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Influence of historical and contemporary habitat changes on the population genetics of the endemic South African parrot *Poicephalus robustus*

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Summary

The Cape Parrot *Poicephalus robustus* is a habitat specialist, restricted to forest patches in the Eastern Cape (EC), KwaZulu-Natal (KZN) and Limpopo provinces of South Africa. Recent census estimates suggest that there are less than 1,600 parrots left in the wild, although historical data suggest that the species was once more numerous. Fragmentation of the forest biome is strongly linked to climate change and exploitation of the forest by the timber industry. We examine the subpopulation structure and connectivity between fragmented populations across the distribution of the species. Differences in historical and contemporary genetic structure of Cape Parrots is examined by including both modern samples, collected from 1951 to 2014, and historical samples, collected from 1870 to 1946. A total of 114 individuals (historical = 29; contemporary = 85) were genotyped using 16 microsatellite loci. We tested for evidence of partitioning of genotypes at both a temporal and spatial scales by comparing shifts in allelic frequencies of historical (1870–1946) and contemporary (1951–2014) samples across the distribution of the species. Tests for population bottlenecks were also conducted to determine if anthropogenic causes are the main driver of population decline in this species. Analyses identified three geographically correlated genetic clusters. A southern group restricted to forest patches in the EC, a central group including birds from KZN and a genetically distinct northern Limpopo cluster. Results suggest that Cape Parrots have experienced at least two population bottlenecks. An ancient decline during the mid-Holocene (~ 1,800-3,000 years before present) linked to climate change, and a more recent bottleneck, associated with logging of forests during the early 1900s. This study highlights the effects of climate change and human activities on an endangered species associated with the naturally fragmented forests of eastern South Africa. These results will aid conservation authorities with the planning and implementation of future conservation initiatives. In particular, this study emphasises the Eastern Cape mistbelt forests as an important source population for the species and calls for stronger conservation of forest patches in South Africa to promote connectivity of forest taxa.

Introduction

Examining spatial patterns of genetic diversity is an important component of conservation planning and implementation (McNeely *et al.* 1990, DeSalle and Amato 2004, Arif and Khan 2009). Interpreting the processes underlying spatial patterns observed in genetic data is difficult, as the genetic diversity present in a population is a product of both long-term processes, such as global climate change, as well as more recent anthropogenic factors such as habitat loss, pollution,

introduced species, disease, poaching and over- exploitation of natural resources (Bouzat *et al.* 1998, Bickham *et al.* 2000, Daszak *et al.* 2000, Bouzat 2010, Dussex *et al.* 2015). Both long-term and more recent human-associated factors can fragment habitat and reduce population size which can lead to populations with lowered genetic variation, evolutionary potential and mean population fitness (Shaffer 1981, Couvet 2002, Spielman *et al.* 2004, Bruggeman *et al.* 2010, dos Anjos *et al.* 2011). The roles played by these processes in shaping extant populations is an important consideration in conservation, as interventions that target single threats often have limited success (Brook *et al.* 2008). However, linking reduction in genetic variation to a specific cause is often difficult. Many studies use genetic data collected from contemporary populations to infer historical phylogeographic patterns (Taberlet *et al.* 1998, Callens *et al.* 2011, Kotlík *et al.* 2014). The use of genotypes from historical archival samples in conjunction with appropriate statistical tools can, however, allow for a more direct measure of the change in demographic and/or phylogeographic patterns in populations over time.

Differences in the genetic diversity over time provides valuable information on whether modern genetic patterns are more heavily influenced by long-term processes such as climate change, or if the decline in genetic diversity is more recent, caused primarily by population fragmentation and population decline driven by human-associated factors (Pliocene musk ox *Ovibos moschatus*: Campos *et al.* 2010; late quaternary megafauna: Lorenzen *et al.* 2011; Hawaiian Petrel *Pterodroma sandwichensis*: Welch *et al.* 2012; Ethiopian wolf *Canis simensis*: Gottelli *et al.* 2013; Kea *Nestor notabilis*: Dussex *et al.* 2015). Some studies have shown no change in phylogeographic patterns across temporal scales (kangaroo rat *Dipodomys panamintinus*: Thomas *et al.* 1990; red fox *Vulpes vulpes*: Teacher *et al.* 2011), while others show significant shifts in the distribution of genetic diversity over relatively short time-scales (brown bear *Ursus arctos*: Leonard *et al.* 2000; tiger beetle Coleoptera, Cicindelidae: Goldstein and Desalle 2003; mice *Peromyscus leucopus noveboracensis*: Pergams *et al.* 2003; hawk moth *Hyles euphorbiae*: Mende and Hundsdoerfer 2013). The South African endemic Cape Parrot *Poicephalus robustus* Gmelin 1788 provides a suitable model system for studying the relative impact of long-term climate change versus the more recent impact of anthropogenic factors on genetic variation. The species is listed as 'endangered' in the Red Data Book of Birds of South Africa, Lesotho and Swaziland (Taylor 2014) and is protected under South African legislation (Minister of Environmental Affairs and Tourism 2007).

