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Title

Temporal monitoring of the Floreana Island Galapagos giant tortoise captive breeding program

Running title

Floreana tortoise captive breeding

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Abstract

Captive breeding programs benefit from genetic analyses that identify relatedness between individuals, assign parentage to offspring, and track levels of genetic diversity. Monitoring these parameters across breeding cycles is critical to the success of a captive breeding program as it allows conservation managers to iteratively evaluate and adjust program structure. However, in practice, genetic tracking of breeding outcomes is rarely conducted. Here, we examined the first three offspring cohorts (2017 – 2020) of the genetically-informed captive breeding program for the Floreana Island Galapagos giant tortoise, *Chelonoidis niger*. This captive breeding program is unique as the Floreana tortoise has been extinct since the 1800s, but its genome persists, in part, in the form of living hybrids with the extant Volcano Wolf tortoise, *Chelonoidis becki*. Breeding over the study period took place at the Galapagos National Park Directorate breeding facility in four corrals, each

containing three females and two males. Using 17 microsatellite markers, we were able to assign parentage to 94 of the 98 offspring produced over the study period. We observe that despite the addition of more founders since the pilot breeding program, the effective population size remains low, and changes to the arrangements of breeding corrals may be necessary to encourage more equal reproductive output from the males. This study demonstrates the value of hybrids for species restoration and the importance of continually reassessing the outcomes of captive breeding.

Key words

Chelonoidis niger, Conservation genetics, ex situ management, microsatellite

Introduction

Past and present human activities, such as poaching, habitat degradation, and invasive species introduction, continue to threaten species with extinction (Díaz et al. 2019). Captive breeding programs are an increasingly prominent intervention strategy that aim to increase the size of existing populations and/or re-introduce populations where they have been extirpated (Bowkett 2009; Rahbek 1993). Scientific management and careful planning are essential for captive breeding programs since by their very nature small, closed populations are prone to evolutionary processes (i.e., genetic drift, inbreeding) that can reduce fitness (Allendorf 1986; Lacy 1987). Thus, monitoring genetic diversity, relatedness, and parentage of offspring within captive breeding programs is essential (Cook and Sgrò 2018; Russello and Jensen 2018; Schulte-Hostedde and Mastromonaco 2015). For example, in the Attwaters prairie chicken *(Tympanuchus cupido attwateri),* moving from a pedigree based to a genetically informed captive program led to a reduction of relatedness between breeders and improved chick survival (Hammerly et al. 2013). While the importance of genetically informed captive breeding programs is becoming more recognized in conservation science today (Fienieg and Galbusera 2013; Norman et al. 2019), the need for temporal monitoring and continual re-assessment has not yet been fully appreciated. Longitudinal monitoring of the outcomes of breeding (e.g.,

parentage and reproductive skew) across breeding cycles can provide valuable insights and allow for adaptive management (Attard et al. 2016; Galla et al. 2022). Ongoing monitoring is especially important in the case of critically endangered species, where maximizing the effective population size is essential to ensuring the health of populations (Allendorf 1993).

The Galapagos giant tortoises (*Chelonoidis sp.)* are an island radiation of 14 formally described species that have faced, and continue to face, serious conservation challenges; at least three species are extinct, while all living species are listed as Vulnerable, Endangered or Critically Endangered (IUCN 2022, Figure 1). Current population sizes are only about 10% of their historical size in the 1800s (Tapia et al. 2021) largely due to overharvesting as a food source and the introduction of invasive species, such as rats and goats (Harper and Carrion 2011; Pritchard 1996; Townsend 1925). Tortoises are ecosystem engineers and provide critical ecosystem services, such as seed dispersal, and their reduced population sizes or extirpation from islands has resulted in ecosystem shifts and habitat degradation for other species (Bush et al. 2022; Gibbs et al. 2010; Hunter and Gibbs 2014). In addition to their ecological role, the giant tortoises are important cultural icons and a major tourism draw to the Galapagos, making them of high conservation priority (Benitez-Capistros et al. 2016).

