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TEMPORAL GENETIC DIVERSITY AND EFFECTIVE POPULATION SIZE OF THE REINTRODUCED APLOMADO FALCON (*FALCO FEMORALIS*) POPULATION IN COASTAL SOUTH TEXAS

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ABSTRACT.—Reintroductions are an important tool in conservation for preserving and enhancing biodiversity and preventing extinction, and post-release monitoring is essential to evaluate and inform conservation management and maximize recovery success. By quantifying genetic diversity levels and effective population size (N_e) over time, managers can gauge to what degree additional efforts are needed to increase the likelihood of population persistence. The endangered Northern Aplomado Falcon (*Falco femoralis septentrionalis*) population in South Texas was reestablished and supplemented with captive-bred individuals originating from 27 founders collected in eastern Mexico (San Luis Potosí, Veracruz, Tabasco, and Chiapas). A total of 927 Aplomado Falcons were released at 23 locations along the southern coast of Texas between 1985 and 2004, and in 2012 and 2013. To assess the species' reintroduction and recovery, we applied a genetic monitoring approach using sampled nestlings ($n = 267$) from a total of 108 nests in 2004–2005 and 2012–2016. Based on ten microsatellite loci, levels of genetic diversity (i.e., allelic richness and heterozygosity) remained stable over the sampled time period, with no indication of inbreeding. Diversity levels were comparable to a subset of samples collected from the captive population founders ($n = 11$). Similarly, individuals from the South Texas population showed strong admixture with the founding population, and levels of both N_e and of effective breeding (N_b) showed no signs of decline over the sampled time period. To what degree overlapping generations and the release of additional Aplomado Falcons during the sampled time period limited our assessment of the South Texas population is not fully known. Continued monitoring across multiple generations is advisable to assess the population's ability to persist.

KEY WORDS: *Aplomado Falcon*; Falco femoralis; *effective population size*; *extra-pair young*; *genetic diversity*; *microsatellite DNA*; *reintroduction*; *temporal analysis.*

DIVERSIDAD GENÉTICA TEMPORAL Y TAMAÑO POBLACIONAL EFECTIVO DE LA POBLACIÓN REINTRODUCIDA DE *FALCO FEMORALIS* EN LA COSTA SUR DE TEXAS

RESUMEN.—Las reintroducciones son una herramienta importante en conservación para preservar y mejorar la biodiversidad y prevenir la extinción de especies. En esto casos, el seguimiento posterior a la liberación es esencial para evaluar y dirigir la gestión de la conservación y maximizar el éxito de la recuperación. Mediante la cuantificación de los niveles de diversidad genética y del tamaño poblacional efectivo (N_e) a lo largo del tiempo, los gestores pueden estimar hasta qué grado se necesitan esfuerzos adicionales para aumentar la probabilidad de persistencia de la población. La población amenazada de *Falco femoralis septentrionalis* en el sur de Texas fue reestablecida y suplementada con individuos criados en cautividad originados a partir de 27 progenitores fundadores recolectados en el este de México (San Luis Potosí, Veracruz, Tabasco y Chiapas). Un total de 927 individuos fueron liberados en 23 lugares a lo largo de la costa sur de Texas entre 1985 y 2004, y en 2012 y 2013. Para evaluar la reintroducción y recuperación de la especie, aplicamos un enfoque de seguimiento genético usando muestras de polluelos ($n = 267$) provenientes de un total de 108 nidos en 2004–2005 y 2012–2016. Basados en diez loci micro-satelitales, los niveles de diversidad genética (i.e., riqueza alélica y heterocigosidad) permanecieron estables a lo largo del periodo de tiempo muestreado, sin

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indicación de consanguinidad. Los niveles de diversidad fueron comparables a un subconjunto de muestras tomadas de los fundadores de la población cautiva ($n=11$). De modo similar, los individuos de la población del sur de Texas mostraron una fuerte mezcla genética con la población fundadora, y los valores tanto de N_e como de reproducción efectiva (N_b) no mostraron signos de disminución a lo largo de período de tiempo muestreado. No sabemos completamente hasta qué grado la superposición de generaciones y la liberación de individuos adicionales de *F. f. septentrionalis* durante el período de tiempo muestreado limitó nuestra evaluación de la población del sur de Texas. Se recomienda el seguimiento continuo a través de múltiples generaciones para evaluar la capacidad de persistencia de la población.

[Traducción del equipo editorial]

INTRODUCTION

Genetic diversity allows populations to adapt to changing environments and reduces potential for inbreeding depression (Allendorf et al. 2012, Lai et al. 2019). Management of genetic diversity is therefore an important component of increasing the likelihood of recovery of threatened and endangered species (Harrisson et al. 2014, Willoughby et al. 2015, Funk et al. 2019). Ensuring adequate levels of genetic diversity is especially important when using translocated or captive-reared individuals to establish a new population. Estimates of effective population size (N_e) as originally described by Wright (1931, 1938) provide a measure for gauging the likelihood of a population's ability to maintain genetic diversity over time (Hare et al. 2011, Jamieson and Allendorf 2012), and can be used as a practical measure for the conservation and management of wild populations (Schwartz et al. 2007). N_e represents the size of an idealized panmictic population where each adult has an equal expectation of generating viable offspring with the same gene frequency drift or inbreeding as the observed population (Wright 1931), or simply the number of breeding adults within a population. Because it is not always fully known if all released individuals contribute to the breeding population, managers can assess to what degree additional efforts are required to mitigate further genetic diversity loss by monitoring N_e over time.

