studied [37] using six microsatellites (Table 2).

Table 2. Population origin, sample size (N), number of alleles (A), and expected (He) and observed (Ho) heterozygosities of microsatellite loci in the two subspecies of scarlet macaws studied to date.

Species/Population	Origin	N	Na	He	Ho	Reference
A. macao cyanoptera (Mesoamerican scarlet macaw)						
Founders (Xcaret)	Captivity	49	7.5	0.715	0.729	This study
Breeding individuals	Captivity	166	8.3	0.763	0.715	This study
La Selva Maya, Guatemala	Wild	37	7.1	0.696	0.713	[33]
A. m. macao (scarlet macaw)						
Costa Rica (CP)	Wild	41	6.7	0.63	0.61	[34]
Costa Rica (SP)	Wild	55	8.1	0.68	0.65	[34]
Perú	Wild	25	10.3	0.833	0.84	[16]
Brazil	Wild	28	11.8	0.846	0.842	[32]

Moderate levels of genetic diversity were found in the Costa Rican wild populations, similar to those of Guatemala, with indications of imbalance possibly due to genetic erosion caused by anthropogenic factors that are demographically affecting them. More stable populations in the Amazon (Peru and Brazil) have more genetic variability, leading to the interpretation that the scarlet macaw is a generalist species and until recently was widely distributed, exhibiting high genetic diversity in relation to other species of more specialized macaws with less diversity [35,38].

Comparing these data on wild populations with those of the Xcaret founders and breeding pairs, heterozygosity and number of allele values are very similar, but wild populations in Guatemala (*cyanoptera*) and Costa Rica (subspecies *macao*) have slightly lower values, whereas those of *macao* populations in Brazil and Peru, where populations are still large, are slightly higher. This similarity in the genetic variability values of the founding and reproductive populations of Xcaret with those of wild populations indicates that the ex situ population maintains levels of genetic diversity comparable to wild populations of the subspecies. However, these comparisons are not tested statistically.

Relatively large population sizes [39], high dispersal [40], and longevity [33,41] may have helped buffer scarlet macaw populations against genetic erosion. In fact, the Xcaret population is now two times larger than the Mayan Forest population, as estimated by Boyd and McNab [11].

Between the captive groups of founders and breeding pairs, there is a lack of population structure, which was evident with the PCoA and the Bayesian analysis, in which admixture is evident in the genetic composition of the individuals; hence, there is no genetic differentiation between founding and current breeding pairs in the captive population. However, the statistics obtained with STRUCTURE indicate that there are five genetic lineages that may be the result of a previous genetic structure in the wild populations where the founders came from. A similar population structure was found with the DAPC analysis, a method that emphasizes the differences between clusters. Of the eight clusters identified, five were not distinguishable in the graphic but three others were, perhaps reflecting a historical population structure from the original wild populations. The genetic

diversity of the ex situ populations is determined by the gene pool of the founders and their reproductive success [42]. Using mitochondrial DNA sequence data, Schmidt et al. [13] showed there was geographic clustering of haplotypes that might suggest genetic structuring of the wild populations of *A. m. cyanoptera* whom the founders of the Xcaret population came from.

Ex situ conservation programs strive to maintain genetic diversity that is comparable to a wild population by capturing a sufficient number of founders and managing matings to select individuals with underrepresented genes [43]. The objective of any reintroduction program is to provide enough genetic diversity to circumvent negative effects of natural selection within the new population, assuming that the reintroduced population can grow rapidly. If a source population has low genetic diversity, the reintroduced population that is derived from it will have similarly low diversity [44]. Our data show that the captive macaw population at Xcaret has captured and maintained similar levels of genetic variability to wild macaw populations of the Selva Maya. An important next step would be to compare the breeding population to the wider natural population. This analysis might reveal that there are unsampled populations in the wild such as The Chimalapas (Oaxaca, or Selva Maya W in [36]); as such, the captive breeding program may be improved by bringing in further birds from the wild to increase the genetic representation of the captive breeding population. Another possibility would be that representatives of this western population survive in captivity in some other aviaries and could be traced.

With limited pedigree information, at least initially, the reproductive management of the captive reproductive population has followed the strategy of reducing kinship to favor reproductive success and, unintentionally, conserving maximum genetic diversity within the population [45]. Hence, with the reproductive management carried out in Xcaret, it has been possible to maintain the gene pool of the founders in the reproductive population. Therefore, it is not surprising that an underlying structure was also found with microsatellites with five clusters that are well marked but intertwining.

The inbreeding coefficient (F) was low (<0.03) for both groups (founders and breeding pairs), which indicates that matings between closely related individuals is infrequent in both populations; however, the pairwise relatedness value in the breeding population showed a slight increase. Although this increase was not significant, it could indicate that ongoing careful management of breeding pairs will be required to avoid inbreeding. Although the breeding pairs group have generally avoided inbreeding, and the population has maintained levels of genetic variability comparable to those found in wild populations, it is recommended to identify individuals with a high degree of kinship in order to prevent breeding attempts by closely related individuals. The kinship information obtained in this study with the breeding pairs, complemented by pedigree information for the descendants of these pairs, should be used for further growth of the captive colony, as well as for the selection of group compositions for reintroduction projects with the aim of providing maximum genetic variability in the new populations, avoiding bottlenecks and promoting the success of reintroductions from a genetic viewpoint.

In order to secure the long-term persistence of reintroduced populations, it is also important that ex situ breeding programs endeavor to minimize time in captivity. If programs exceed the limit of 10–15 generations, relaxed selection could incur genetic costs [46]. With these guidelines, the Xcaret and its allies' opening of reintroduction projects of the subspecies in two separate sites in Mexico (Palenque, Chiapas and Los Tuxtlas, Veracruz) is timely.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14010054/s1, Figure S1: Genetic clusters obtained from the STRUCTURE analysis; Table S1: Genotypic linkage disequilibrium; Table S2: Summary ofor Hardy-Weinberg Equilibrium; Table S3: Evanno summary; Table S4: RMSD in KinInfor; Table S5: Relatedness estimates in Coancestry; Table S6: Ritland's relatedness estimator; Table S7 Table of alleles.

Author Contributions: P.E.-P. designed the study, coordinated the work, and participated in the analysis and interpretation of results as well as the writing of the manuscript. N.M.-F. performed the laboratory assays, analyzed the data and participated in the interpretation of results and writing of the manuscript. P.R.-E. carried out part of the laboratory assays and participated in the interpretation of results and writing of the manuscript. G.L.-M., K.S.-G. and R.R.-F. maintained the reproductive population, helped collect samples, and participated in the interpretation of results and writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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