A truly unique conservation situation has arisen for *C. niger*, the Floreana Island giant tortoise. *Chelonoidis niger* officially went extinct in the mid-1800s (Broom 1929; Steadman 1986), but due to a ‘happy accident’ the genome of the species persists in the form of wild hybrids with an extant species, *Chelonoidis becki.* These hybrids live among the endemic *C. becki* population on Volcano Wolf, Isabela Island (Garrick et al. 2012; Poulakakis et al. 2008). Their initial hybridization is thought to have happened within the last 200 years and had been human mediated, as there is no natural dispersal route between islands, but ships' logbooks detail the collection and depositing of tortoises between islands (Pritchard 1996). Since the discovery of these hybrids in 2008, there has

been much hope for captively breeding a genetically similar tortoise to that of the extinct *C. niger* that could be used to repopulate Floreana Island. Given Floreana Island has been lacking tortoises and their associated ecosystem services (e.g., seed dispersal and trampling of vegetation, Hunter et al. 2021) for two centuries, there is an urgent need to return tortoises to the island to resume their role as ecosystem engineers. Since hybrids with partial *C. niger* ancestry exist, they are the perfect candidates to be founders of the new Floreana population and may even have genetic variants associated with adaptations to the local environment. A small pilot captive breeding program was established in 2011 using nine founders with partial *C. niger* ancestry that were already in captivity (Miller et al. 2018; Russello et al. 2010). The breeders were housed in a single corral with mating occurring at random. After three breeding seasons, the 130 offspring successfully hatched were genotyped at 12 microsatellite loci to determine parentage and assess reproductive skew (Miller et al. 2018).

The importance of monitoring parentage and reproductive skew was highlighted in the captive breeding program for another Galapagos giant tortoise, *C. hoodensis*, endemic to Española Island. This species narrowly escaped extinction when in the 1960s only nine females and three males remained (Cayot 2021). Over the ensuing decades the captive breeding program produced and repatriated over 1,200 offspring to Española Island. In 2004, when advances in technology allowed the program to be genetically assessed, it was found that one of the males had fathered significantly more offspring than the other two, and this skew had reduced the male sex specific effective population size ($N_{e.m}$) to just 1.8, with an overall effective population size (N_e) of just 5.7 (Milinkovitch et al. 2004). As a direct result of these findings, arrangements in the captive breeding program were changed to encourage more equal reproductive output. These changes were successful in increasing the overall N_e to 7.8 in subsequent years, although reproductive output remained

skewed, potentially due to social dominance of some individuals or differences in fertility (Milinkovitch et al. 2013).

Building upon the lessons learned from the Española tortoise captive breeding program, in this study we continue the monitoring of the Floreana hybrid breeding program. After the pilot phase, in 2015 additional founders were added (now totaling 20), and four breeding groups were designed based on genotypes from 21 microsatellite loci according to a strategy that would minimize relatedness among parent-pairs (Quinzin et al. 2019). This phase of the program will be referred to as version 1 (V1) hereon. Breeding has followed this plan since 2017, resulting in 98 successfully hatched offspring over three breeding seasons. Here, we assess parentage of these offspring to monitor for reproductive skew and calculate genetic diversity measures. Through this ongoing monitoring of the breeding program, we are assuring that the desired outcome is being achieved, namely the breeding of a genetically diverse population of young tortoises with *C. niger* ancestry that can repopulate Floreana Island.

Methods

Existing Breeding Arrangements

The V1 breeding program consists of four corrals, each containing two males and three females (Supplemental Table 1) that are housed together year-round. The individuals had been assigned to particular groups based on the analyses in Quinzin et al. (2019) and were not rotated among corrals during the time period of this study. During the nesting season the corrals are checked daily for new nests, with the eggs removed and taken to be artificially incubated.

Sample Collection and Genotyping

Blood samples from the 20 breeders in V1 were collected from the brachial vein of the front leg (Miller et al. 2017; Russello et al. 2010). Small tissue biopsies were obtained from the 98 successfully hatched offspring (20 hatched 2017/2018, 43 hatched 2018/2019, 35 hatched 2019/2020) and stored at ambient temperature in Longmire Lysis buffer (Longmire et al. 1997) until arrival in the lab, after which they were stored at 4 °C. DNA was extracted from the tissues using Qiagen DNeasy kits following the manufacturer's protocol. PCR products were separated by electrophoresis using an Applied Biosystems (AB) 3730xl capillary sequencer, with allele peaks identified in GeneMarker (Hulce et al. 2011). Genotypes for both the breeders and offspring were called using bins through the R package MsatAllele (Alberto 2009), R version 3.6.2 (R Development Core Team 2019) and then manually verified by eye. All DNA samples were genotyped at the same 21 loci as Quinzin et al. (2019). However, since 4 loci did not perform consistently across samples, they were excluded from downstream analyses which were based on 17 loci (Supplementary Table 2).