Ongoing conservation efforts focused on reestablishing a self-sustaining Northern Aplomado Falcon (*Falco femoralis septentrionalis*) population in the United States of America (USA) would benefit from knowledge of levels of genetic diversity and estimates of N_e over time compared to the Northern Aplomado Falcon founding population. The Northern Aplomado Falcon (hereafter Aplomado Falcon) historically inhabited areas in the Texas Coastal Plains and the Chihuahuan Desert in West Texas, southern New Mexico, and southeastern Arizona. The subspecies has declined significantly in abundance over the past century, with the last breeding pair in the USA (prior to captive release efforts initiated in the mid-1980s) observed in the wild in 1952 (Hunt et al. 2013). Although the species' overall geographic distribution extends south through Mexico to Argentina, the distribution of the most northern subspecies (*F. f. septentrionalis*) is from Guatemala to the southern USA (Keddy-Hector 2019, Keddy-Hector et al. 2020). This subspecies has been identified by the US Fish and Wildlife Service (USFWS) as federally endangered (51 FR 6686) since 1986.

To reestablish the Aplomado Falcon in the USA, 25 wild nestlings (from at least 15 different nests) and two adults donated by falconers (one originating from a nest in Tabasco and the other of unknown origin) were obtained between 1977 and 1988 from the four Mexican States of San Luis Potosí, Veracruz, Tabasco, and Chiapas (see Supplemental Material 1). The captive population was created with the intention to propagate and release captive-hatched individuals in an effort to restore the species to its original range within the southern USA. The first captive-hatched Aplomado Falcons were produced in 1982 (Cade et al. 1991) and the first were released to the wild in 1985 (Hunt et al. 2013). All released Aplomado Falcons were offspring of the 27 founders or their subsequent generations that were reared and bred in captivity.

Although substantial effort was made in an attempt to establish a breeding population in West Texas and New Mexico, only one nesting pair was observed in New Mexico after 2012 despite the release of 637 captive-hatched Aplomado Falcons in West Texas between 2002 and 2011, and more than 337 in New Mexico between 2006 and 2012 (Hunt et al. 2013, USFWS 2014). The exact reason the released Aplomado Falcons failed to establish is not fully known, but multiple years of severe drought and deterioration of ground cover likely decreased prey availability, which in turn likely decreased the

population's productivity during the reintroduction efforts (Hunt et al. 2013, Sweikert and Phillips 2015). In contrast, the release efforts in coastal South Texas have been more successful. A total of 927 Aplomado Falcons were released at 23 locations along the southern coast of Texas between 1985 and 2004, and in 2012 and 2013 (Jenny et al. 2004, Hunt et al. 2013). These resulted in the establishment of two general breeding areas. Between 2008 and 2013, 15 to 20 mated pairs were observed each year north of Brownsville (herein identified as Laguna Atascosa National Wildlife Refuge, LANWR) and 13 to 17 pairs on Matagorda Island, San Jose Island, and Mustang Island near Rockport, Texas (herein identified as Matagorda Island National Wildlife Refuge, MINWR; Hunt et al. 2013). Since 2013, the South Texas population grew annually through 2017, when 39 territorial pairs were identified within the LANWR and MINWR populations.

Nestling survival within the South Texas population appeared to have benefited greatly from the use of raised artificial nest platforms with barred boxes over the nest that prevent predation of young by Great Horned Owls (*Bubo virginianus*), the primary predator of Aplomado Falcons (Jenny et al. 2004, Hunt et al. 2013). Reproductive rates in 2012 and 2013 for the LANWR population averaged 1.60 and 1.93 fledglings per nesting attempt, respectively, and 1.85 and 2.00 fledglings per nesting attempt, respectively, for the MINWR population (Hunt et al. 2013). Current efforts are focused on establishing additional breeding territories in South Texas, with increased attention on habitat restoration and installation of additional artificial nest platforms (e.g., McClure et al. 2017a); there are no immediate plans to release additional captive-bred falcons. Other than the number of known breeding pairs at artificial nest platforms, no information is available concerning the South Texas population's effective population size (N_e) and its genetic variability compared to its founding population.

Post-release demographic monitoring of translocated and reestablished populations is essential to ensure population persistence (e.g., Ewen and Armstrong 2007, Seddon et al. 2007, McClure et al. 2017b). Here we applied a population genetic approach using multi-locus genotypic data generated from microsatellite DNA to monitor temporal trends in population genetic diversity and effective population size (N_e) of the Aplomado Falcon population in South Texas between 2004 and 2016. We predicted that levels of genetic diversity and N_e of the South Texas Aplomado Falcon population were similar to those of the founding population, given the recency with which individuals from the captive propagation program were released. The results from this study provide a baseline measure for future monitoring efforts to help gauge progress toward achieving a viable self-sustaining Aplomado Falcon population in the USA.