Cape Parrots are habitat specialists feeding and nesting predominantly in yellowwood (*Afrocarpus* spp. and *Podocarpus* spp.) forests (Wirminghaus *et al.* 2001a, 2001b, 2002, Downs and Symes 2004, Perrin 2009). The Cape Parrot is particularly mobile for a forest bird as it is a large species and a strong flyer, which is somewhat atypical of forest fauna. Despite this, the current distribution of the Cape Parrot is fragmented, and the species is restricted to forest patches in the Eastern Cape (EC) and KwaZulu-Natal (KZN) (Figure 1), with a relict population in Limpopo province (Wirminghaus 1997). Historical records show that populations of these parrots have drastically contracted over the last century, especially in the northern parts of KZN and along the escarpment of Mpumalanga (Wirminghaus *et al.* 2000, Symes *et al.* 2004, Downs 2005a, Downs *et al.* 2014. Large flocks of Cape Parrots were frequently observed during the early 1950s (Wirminghaus *et al.* 1999); these numbers declined sharply after 1950. This decline can in part be linked to eradication of "pest' parrots during the early 1900s (Symes 2010). In recent times, large flocks are rarely seen except when parrots congregate at agricultural pecan orchards during periods of food shortages (Downs *et al.* 2014). Illegal harvesting of individuals for the pet trade and psittacine beak and feather disease (Wirminghaus *et al.* 1999, 2000) both play a role in the decline of Cape Parrots, but the greatest threat to the species is habitat destruction (Downs 2015a). Habitat loss is one of the main extinction drivers in wild parrots globally (Collar 2000, Pain *et al.* 2006). Despite the Cape Parrot experiencing a range decline of 59% in the past quarter century alone (Cooper *et al.* 2017), recent census data suggests that the population is not in decline (Downs *et al.* 2014).

As the smallest biome in southern Africa, forests have a long history of human habitation and utilisation. The principal threat in recent times has been exploitation for timber, with

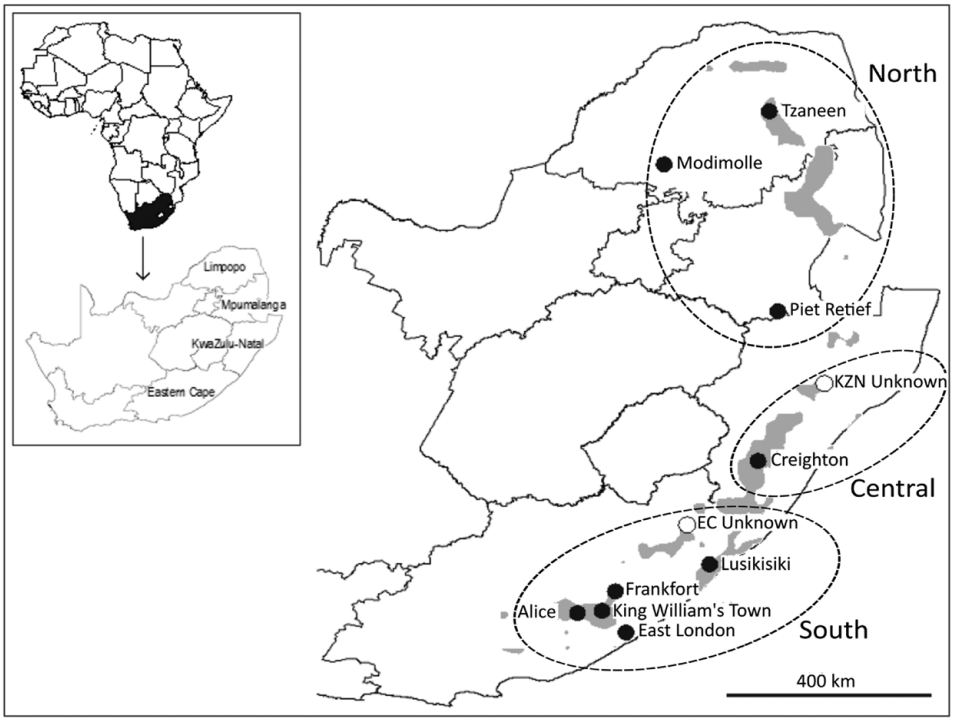

Figure 1. Sampling sites for Cape Parrot *Poicephalus robustus* in South Africa. Grey shading indicates the extent of current forest cover in the region (Mucina and Rutherford 2006). The black circles indicate sampling sites with detailed locality information, with the white circles representing samples from the Eastern Cape (EC Unknown) and KwaZulu-Natal (KZN Unknown) that do not have precise locality information. The dashed ellipses around the localities indicates the three main sampling regions (South, Central and North) used in this study.