Genetic diversity

Across all loci for the breeders and offspring separately, the total number of alleles and polymorphism information content (PIC) were calculated in CERVUS v.3.0.7 (Kalinowski et al. 2007) and the mean number of alleles (N_a), expected heterozygosity (H_e), observed heterozygosity (H_O), and the inbreeding coefficient (G_{IS}) were calculated in Genodive v3.0 (Meirmans 2020). The significance of the differences in these measures between the breeders and offspring was assessed using permutation tests with 999 permutations. To directly compare the diversity measures estimated for the V1 program and pilot phase and ensure comparability with the results, we re-estimated the diversity measures using the genotypic data from Miller et al. (2018) available on Dryad (see data availability statement).

Parentage assignment

Parentage assignments of offspring were determined using CERVUS v.3.0.7 (Kalinowski et al. 2007) following Miller et al. (2018). A parent-offspring combination was chosen if the pair had a LOD score difference of 3 when comparing the first and t the second most likely parent-offspring combination, indicating that the top parent pair is >20x more likely to be the true parent. We also tested for parentage assignment using COLONY v.2.0.6.8 (Jones and Wang 2010), to allow a total evidence approach to be taken to assigning parentage. After assigning a consensus parentage between the methods, the constructed pedigree of parent-offspring relationships was used to estimate N_e (see Supplementary Materials for N_e equations used). We also estimated the theoretical maximum N_e if all breeders had equally contributed offspring.

Evaluation of assortative mating

Relatedness values among all potential V1 breeders were calculated using the R package *related* v1.0 (Pew et al. 2015). To test for evidence of assortative mating, we compared the relatedness of individuals who did breed to those who did not breed using a Wilcoxon rank-sum test (Wilcoxon 1945). We then examined if relatedness values were correlated to the number of offspring produced by a pair who did breed using Pearson's product-moment correlation test using the *cor.test* function implemented in R.

Results

Breeding Program Diversity

The dataset of genotypes for the V1 breeders and offspring had little missing data, with all individuals genotyped at the minimum threshold of seven loci, a genotyping success rate of 96.7%. There was no significant difference in diversity measures between the breeders and offspring (Table 1). Genetic diversity across the breeders and offspring was high, with 5.65 alleles per locus on

174 average and mean PIC of 0.64 (Supplementary Table 2). Inbreeding in the breeding population was
175 low, and did not increase in the offspring.
176
177 *Parentage assignment*
178 We were able to unambiguously assign parentage to 94 of the 98 offspring (Figure 2). For the four
179 individuals excluded from further analysis; one had a low LOD score and a mismatch in mother
180 assignment from COLONY and CERVUS, one had a low LOD score and COLONY had assigned
181 mother as not present in dataset, one had both parents assigned by COLONY as not present in the
182 dataset and one had a father assigned as not present in the dataset by COLONY along with a two
183 allele mismatch (Supplementary Tables 3 – 4). Analysis of most likely fathers revealed five of the
184 possible eight fathers has offspring. Of those males that did breed, I64 was the most prolific male,
185 siring 28.7% of offspring, followed by Z4 and Z18 who contributed similarly at 26.6% and 23.4%,
186 respectively. The lowest male contributors to offspring were H210, who contributed 17% of total
187 offspring, and H2 which only contributed 4.3%. Among the females, all 12 has offspring. Individual
188 Z9 was the most successful female, contributing 23.4% of all offspring, and Z7 and BR106 were the
189 least successful, contributing only one offspring each (Supplementary Table 3).
190
191 Over the three breeding seasons, 12 parent-pairs were identified. Parent-pair analysis revealed that in
192 almost all corrals, each female sired offspring with only one male, apart from in corral 4 where two
193 males were successful breeders. In most corrals males sired offspring with multiple females in the
194 same year (Figure 2). The most prolific parent pair, female Z9 and male I64, contributed 23.4% of
195 offspring and the least successful contributed 1.1% (Supplementary Table 3 and 4).
196