Table 1. Number of Aplomado Falcon samples collected by year and location within South Texas (numbers in parentheses indicate the number of nests sampled). Laguna Atascosa National Wildlife Refuge, LANWR; Matagorda Island National Wildlife Refuge, MINWR.

		No. of Samples (No. of Nests)	
Year	No. of Samples	LANWR	MINWR
2004	38	22 (9)	16 (7)
2005	44	20 (9)	24 (9)
2012	28	15 (7)	13 (5)
2013	26	18 (8)	8 (3)
2014	32	16 (7)	16 (8)
2015	39	17 (7)	22 (11)
2016	40	22 (10)	18 (8)

Methods

Tissue Collection and DNA Extraction. We obtained muscle tissue from 11 of the 27 founders of the captive Aplomado Falcon population. The carcasses were kept frozen (–20°C) prior to sampling for this study. For the South Texas population, we obtained blood samples from a total of 267 Aplomado Falcon nestlings (11 to 18 nests per year between 2004 and 2016) in two coastal breeding areas, LANWR and MINWR (Table 1). We extracted DNA from all samples using the DNeasy Blood and Tissue Kit following manufacturer's protocols (Qiagen, Inc., Germantown, MD, USA).

Microsatellite DNA Genotyping. We screened a total of 19 microsatellite loci previously described for Orange-breasted Falcon (*F. deiroleucus*; Beasley et al. 2014) for polymorphism in Aplomado Falcon using PCR methods as described elsewhere (Beasley et al. 2014). We genotyped samples using an ABI 3130xl Genetic Analyzer (Applied BioSystems, Foster City, CA, USA) and the program GeneMarker v.1.6 (SoftGenetics, LLC, State College, PA, USA). Based on a subset of ten unrelated individuals, ten microsatellite loci (Fade-10, Fade-13, Fade-14, Fade-15, Fade-16, Fade-20, Fade-22, Fade-31, Fade-33, Fade-48) possessed multiple alleles in Aplomado

Falcon, resulting in unambiguous allele calls. We genotyped all remaining samples with the ten polymorphic loci.

Pairwise Relatedness. Data sets containing a high proportion of close relatives can influence the ability to obtain accurate estimates of genetic diversity and population structure (Rodriguez-Ramilo and Wang 2012, de Jager et al. 2017, but see Waples and Anderson 2017). To identify unrelated individuals, we calculated pairwise genetic estimates of relatedness among all sampled individuals based on Wang's relatedness coefficient (r_w; Wang 2002) with the R package *related* (Pew et al. 2015; see also Wang 2011) in R v.3.4.0 (R Core Team 2017). We conducted simulation analyses to determine which of seven different methods for calculating relatedness with COANCESTRY was most appropriate given the characteristics of the generated microsatellite data set (Csillery et al. 2006, Taylor 2015). We simulated data sets of 100 pairs for four relatedness categories (parent–offspring, full-sibling, half-sibling, and unrelated), with allele frequencies calculated from the study population's allele frequencies. We then used seven estimators to calculate relatedness, and compared the estimates from the simulations to those expected based on diploid inheritance patterns (parent-offspring = 0.50, full-siblings = 0.5, half-siblings = 0.25, and unrelated = 0.0) using their correlation coefficients. The Wang (2002) estimator was identified with the highest correlation coefficient (Pearson's $r = 0.83$), and used in subsequent analyses (see also Supplemental Material 2, Fig. S1).

All nestling samples obtained from the same nest should indicate full-sibling relationships based on their pairwise relatedness value ($r = 0.5$) when assuming monogamy, whereas half-sibling relationships or extra-pair young (EPY; $r = 0.25$) may occur due to extra-pair paternity (EPP) when young have different sires or when intraspecific brood parasitism occurs. Our ability to accurately estimate relatedness between individuals using genetic data, however, is dependent on a number of factors including the number and level of polymorphism of the microsatellite loci (Van de Casteele et al. 2001, Blouin 2003, Csillery et al. 2006). Considerable sampling variance often exists around point-estimates of relatedness using microsatellite genotypic data (e.g., Supplemental Material 2, Fig. S1), and therefore, measures must be taken to account for the potential uncertainty (Taylor 2015).

We compared pairwise relatedness values for all young within nests surveyed in South Texas to verify full-sibling relationships ($r_w \approx 0.5$) based on their 95% CIs calculated using bootstrapping over loci (1000 replicates). For cases where young from the same nest possessed upper 95% CI estimates <0.45, we attempted to identify nests within the same subpopulation (LANWR or MINWR) with young that could be half-siblings ($r_w \approx 0.25$). All young within the nest would also need to show a similar relationship with the other young, and shared paternity for nest sites within close geographic proximity was assumed more likely than for nests farther away. When more than three nestlings were present in a nest, we also determined whether allele combinations would allow us to reject full-sibling relationship based on diploid inheritance pattern. Using the above criteria, we feel confident that the young identified as half-siblings within the same nest represent the minimum number of EPY cases within the population.