deforestation in South Africa reaching a peak during the colonial era (1850–1910; Lawes *et al.* 2007), with less rigorous removal of *Podocarpus* ssp. during the mid-1920s to 1930s (King 1941) and some forest areas being logged up to 1940 (Rycroft 1942, McCracken 1986). The availability of food and nesting sites for Cape Parrots is directly affected by forest fragmentation, as patch size has an influence on *Afrocarpus*/*Podocarpus* fruit availability, with the larger patches having more trees with longer fruiting periods (Hart *et al.* 2013). Cape Parrots are secondary cavity nesters (Wirminghaus *et al.* 2001a, Downs 2005b) and prefer to nest in pre-existing cavities in tall, dead *Afrocarpus*/*Podocarpus* trees (Wirminghaus *et al.* 2001a, Downs and Symes 2004). Potential nest sites are often destroyed during logging operations, which target the old, large trees (Wirminghaus *et al.* 1999, 2001a, Brightsmith 2005, Downs 2005a). The impact of commercial harvesting of *Afrocarpus*/*Podocarpus* trees, as well as current subsistence harvesting of pole-sized trees (Lawes *et al.* 2007) threaten the South African mistbelt forests. Currently, the forest biome is highly fragmented with few large blocks of intact habitat existing outside of conservation areas.

Although overexploitation of forests in southern Africa is certainly an important driver of fragmentation, this is not solely responsible for the relict nature of South African forests. The distribution of this biome is also strongly linked to prevailing climate (Eeley *et al.* 1999). Beginning in the Pliocene, periods of climate change-driven aridity and increased fire frequency contributed towards the 'natural' fragmentation of the forest biome in southern Africa (Geldenhuys 1989, Scott *et al.* 1997). The Quaternary global interglacial-glacial cycles prompted

the contraction and expansion of the forest biome in Africa (Miller and Gosling 2014). Analysis of pollen taken in cores from the South African east coast have shown an increase in *Podocarpus* containing forests between c.6,500 and 3,700 years before present (BP). In contrast, c.3,600–3,500 years BP, pollen data indicate a rapid decrease in *Podocarpus*, with cores dominated by Poaceae and Ateraceae pollen, indicative of a drier grassy environment (Neumann *et al.* 2010). Climate change during the Pliocene and Pleistocene has been suggested as the major (although not exclusive) determinate of the faunal biogeographical pattern within other biomes in South Africa (for example, Cape Floristic Region: Tolley *et al.* 2006, Makokha *et al.* 2007, Tolley *et al.* 2008, 2009, Swart *et al.* 2009).

In this study, we examine the genetic structure of the South African endemic Cape Parrot population. We compare the historical (many generations ago) and contemporary (one to few generations ago) population structure by making use of temporal sampling. Although our historical (1870–1946) sample size is small, these samples represent the Cape Parrot population before severe anthropogenic landscape transformation. Contemporary (1951–2014) samples were used to examine the current population structure of the species. The date of 1950 was selected for the separation of historical and contemporary samples, following the observed population decline between 1900 and 1950 (Wirminghaus *et al.* 1999, Downs *et al.* 2014). Multilocus microsatellite data were used to estimate changes in population structure over time. This study also aimed to provide a better understanding of the subpopulation structure and connectivity between the contemporary populations. Given the current distribution of suitable habitat, the populations from the three provincial regions (EC, KZN and Limpopo) could be considered as separate management units. Estimating connectivity of disjunct populations will provide conservation authorities with the necessary information needed to make and implement the appropriate conservation measures to protect the Cape Parrot.