The N_e of the V1 breeders was calculated to be 10.9, while $N_{e.f}$ was 7.9 and $N_{e.m}$ was 4.2. The theoretical maximum N_e (i.e., if all breeders had contributed equally to the 94 offspring) was 21.2 overall, with $N_{e.f}$ = 13.6 and $N_{e.m}$ = 8.7.

Relatedness and evidence of assortative mating

The Wang relatedness estimator was chosen as the most appropriate for this data (Supplementary Table 5, Supplementary Figure 1). The overall relatedness between pairs who did breed was not different than between pairs who did not breed (Wilcoxon rank-sum test W = 968, p-value = 0.589, Supplementary Figure 2). Additionally, in each corral, there was no significant difference in relatedness between breeding and non-breeding pairs (Supplementary Table 6). For those individuals who did breed, more related pairs produced significantly fewer progeny (Pearson's product-moment correlation r = -0.719, t = -3.27, df = 10, p-value = 0.0083). The males that did not breed have nine private alleles compared to the females and males that did produce offspring.

Discussion

The importance of genetic monitoring of captive breeding has already been demonstrated for the Española giant tortoise species (Milinkovitch et al. 2013; Milinkovitch et al. 2004). In this study we evaluated the progress of the scientifically managed V1 program for *C. niger* after three years of breeding, in terms of number of offspring produced by each breeder, the effective population size and genetic diversity of the overall population and relatedness between parent-pairs. This evaluation enables a temporal comparison of these factors to that of the pilot study and the chance to adjust future captive breeding efforts based on observations across the pilot and V1.

The primary goal of the Floreana tortoise program is to re-establish a healthy, wild population on Floreana Island that can survive and reproduce without ongoing intervention. To achieve this, it is

essential that the founders of the population are bred in such a way as to maximize the genetic diversity transmitted to the offspring cohorts, to limit the bottleneck as much as possible (Lacy 1994). Using the microsatellite alleles as markers for broader genome-wide diversity allows us to track the allelic diversity between the breeders and offspring, providing important information on how successfully diversity is being transmitted. Here we find that there are no significant differences in the N_a, heterozygosity, or inbreeding measures (G_{IS}) between the breeders and offspring (Table 1), all of which are positive signals. However, we also found that there are more alleles in the breeders than in the offspring (96 vs 85), due to three males that have not bred having unique variants that have not yet been passed on. The fact that 9% of the allelic variants in the breeding population have not been transmitted to the offspring is a concerning sign that the bottleneck is being unnecessarily exacerbated. Now that this issue has been identified, changes in the mating groups can be implemented to alleviate and possibly eliminate this issue. By maximizing the number of alleles in the offspring, the reintroduced population will have the greatest possible range of genetic variants for selection to act upon in response to local conditions.

In addition to tracking the alleles that may be lost due to bottlenecking, it is important to monitor the N_e. One way that N_e can be maximized in a captive population is to ensure that all breeders have an equal contribution of offspring (Allendorf 1993). Unfortunately, this is not what we are observing in the Floreana program where unequal fitness is observed for both males and females. For males we observed an $N_{e.m}$ of 4.2, which is only half of the theoretical maximum under equal breeding (8.7), due to the fact that, despite having two males in each corral, females had offspring with just one of them across the three years under study (Figure 2). Similar unequal fitness is present across the 12 female breeders. Although they all had offspring, the number ranged from a single one to 22, resulting in an $N_{e.f}$ of 7.9, well below the theoretical maximum of 13.6.