Genetic Diversity. We created two data sets to assess choice of method for reducing relatedness among samples prior to calculating genetic diversity metrics. One data set consisted of a single individual per nest per year, and the second data set was created using the program *Friends-and-Family* (de Jager et al. 2017) with a relatedness cutoff value of 0.45 based on the Wang (2002) estimator. Although de Jager et al. (2017) recommended a cutoff value of 0.25, that parameter resulted in insufficient sample sizes for population genetic analyses. Instead, we follow recommendations of Waples and Anderson (2017) who showed that using a less stringent cut-off value of 0.5 could result in more reliable output in population genetic analyses.

For each data set, we tested microsatellite genotypes for linkage disequilibrium and departure from Hardy-Weinberg Equilibrium (HWE) for each study site (founders, LANWR, and MINWR) and locus with the program GDA v. 1.1 (Lewis and Zaykin 2001). We corrected for multiple simultaneous comparisons using sequential Bonferroni corrections (Rice 1989). We also calculated total number of alleles (A), allelic richness (AR), mean observed (H_o) and expected heterozygosity (H_e), and inbreeding coefficient (f) values using the R package DIVERSITY v.1.9.89 (Keenan et al. 2013) in R v.3.3.2 (R Core Team 2016). We calculated AR to control for differences in the number of alleles among populations that differed in sample size (Leberg 2002), and constructed 95% CIs by means of 1000 bootstrap replications to estimate statistical significance of AR estimates between populations and temporal peri-

ods. We considered values for f as significant if their 95% CI did not overlap with zero.

Population Genetic Differentiation. We used principal coordinate analysis (PCoA) implemented in the Excel-based genetic analysis program GenAlEx v. 6.503 (Peakall and Smouse 2012) to visualize whether any pattern existed among individual samples that corresponds with temporal sampling period and study site location. We produced a standardized genetic distance PCoA using pairwise genetic distances among all individuals with the codom-genotypic option as implemented in GenAlEx (Peakall and Smouse 2012). The genetic distances were based on the number of alleles shared by individuals and their respective heterozygosity level (Smouse and Peakall 1999).

To assess genetic differentiation among temporal sampling periods and study sites, we used the Bayesian clustering program STRUCTURE v.2.3.4 (Pritchard et al. 2000) to identify the most likely number of clusters (K) and assign individuals to the inferred population cluster(s). We used an admixture model with correlated allele frequencies and a burn-in of 2.5×10^5 followed by 5×10^5 permutations for K = 1 to 10 with 10 iterations for each K. We conducted analyses without the LOCPRIOR model. We calculated the membership coefficient (q), which represents a measure of proportional individual membership to different inferred population clusters allowing an assessment of admixture from two or more population clusters. We used the web-based program STRUCTURE HARVESTER v. A.1 (Earl and vonHoldt 2012) and the Evanno ΔK method (Evanno et al. 2005) to determine the mostly likely number of genetically distinct clusters (K). We used the program CLUMPP v. 1.1.2 (Jakobsson and Rosenberg 2007) to compile replicate run results from STRUCTURE, and used the program STRUCTURE PLOT v.2.0 to visualize the results (Ramasamy et al. 2014).

We also used GenAlEx to calculate pairwise estimates of population differentiation based on F_{ST} and Jost's D (D_{est}; Jost 2008) among temporal sampling periods and study sites. D_{est} has been shown to be an effective estimator for describing allelic differentiation among populations and less affected by a DNA marker's level of polymorphism, which can limit maximum differentiation estimates as shown with F_{ST} (Jost 2008, Meirmans and Hedrick 2011). We used 1000 random permutations to test for departure from the null hypothesis of no genetic differentiation (Peakall and Smouse 2012).

Effective Population Size. We calculated estimates of effective number of breeders (N_b) and contemporary effective population size (N_e) for each sampled time period and study site, respectively. N_b is fairly quick to assess because only one season (or cohort) of data collection is required, allowing managers to compare the number of breeders that produced each cohort across multiple years to gauge breeding stability over time. In contrast, estimates of N_e represent the harmonic mean size of a breeding population over a single generation where fluctuations in size are less obvious. Estimates of N_e provide a valuable measure for conservation because the magnitude of N_e influences the rate of random genetic drift, loss of genetic diversity, and effectiveness of selection and migration (Charlesworth 2009). Our ability to estimate N_e accurately with wild populations, however, can be complicated due to overlapping generations with individuals that reproduce multiple times during their lifespan (Luikart et al. 2010, Waples et al. 2014).

Our sampling design allowed us to assess N_e for each of the study populations and N_b for each consecutive breeding cohort or year (from 2004–2005 and 2012–2016) within the South Texas population. To estimate N_b, we used two single-sample estimator methods that differed based on their use of sib-relationships among samples. The first method was the sibship assignment maximum likelihood method described in Wang (2009) that estimates N_b from frequencies of full- and half-sibling dyads from randomly sampled sets of offspring. This method has been shown to provide accurate estimates of N_b of the parent generation that produced the cohort, if samples are collected from a single cohort at the same life stage (e.g., offspring prior to fledging; Wang 2009, 2016). We estimated $N_{b(sib)}$ and 95% CIs for each cohort using the program Colony2 (Jones and Wang 2010) with the "very long" duration option under the full likelihood model with medium precision and a uniform prior for sibship size while allowing for polygamous females (see Results).