Materials and methods

Specimen sampling

Historical samples

Historical Cape Parrot samples were sourced from four natural history collections. Only specimens with collection dates and locality information (at least a provincial allocation) were selected for inclusion in the study. In total 29 archival Cape Parrot samples collected between 1870 and 1946 from six localities were selected for inclusion in the historical dataset (Alice, Eastern Cape, $n = 2$; King William's Town, Eastern Cape, $n = 6$; Frankfort, Eastern Cape, $n = 3$; Modimolle, Limpopo, $n = 4$; Piet Retief, Mpumalanga, $n = 2$; Table S1 in the online Supplementary material). Samples collected from Piet Retief and Modimolle fall outside the current Cape Parrot distribution (Figure 1). These records suggest that either these specimens represent migrants or that during the late 1800s and early 1900s, when they were collected, forests, and hence Cape Parrots, were more widely distributed. Eight samples had only provincial allocations. These samples were grouped as EC unknown ($n = 4$) and KZN unknown ($n = 4$). Although these are indicated on the map (Figure 1), it should be noted that the exact sampling locality is unknown.

Contemporary samples

Contemporary samples were sampled between 1951 and 2014 from wild caught Cape Parrots from across their distribution range ($n = 75$; Figure 1). Sixty samples were collected from four localities in the EC province of South Africa (Alice, $n = 41$; King William's Town, $n = 11$; East London, $n = 3$; Lusikisiki, $n = 3$; EC unknown, $n = 2$), 19 samples from Creighton (KZN), one unknown KZN sample and five samples from the relic population in the Tzaneen area (Limpopo). Although the sample size of the Limpopo population is small, this sample does represent a significant portion of the census population ($n = 80$–100; Downs *et al.* 2014).

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Laboratory procedures

Genomic DNA was extracted from archival toe pads, muscle tissue, whole blood and feather samples using the methods described in Coetzer *et al.* (2015). All archival samples were re-extracted and reamplified at least three times to ensure that genotypes were consistently scored and to check for contamination. Work with archival samples was performed separately from the contemporary samples, after cleaning the work benches and equipment used. Specimens were genotyped using a panel of 16 microsatellite markers (*Prob01*, *Prob06*, *Prob09*, *Prob15*, *Prob17*, *Prob18*, *Prob23*, *Prob25*, *Prob26*, *Prob28*, *Prob29*, *Prob30*, *Prob31*, *Prob34*, *Prob35* and *Prob36*) designed specifically for *P. robustus* (Pillay *et al.* 2010). The microsatellite panel was divided into six multiplex sets (Multiplex 1: *Prob06*, *Prob15* and *Prob26*; Multiplex 2: *Prob30* and *Prob36*; Multiplex 3: *Prob18*, *Prob25* and *Prob31*; Multiplex 4: *Prob01*, *Prob09* and *Prob17*; Multiplex 5: *Prob23* and *Prob28*; Multiplex 6: *Prob29*, *Prob34* and *Prob35*). The PCR reaction mixtures and cycling protocol were the same as in Coetzer *et al.* 2015. To check for genotyping consistency, we reamplified 20% of contemporary samples.

Analysis of genetic variation

To better elucidate temporal changes in populations of Cape Parrots, historical (1870–1946) and contemporary (1951–2014) samples were analysed separately. Analyses were also conducted on the combined dataset (1870–2014). Tests for deviation from Hardy-Weinberg equilibrium (HWE) and estimates of genotypic disequilibrium (GD) between pairs of loci within sampling regions were performed using Genepop 4.2 (Rousset 2008). Null allele frequencies were estimated using ML-NullFreq (Kalinowski and Taper 2006) and FreeNA (Chapuis and Estoup 2007). GenAlEx 6.5 (Peakall and Smouse 2012) was used to assess genetic diversity by calculating the number of alleles (NA), number of private alleles (P_A) observed heterozygosity (H_O) and unbiased expected heterozygosity (uH_E). Allelic richness (Ar) and inbreeding coefficient (F_{IS}) was calculated in FSTAT (Goudet 2001). To assess the genetic differences between the contemporary and historical data, genetic diversity values were compared per locus using the Wilcoxon signed-rank test calculated in R (R Core Team 2015) and applying a standard Bonferroni correction for multiple comparisons (Rice 1989).

Population structure analysis

Bayesian clustering analysis was performed on contemporary and historical data independently using the program STRUCTURE v2.3 (Pritchard *et al.* 2010). Analyses were also conducted on the combined dataset, with the rationale that if the contemporary structure corresponded to the historical one, the historical and contemporary samples from the same geographical region should cluster together. On the other hand, if the contemporary and historical structures differ, samples from the same region should not cluster well or are expected to have a more admixed membership. To estimate the number of genetic clusters (K), simulations were conducted with K ranging from 1 to 10 for the contemporary and historical datasets and K ranging from 1 to 12 for the combined dataset. Twenty independent runs were performed for each K value, using 5×10^5 Markov chain Monte Carlo (MCMC) replicates per run and a burn-in of 5×10^4 iterations. In each run the correlated allele frequencies and the admixture ancestry model were selected. The LOCPRIOR was used, incorporating population locality information to help identify genetic patterns in the presence of low sample size or close relationships between populations (Hubisz *et al.* 2009). STRUCTURE HARVESTER (Earl and vonHoldt 2012) was used to estimate the optimal number of genetic clusters using the method implemented by Evanno *et al.* (2005). ClumpAK (Kopelman *et al.* 2015) was used to estimate the probability of membership (Q-values) for each individual and each genetic cluster.