246

247 Unequal fitness was also observed during the pilot phase, with an overall N_e of 4.4 and $N_{e.m}$ and $N_{e.f}$
248 of 1.8 and 2.9, respectively (Miller et al. 2018). The observed increases in N_e (10.9), $N_{e.m}$ (4.2) and
249 $N_{e.f}$ (7.9) estimated in this V1 phase strongly suggests that increasing the number of breeders has had
250 a positive impact on N_e. Although there is a relative increase of these estimates between the pilot and
251 V1 phase, it is also true that in absolute terms these N_e values are small and well below what is
252 generally considered necessary to avoid inbreeding and genetic drift over evolutionary times
253 (estimated to be 50 and 500, respectively, Franklin 1980). However, although it is important to
254 maximize N_e to its greatest extent possible, it is also true that for this particular captive program all
255 offspring will be repatriated to the wild and not included as breeders in the captive program (Quinzin
256 et al. 2019). Thus, N_e is not expected to stay low over multiple generations, as the wild population is
257 expected to grow rapidly.

258

259 The genetic outcomes of the captive breeding program can be modulated by changing the design of
260 the breeding groups, but the role of tortoise behaviour must also be considered. Important differences
261 arose between the pilot and V1 programs. In the pilot version of the program, all three males sired
262 offspring, despite being housed within the same corral. However, during V1, in most corrals, a single
263 male was responsible for siring all offspring, despite females having the choice of two males. The
264 finding that there was a "dominant" male in each corral was a surprise, and even more so, because
265 two of the males with no offspring in V1 (BR610 and BR 877) were very prolific during the pilot
266 phase with over 50 offspring each (Miller et al. 2018). It is unlikely that this behaviour is the result
267 of assortative mating, since there is no difference in the relatedness between pairs that produced
268 offspring and those that did not. It is possible that the reorganization of the breeding groups from the
269 pilot phase to V1, along with the introduction of the additional breeders, upset the social dynamics

among the tortoises, leading to the differences in mating observed in the pilot and V1 phases. Limited data are available on the mating behaviour of Galapagos giant tortoises (Kubisch and Ibargüengoytía 2021), so ongoing monitoring of the program may provide novel insights as breeding outcomes are tracked over time. One result that is particularly worth tracking is the positive trend in more related parent-pairs producing fewer offspring.

The *C. niger* captive breeding program will be expanding again in the near future. An expedition to Volcano Wolf in January 2020, led by the Galapagos National Park and Galapagos Conservancy as part of the Giant Tortoises Restoration Initiative brought 31 new potential breeders (21 females, 10 males) into captivity (W. Tapia, unpublished data). As part of establishing version 2 (V2) of the breeding program, these individuals will be assessed for ancestry to the Floreana species and included as potential breeders if they possess significant Floreana ancestry. Thus, there is the potential to almost double the number of breeders in the program for the next breeding season, which should lead to positive increases in overall diversity and N_e. To accommodate this larger population of breeders, the breeding center has constructed additional corrals, allowing up to 11 breeding groups to be housed. Based on our observations of the breeding outcomes of the pilot and V1 phases, we can make suggestions for the next phase that may help to equalize the fitness among males and ensure the bread of genetic variation in the breeders is transmitted to the offspring cohorts. Recognizing the female preference to breed with a single male in each corral, we suggest the males that have fathered the most offspring so far (I64, H210, Z18 and Z4) be removed for the next few seasons, to allow the other males the opportunity to breed. We further recommend that only a single male is housed within each corral at a time, perhaps rotating males in and out of breeding every few years. In some species, male-male competition is necessary to induce successful breeding (Lance and Rostal 2002), but we do not believe this is the case for Galapagos tortoises. In the captive breeding program for the Española tortoise, corrals with a single male were still successful in producing offspring (Cayot

2021). Breeding groups will need to be re-evaluated with this design in mind, and to incorporate the new potential breeders. For V1, breeding groups were designed to minimize the relatedness using the software SWINGER (Sandoval-Castillo et al. 2017), and this procedure along with the forward in time simulations in Quinzin et al. (2019) will need to be repeated.

Despite representing a robust genetic panel for assessing parentage, the 17 microsatellite markers used in this study are not ideal for assessing the level of *C. niger* ancestry in breeders or offspring, as they represent fewer than 1 locus per chromosome. Work is underway to collect whole genome sequence data from a reference panel of pure *C. niger* museum specimens to enable the development of a suite of genome-wide SNP markers for identifying and quantifying *C. niger* ancestry in the breeders and offspring. Using these new markers, we hope to be able to include ancestry levels more directly in the development of breeding groups for V2, so that breeding can be planned to minimize relatedness between breeders while also increasing *C. niger* ancestry in the offspring. In other non-model organisms SNPs have been shown to provide more precise estimates of population-level diversity than microsatellites and can enable studies on genomic local adaptations (Zimmerman et al. 2020). Future studies on the hybrid individuals presented here could also use SNPs to identify local adaptations of the *C. niger* tortoise that may help in the overall success of hybrid repatriation to Floreana island.