The second method we used to estimate N_b was the linkage disequilibrium (LD) method as implemented in LDNe v. 1.31 (Waples and Do 2008) using the program NeEstimator v2 (Do et al. 2014). The LD method estimates N_b (or N_e depending on timing of sampling) based on measuring the nonrandom association of alleles at different loci (Hill 1981). Similar to the sibship assignment method, we obtained $N_{b(LD)}$ estimates for each individual

reproductive cohort, but we used a data set based on a single randomly selected individual per nest since the method assumes random mating. We excluded alleles with frequencies <0.02 (Waples 2006), and obtained 95% CIs using the jackknife option (Waples and Do 2008). We applied a correction to each estimate of $N_{b(LD)}$ to address potential bias due to age-structured populations with overlapping generations (Waples et al. 2013, 2014) based on two life-history parameters corresponding with adult lifespan (AL = 15) and age at maturity ($\alpha = 1$; see Table 3 in Waples et al. 2014) using banding records obtained directly from the South Texas population (B. Rolek pers. comm.). We also used the same life-history parameters to estimate $N_{e(LD\text{-}Adj2)}$ for each $N_{b(LD\text{-}Adj2)}$ estimate using methods described in Waples et al. (2014), and calculated a separate $N_{e(LD\text{-}pool)}$ estimate after pooling all samples into a single data set.

RESULTS

Pairwise Relatedness. Five percent (3 of 55) of the pairwise relatedness estimates (r_w) among founders were ≥0.25 (i.e., relationships equivalent to half-siblings and above). One pairwise comparison indicated a full-sibling relationship ($r_w \approx 0.5$), and the other two were half-siblings ($r_w \approx 0.25$). Approximately 13% (3856 of 30,381) of the pairwise comparisons among all sampled individuals from the two management areas in South Texas (LANWR and MINWR) possessed $r_w \geq 0.25$. Mean r_w among management areas was 0.085 (SD = 0.136). The percent of pairwise comparisons with $r_w \geq 0.25$ (or the equivalent of half-siblings, grandparent-grandnestling, aunt/uncle-nephew/niece and higher) between the sampled founders and among the temporal South Texas population ranged from 5.2% in 2013 to 8.8% in 2012 with no consistent pattern observed over time (Fig. 1).

Among the 108 sampled nests in South Texas, we failed to reject full-sibling relationships ($r_w \approx 0.5$) for the majority of young within a nest. However, at least seven nests ($n = 2$, 2004, LANWR and MINWR; $n = 2$, 2005, LANWR and MINWR; $n = 2$, 2013, LANWR; $n = 1$, 2016, LANWR) possessed relatedness estimates suggesting half-sibling relationships, three of which were confirmed based on diploid allele inheritance patterns (LANWR-2004, LANWR-2013, LANWR-2016), suggesting extra-pair paternity or intraspecific brood parasitism. For six of the seven nests with young that had half-sibling relationships, nestlings in a neighboring nest within the same management area were also identified as their half-siblings ($r_w \approx 0.25$). An additional eight nests held nestlings that may also have been half-siblings, but their upper 95% CI estimates of relatedness among within-nest comparisons were $0.45 \leq r_w \leq 0.50$.

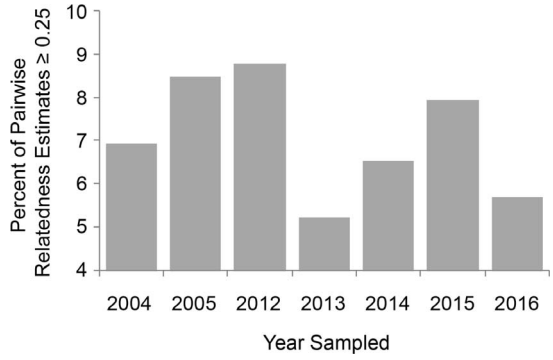

Figure 1. Percent of pairwise relatedness estimates (r_w) between Aplomado Falcon founders and each of the temporal South Texas Aplomado Falcon breeding areas (LANWR and MINWR combined) that were ≥ 0.25 (or half-sibling, grandparent-grandnestling, aunt/uncle-nephew/niece and higher order relationships).

We were able to investigate turnover of breeders at nest sites for 38 cases that included young sampled from the same nest site in at least two consecutive years over the duration of this study (2004–2005 and 2012–2016). Pairwise r_w values among young from 31 of those 38 cases (82%) failed to reject that the same breeding pair occupied the same nest site over consecutive years (i.e., all young $r_w \geq 0.30$). In the six cases where nestlings were sampled from the same nest site for three consecutive years, pairwise r_w values between years suggested they were all occupied by the same breeding pair for each nest site. Overlapping generations within the study population and insufficient precision in our estimates of r_w to distinguish between half- and full-sibling relationships combined to preclude confident identification of breeding pairs that may have moved to different nest site locations between sampled years.