Conclusions

Overall, this study highlights the importance of continually reassessing genetic parameters throughout a captive program. During V1, we have observed that only one of the two males in three of the corrals have fathered offspring, reducing the overall N_e of the program. This result contrasts with the pilot phase where multiple males within the corral were fathering offspring. Based on this new result, we can update the breeding groups and arrangements to encourage the other males to

breed, potentially by only housing one male in each corral at a time. Given the small number of breeders available for this program, it is important that breeding be fine-tuned to maximize the N_e and genetic diversity. As the captive breeding program is only intended to exist for a single generation, with the offspring being repatriated to Floreana Island to establish a wild population, this program has some unique microevolutionary considerations. More generally, this case serves as an example of how ex situ programs can and should be monitored and reported (Gant et al. 2020).

Supplementary material

Supplementary text

Supplementary figures 1 – 2

Supplementary tables 1 – 6

Funding

This work was funded by the Galapagos Conservancy and Oak Foundation.

Acknowledgments

We thank the Galapagos National Park Directorate for its work with the Giant Tortoise Restoration Initiative undertaken in conjunction with the Galapagos Conservancy. We thank Johanna Weston for her comments on a previous draft, and the editors and reviewers for their suggestions. The samples from the juveniles and 2020 potential breeders were collected and transported under CITES permit 20US209142/9, Galapagos National Park Directorate Permit PC-01-19, permit MAE-DNB-CM-2016-0060-M-0003 from the Ecuador Ministry of the Environment, and following Yale Institutional Animal Care and Use Committee (IACUC) permit number 2020-20346.

Data Availability

New microsatellite genotypes generated for this study will be submitted to Dyrad upon manuscript acceptance. Data used to reproduce the results of Miller et al. 2018 was obtained from Dyrad (http://dx.doi.org/10.5061/dryad.pd45719).

345 **Figure Captions**

346 Figure 1: LHS: Map of the Galapagos archipelago. Island names are in capital letters, volcano names
347 are indicated on Isabela Island and species names are given in italics. Blue squares indicate where
348 the hybrids were originally identified (Volcano Wolf), Floreana Island where the *C. niger* tortoise is
349 native to and the captive breeding center on Santa Cruz Island. Map tiles by Stamen Design, under
350 CC BY 3.0. Data by OpenStreetMap, under ODbL. Top RHS: hatchlings at the GNPD during the
351 captive breeding program (photo by E. Jensen). Bottom RHS: partial Floreana ancestry adults with
352 saddleback morphology in the breeding corral (photo W. Tapia).

353

354 Figure 2: Offspring assignment counts for each parent pair across the three breeding seasons.

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Supplementary Material for

Temporal monitoring of the Floreana Island Galapagos giant tortoise captive breeding program

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Supplementary Materials

Estimates of Effective Population Size (N_e)

$$N_{e.f} = \frac{N_f \bar{k}_f - 1}{\bar{k}_f - 1 + V_{k.f}/\bar{k}_f} \qquad N_{e.m} = \frac{N_m \bar{k}_m - 1}{\bar{k}_m - 1 + V_{k.m}/\bar{k}_m}$$

In the above equations N_f and N_m are the number of female and males that bred, $\bar{k}_f$ and $\bar{k}_m$ are the average number of offspring for females and males and $V_{k.f}$ and $V_{k.m}$ are the variance in the number of offspring. Values were calculated across all three breeding seasons. $N_{e.f}$ and $N_{e.m}$ estimated were then used to calculate the overall estimate of N_e across the cohort (Wright 1930):

$$N_e = \frac{4 N_{e.f} N_{e.m}}{N_{e.f} + N_{e.m}}$$

Supplemental Table 1: Pit tag identifier and shortened ID for male and female potential breeder tortoises, indicating the corral they were housed in throughout the three breeding seasons.

Corral	Pit Tag	ID	Sex
1	052383072	I64	M
1	016026877	BR877	M
1	052108873	Z19	F
1	026623636	Z9	F
1	052109085	Z7	F
2	052605592	H210	M
2	015809610	BR610	M
2	048079350	Z21	F
2	051560585	Z14	F
2	050338526	BR106	F
3	052597257	I94	M
3	000362301	Z18	M
3	051539082	Z11	F
3	052327006	Z17	F
3	052606031	I8	F
4	010776088	Z4	M
4	026069063	H2	M
4	050867066	E11	F
4	012112542	BR542	F
4	045285378	G8	F

Supplemental Table 1: List of loci used in parentage analysis with total number of alleles (k), observed heterozygosity (H_O), expected heterozygosity (H_e), and Polymorphic Information Content (PIC).

Locus	**k**	**H_O**	**H_e**	**PIC**
AC039	6	0.791	0.737	0.693
AC063	5	0.697	0.630	0.577
AC149	4	0.687	0.560	0.497
AC190	5	0.802	0.765	0.724
AC251	6	0.781	0.765	0.724
AGG68	2	0.435	0.379	0.306
Gal100	8	0.855	0.807	0.775
Gal127	6	0.581	0.678	0.646
Gal136	7	0.846	0.778	0.744
Gal158	3	0.681	0.613	0.539
Gal194	7	0.821	0.767	0.727
Gal21	9	0.882	0.803	0.770
Gal263	9	0.788	0.828	0.799
Gal45	4	0.573	0.639	0.568
Gal75	5	0.754	0.683	0.628
Gal94	7	0.845	0.780	0.740
GGA45	3	0.579	0.555	0.459
Mean	5.647	0.729	0.692	0.642

Supplemental Table 3: Number of offspring produced by each female and percentage of total offspring for each of the three breeding seasons.

Mother ID	2017 - 2018	2017 - 2018 (%)	2018 - 2019	2018 – 2019 (%)	2019 - 2020	2019 – 2020 (%)	Grand Total	Grant Total (%)
BR542	4	20	7	16.67	1	3.12	12	12.77
Z9	6	30	12	28.57	4	12.5	22	23.40
G8	3	15	4	9.52	6	18.75	13	13.83
Z21	0	0	0	0	5	15.63	5	5.32
E11	4	20	0	0	0	0	4	4.26
Z11	0	0	0	0	3	9.38	3	3.19
Z14	1	5	3	7.14	6	18.75	10	10.64
Z19	0	0	3	7.14	1	3.12	4	4.26
Z7	0	0	1	4.76	0	0	1	1.06
BR106	0	0	0	0	1	3.12	1	1.06
I8	2	10	10	23.81	0	0	12	12.77
Z17	0	0	2	2.38	5	15.63	7	7.45
Total	20	100	42	100	32	100	94	100

Supplemental Table 4: Number of offspring produced by each male and percentage of total offspring for each of the three breeding seasons.

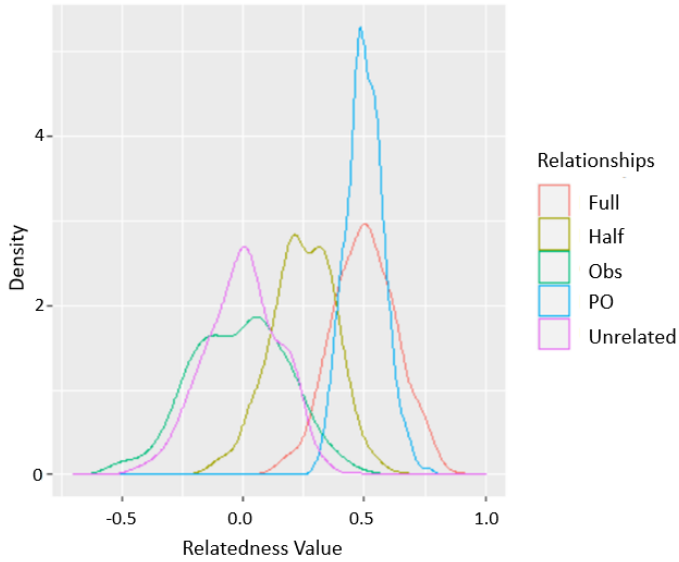
Father ID	2017 -2018	2017 – 2018 (%)	2018 - 2019	2018 - 2019 (%)	2019 - 2020	2019 - 2020 (%)	Grand Total	Grand Total (%)
Z4	7	35	11	26.19	7	21.87	25	26.60
BR610	0	0	0	0	0	0	0	0
BR877	0	0	0	0	0	0	0	0
H2	4	20	0	0	0	0	4	4.26
Z18	2	10	12	28.57	8	25	22	23.40
I64	6	30	16	38.10	5	15.63		28.72
H210	1	5	3	7.14	12	37.5	16	17.02
I94	0	0	0	0	0	0	0	0
Total	20	100	42	100	32	100	95	100