Genetic Diversity. All loci per population and temporal period were in Hardy-Weinberg Equilibrium after adjusting the significance level due to multiple comparisons (Rice 1989). Genetic diversity measures and population mean relatedness values were similar between the founders and the South Texas temporal sampled cohort population, and no significant change in diversity was observed over time (Table 2). Similar results were obtained with

Table 2. Microsatellite DNA (10 loci) diversity estimates for each population temporal period. Estimates based on data set 1 (single young per nest; see Supplemental Material Table S1 for data set 2 estimates). *n*, sample size; *A*, number of alleles; *AR*, allelic richness (95% CI); H_o, observed heterozygosity (± SE); H_e, expected heterozygosity (± SE); F_{is}, inbreeding coefficient (95% CI); *r*, mean pairwise relatedness.

YEAR	*n*	*A*	*AR*[a]	H_o	H_e	F_{is}	*r*
Founders	11	63	5.3 (4.5–5.9)	0.63 ± 0.09	0.70 ± 0.05	0.093 (-0.025-0.185)	–0.044
South Texas							
2004	16	61	5.0 (4.2–5.6)	0.60 ± 0.05	0.66 ± 0.06	0.091 (–0.031–0.212)	0.015
2005	18	62	5.3 (4.6–5.8)	0.68 ± 0.06	0.68 ± 0.06	–0.006 (–0.087–0.074)	0.010
2012	12	58	5.1 (4.3–5.7)	0.68 ± 0.06	0.70 ± 0.05	0.017 (–0.010–0.127)	–0.021
2013	11	56	5.0 (4.4–5.5)	0.73 ± 0.06	0.70 ± 0.06	–0.043 (–0.169–0.057)	–0.012
2014	15	63	5.4 (4.7–6.0)	0.75 ± 0.05	0.72 ± 0.05	–0.043 (–0.131–0.030)	–0.021
2015	18	57	4.8 (4.2–5.4)	0.68 ± 0.05	0.67 ± 0.05	0.058 (–0.036–0.146)	0.032
2016	18	61	5.0 (4.2–5.7)	0.65 ± 0.05	0.67 ± 0.05	0.032 (–0.070–0.134)	0.024

[a] Controlled for differences in samples size; minimum sample size = 11.

the data set that included only unrelated individuals as determined using Friends-and-Family method (Supplemental Material 2, Table S1).

Population Genetic Differentiation. A PCoA showed that the Aplomado Falcon individuals formed a single cluster with no clear demarcation of populations (Fig. 2). Axes 1, 2, and 3 explained 13.63%, 10.27%, and 8.30% of the variation, respectively, or 32.20% of the total. Similarly, no genetic differentiation was observed among founders and temporal periods within the South Texas population based on the analysis STRUCTURE. Although K = 9 had the highest mean probability and ΔK (Supplemental Material 2, Table S2), all the plots for K = 2 to 10 showed consistent admixture with no clustering of individuals by location or year, and therefore failed to indicate any evidence of population genetic structure among time periods or

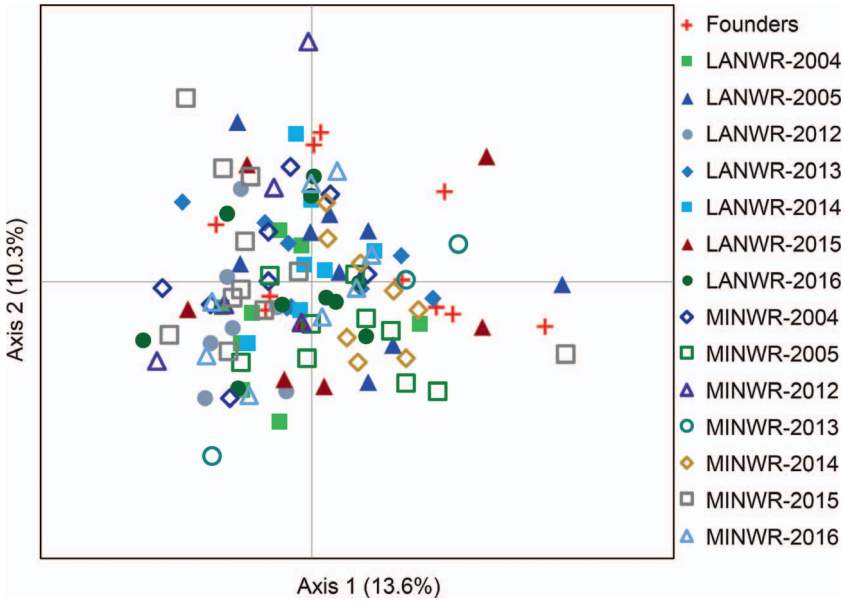

Figure 2. Principal coordinate analysis (PCoA) using one Aplomado Falcon individual per nest for each year sampled and breeding area (Laguna Atascosa National Wildlife Refuge, LANWR; Matagorda Island National Wildlife Refuge, MINWR). Axis labels represent the first two coordinate axes with their respective explained variation. See online version for interpretation of color symbols.

Table 3. Estimates of effective number of breeders (N_b) and population size (N_e) for the South Texas Aplomado Falcon population over time based on the sibship assignment (sib) and linkage disequilibrium (LD) methods. Estimates of N_b and N_e using LD have been adjusted to correct for biases due to age structure (see Table 3 in Waples et al. 2014) and 95% CIs are provided in parentheses.

YEAR	n^a	$N_{b(sib)}$	n^b	$N_{b(LD\text{-}Adj2)}$	$N_{e(LD\text{-}Adj2)}$
2004	38	32 (19–55)	16	56 (22–∞)	40 (16–∞)
2005	44	38 (23–63)	18	40 (16–∞)	29 (12–∞)
2012	28	25 (14–46)	12	61 (22–∞)	44 (16–∞)
2013	26	22 (13–42)	11	33 (14–∞)	24 (10–∞)
2014	32	24 (14–44)	15	18 (11–∞)	13 (8–∞)
2015	39	28 (17–49)	18	55 (19–∞)	40 (14–∞)
2016	40	39 (24–63)	18	25 (15–∞)	18 (11–∞)

[a] All samples for each breeding cohort were used to estimate $N_{b(sib)}$.
[b] One young per nest for each cohort was used to estimate $N_{b(LD\text{-}Adj2)}$ and $N_{e(LD\text{-}Adj2)}$.

management areas (Supplemental Material 2, Fig. S2). Estimates of population differentiation using F_{ST} and D_{est} provided similar results indicating no consistent population structure among founders and each of the temporally sampled management areas after adjusting the significance level due to multiple comparisons (Supplemental Material 2, Table S3; Rice 1989).

Effective Breeding (N_b) and Population (N_e) Size. Effective number of breeders (N_b) varied among sampled cohorts and populations, with estimates for South Texas ranging from 22 in 2013 to 39 in 2016 with overlapping 95% CIs among all sampled years (Table 3). The number of breeders estimated for each sampled cohort using the LD method ($N_{b(LD\text{-}Adj2)}$) ranged from 18 in 2014 to 61 in 2012 with undefined upper 95% CIs (or identified as infinity), which limited their use for comparative purposes (Table 3).

Adjusted estimates of the effective population size ($N_{e(LD\text{-}Adj2)}$) per generation varied among the sampled cohorts and ranged from 13 in 2014 to 44 in 2012, with all values smaller than their corresponding $N_{b(LD\text{-}Adj2)}$ values for each cohort including three of their seven $N_{b(sib)}$ estimates (Table 3). Using the pooled data set based on a single random young per nest and reproductive cohort for South Texas, $N_{e(LD\text{-}pool)}$ was 21.0 (95% CI = 10.6–40.3) and was comparable to the harmonic mean of $N_{e(LD\text{-}Adj2)}$ obtained for all cohorts (harmonic mean = 24.8). In contrast, the estimate of N_e for the founder population was large ($N_{e(LD\text{-}pool)}$ = 8901.9) with high sampling variance as shown by the extremely wide 95% CI (31.4–∞).

DISCUSSION

Population genetic analyses of the South Texas Aplomado Falcon population between 2004–2005 and 2012–2016 indicate no significant change in genetic diversity or effective breeding size (N_b) over the sampled time period. In fact, the diversity measures were similar to estimates obtained from a subset of individuals ($n = 11$) from the founding population used in the captive propagation and release program. Estimates of N_b and N_e for each reproductive cohort for the South Texas population were similar or slightly higher than the actual number of wild-caught Aplomado Falcons ($n = 25$) used to create the captive population in the early 1990s. These results indicate that the wild population (at least through 2016) appeared stable and comparable in genetic diversity and number since the time of their reintroduction. Further, based on annual nest survey results, there was no indication that a consistent decline in productivity has occurred (P. Juergens and B. Mutch, unpubl. data).

Between 2008 and 2013, the South Texas population averaged 37 occupied territories per year (range: 30–41) and 31 nesting territories (range: 28–34; Hunt et al. 2013). Similar levels were observed in 2014 through the 2017 breeding season (P. Juergens and B. Mutch, unpubl. data), but occupancy levels were lower in 2018, likely due to habitat modification and nest site damage following Hurricane Harvey in August 2017. Survey efforts during 2018 revealed ten territorial pairs of Aplomado Falcons had been lost to Hurricane Harvey: five pairs on Matagorda Island National Wildlife Refuge and five pairs on San Jose Island. The single pair occupying Mustang Island State Park remained. The LANWR population was not affected by Hurricane Harvey but occupancy had declined from 21 pairs observed in 2017. Recent surveys in South Texas indicated that numbers have rebounded, with 28 Aplomado Falcon pairs with established territories (LANWR: 19; MINWR: 9) and 45 nestlings observed during the 2020 breeding season (P. Juergens and B. Mutch, unpubl. data).

Each of $N_{b(sib)}$ and $N_{b(LD\text{-}Adj2)}$ estimates were similar to twice the number of nests sampled for each of the reproductive cohorts and also similar to the harmonic mean estimate of $N_{e(LD\text{-}Adj2)}$ for the South Texas Aplomado Falcon population (Table

3). These differences suggest few additional nests beyond those that were sampled for this study contributed to the breeding population for each cohort. Previous research has shown that artificial nest sites, which constituted the majority of nests sampled for this study, are essential for increasing hatch-year survival and productivity of the current South Texas population (Hunt et al. 2013, McClure et al. 2017a). These results further confirm that the breeding population relies heavily on the artificial structures for nest sites because estimates of N_b tend to exceed the population's N_e.