Supplemental Table 5: Correlations between observed and expected relatedness values for 100 simulated dyads of known relations.

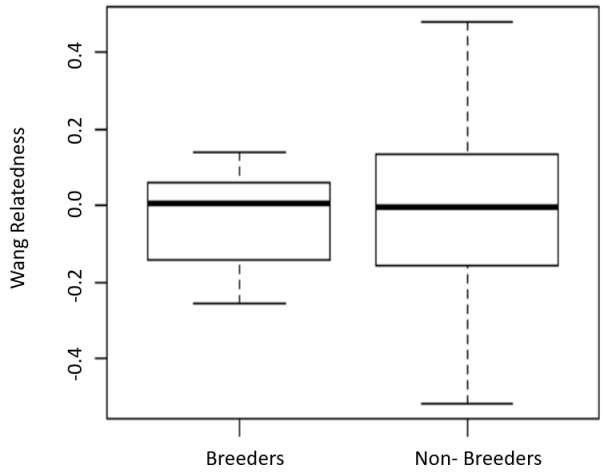
Relatedness Estimator	Correlation Coefficient
Wang	0.837
Lynch and Li	0.828
Lynch and Ritland	0.794
Queller and Goodnight	0.818

Supplemental Table 6: Exploration of difference in relatedness values between breeding and non-breeding pairs in each breeding corral throughout the three breeding seasons.

Corral	Breeding Pair IDs	Non-breeding Pair IDs	Wilcoxon
1	F_Z19 + M_I64 F_Z9 + M_I64 F_Z7 + M_164	F_Z19 + M_Br877 F_Z9 + M_BR877 F_Z7 + M_BR877	W = 6 , p = 0.7
2	F_Z21 + M_H210 F_Z14 + M_H210 F_BR106 + M_H210	F_Z21 + M_BR610 F_Z14 + M_BR610 F_BR106 + M_BR610	W = 4, p = 1
3	F_Z11 + M_Z18 F_Z17 + M_Z18 F_I8 + M_Z18	F_Z11 + M_I94 F_Z17 + M_I94 F_I8 + M_194	W = 2, p = 0.4
4	F_E11 + M_H2 F_BR542 + M_Z4 F_G8 + M_Z4	F_E11 + M_Z4 F_BR542 + M_H2 F_G8 + M_H2	W = 7, p = 0.4



Supplemental Figure 1: Density plot of pairwise relatedness estimates for simulated relatedness categories (full-sibling (full), half-sibling (half), unrelated and parent-offspring (PO)) and the observed distribution among the 20 breeders (Obs).



Supplemental Figure 2: Wang relatedness comparison between breeders and non-breeders.

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Table 1: Allelic diversity (N_a), total number of alleles (k), observed heterozygosity (H_O), expected heterozygosity (H_e), and average inbreeding coefficient (G_{IS}) for the 20 breeders and 98 offspring, from V1 and 9 breeders and 127 offspring from the pilot of the program

	17 loci		**12 loci**	
	V1 Breeders	**Offspring 2017-2020**	**Pilot Breeders**	**Offspring 2011-2015**
N_a	5.65	4.73	6.08	4.89
k	96	85	75	83
H_O	0.76	0.72	0.88	0.81
H_e	0.72	0.69	0.74	0.74
G_{IS}	-0.005	-0.005	-0.020	-0.096