The accuracy of the sibling assignment method for estimating N_b is maximized when sample sizes are near to or greater than the true N_b (Wang 2009, 2016; Ackerman et al. 2017). In this case, we feel that we have achieved that level of confidence because only a minimal increase in estimates of $N_{b(sib)}$ was observed when consecutive cohorts were included in the analysis with increasing sample size, i.e., 2004–2005 ($n = 73$), $N_{b(sib)} = 42$ (95% CI = 30–69); 2012–2014 ($n = 86$), $N_{b(sib)} = 52$ (95% CI = 36–80); 2014–2016 ($n = 111$), $N_{b(sib)} = 53$ (95% CI = 38–79). The sibship assignment method estimates N_b of the parent generation that produced the sampled cohort, and thus, we expect an increase in the estimate as new full- and half-sibling groups are included in the analysis with consecutive cohorts. The magnitude by which $N_{b(sib)}$ increases will depend on the number of adult pairs that breed over consecutive years. These results indicate the need for future efforts to increase the number of artificial nest platforms in the study population (see also McClure et al. 2017a), thereby providing an opportunity to increase the number of breeding pairs and increase overall productivity of the recovering Aplomado Falcon population in South Texas.

Although we documented at least three cases where nestlings were identified as half-siblings based on pairwise estimates of $r_w \approx 0.25$ and diploid allelic inheritance patterns among nestlings (max number of alleles per family group = 4), we do not know if the pattern was due to extra-pair paternity (EPP) or intraspecific brood parasitism. An additional four nests may also include half-siblings based on the upper 95% CI estimate of r_w; however, we failed to reject a full-sibling relationship for the nestlings in those nests based on allelic inheritance patterns either because only two young were sampled at the nest or because insufficient allelic variability existed in the data set. Not having the genotypes of parents at each nest decreased our ability to identify extra-pair young (EPY); this is common among species with small clutch sizes such as the Aplomado Falcon (three-egg clutches are typical; Keddy-Hector et al. 2020).

The rate of EPY observed in the South Texas population was low at only 1–3% of young and 3–6% of nests ($n = 3$ or 7 of 267 sampled nestlings and 3 or 7 of 108 nests depending on the two estimates of EPY in this study). Nonetheless, additional research is warranted to determine if similar patterns exist in other Aplomado Falcon populations elsewhere in their distribution or if the phenomenon is unique to the South Texas reintroduced population. Although the occurrence of EPY in the population can influence genetic variability within a brood and potentially increase reproductive success for a male that breeds with multiple females, indirect benefits, especially to the female, are less understood and can vary depending on the local environment (Brouwer and Griffith 2019). The incidence of EPY in some species is positively correlated with the relatedness of the social partners at the nest (Arct et al. 2015). Although we do not have genotype data of the adults at each nest to investigate this further, the overall frequency of EPY as reported in this study is low and unlikely to have a major effect at the population level from a management perspective.

Given the life history constraints of the Aplomado Falcon (i.e., long generation time and small brood size) and recency of captive propagation and release, continued efforts should be made to collect samples every year to continue monitoring genetic diversity levels. The current data set based on microsatellite genotypes was insufficient to completely assess connectivity between management areas within the South Texas population by using relatedness to assess dispersal patterns. If possible, adult birds should be identified within respective breeding territories based on their banding records, and researchers should also generate single nucleotide polymorphism (SNP) data using next generation sequencing methods to obtain higher precision in estimates of r_w (Kopps et al. 2015, Ackerman et al. 2017, Wang 2017). We were limited in our ability based on the current data set to distinguish between familial relationships such as half-siblings, first cousins, and grandparent-grandnestling, and additional genetic markers (e.g., loci and SNPs) should allow us to identify potential sires of young that were the product of EPP (e.g., Weinman et al. 2015) and further investigate turnover of breeders at nest sites.

SUPPLEMENTAL MATERIAL (available online). Supplemental Material 1. Summary of founding Northern Aplomado Falcon population used for propagation and release of captive-hatched individuals. Supplemental Material 2. Table S1: Microsatellite DNA (10 loci) diversity estimates for each Northern Aplomado Falcon population temporal period. Table S2: Mean LnP(K) for K = 1 to 10 using dataset 1 (single young per nest) with program STRUCTURE. Table S3: Pairwise estimates of a) F_{ST} and b) D_{est} among Northern Aplomado Falcon founders and the South Texas management areas (LANWR and MINWR) sampled in 2004–2005 and 2012–2016 using dataset 1 (single young per nest). Figure S1: Box plots comparing the relatedness estimates of four of seven different estimators for simulated individuals of known relatedness using the program Related (Pew et al. 2015). Figure S2: Assignment of individual Aplomado Falcons to a defined cluster using STRUCTURE for K = 2 to 4.

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