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Due to the low sample size in some of our datasets, an additional population structure analysis for the historical and contemporary datasets was run using the program TESS version 2.3.1 (Chen *et al.* 2007, Durand *et al.* 2009). Both STRUCTURE and TESS are Bayesian clustering methods, but TESS implements a more sophisticated spatially explicit model which allows for the inclusion of individual sampling locality information to better estimate ancestry coefficients. Location coordinates were generated in TESS for samples with only regional information by using the available information for the Cape Parrot distribution range in the province the samples originate from. The samples were also grouped according to the groupings used for the STRUCTURE analysis. The CAR admixture model was used for all analyses, with the default spatial interaction parameter (ψ) setting of 0.6. Multiple clusters (Ks) were tested (K = 2–10), with 20 runs per K. Burn-in was set at 1,000 for a total of 10,000 sweeps. The average Deviance Information Criterion (DIC) scores were calculated to determine the most likely number of genetic clusters. The ancestry coefficient for each individual across all runs were estimated in CLUMPP version 1.1.2 (Jakobsson and Rosenberg 2007). DISTRUCT version 1.1 (Rosenberg 2004) was used to visualise the results.

It has been reported that STRUCTURE can be negatively influenced by unbalanced sampling (Kalinowski 2011). We therefore decided to run STRUCTURE and TESS for a subset of the EC sample set, with only birds collected from Alice and King William's Town, to identify possible substructure which might have been overlooked due to the unbalanced sampling. This was done because the EC dataset (n = 60) is disproportionate in size compared to the KZN and Limpopo datasets and Alice and King William's Town are the largest sample sets within EC. STRUCTURE analysis was run under the admixture model with correlated allele frequencies, for 10,000 replicates and 1,000 burn-in. K was tested from 1 to 5 for 10 iterations each. The analyses were assessed and visualised as described above. The TESS analysis was performed using the CAR admixture model, with ψ set at default (0.6). Multiple clusters (Ks) were tested (K = 2–5), with 10 runs per K. A total of 10,000 sweeps and a burn-in of 1,000 was selected. The most probable K was established as described above.

Principal coordinates analysis (PCoA) of pairwise F_{ST} values (Peakall *et al.* 1995) were estimated using GenAlEx. The pairwise F_{ST} analyses were performed for two grouping schemes. First, samples were grouped according to sampling locality. For the historical data, individuals were assigned to eight groups (Alice, King Williams's Town, Frankfort, EC unknown, KZN unknown, Modimolle, Piet Retief and Tzaneen). Seven groups (Alice, King Williams's Town, East London, Lusikisiki, EC unknown, Creighton and Tzaneen) were analysed for the contemporary dataset, with the one unknown contemporary KwaZulu-Natal sample included in the Creighton sample set for this analysis. The samples from the combined dataset were grouped into 11 groups (Alice, King Williams's Town, Frankfort, East London, Lusikisiki, EC unknown, Creighton, KZN unknown, Modimolle, Piet Retief and Tzaneen). Geographically close populations were then combined into three regional groupings for the historical, contemporary and combined analyses (historical: South (Alice, King Williams's Town, Frankfort, EC unknown), Central (KZN unknown) and North (Modimolle, Piet Retief and Tzaneen); contemporary: South (Alice, King Williams's Town, East London, Lusikisiki, EC unknown), Central (Creighton) and North (Tzaneen); combined: South (Alice, King Williams's Town, Frankfort, East London, Lusikisiki, EC unknown), Central (Creighton, KZN unknown) and North (Modimolle, Piet Retief and Tzaneen).

To further explore the relationship between genetic diversity and geography over time we performed a series of Mantel tests (Mantel 1967) using the ADE4 package (Dray and Dufour 2007) in R. A permutation procedure (10,000 permutations) was used to obtain P-values. Analyses were performed on the three data sets (all specimens, contemporary and historical) testing for a correlation between genetic distance, and geographic distance, and genetic distance and difference in age of samples. Pairwise genetic distance matrices were constructed in GenAlEx.

Demographic history

Three methods were used to detect the genetic signature of population bottlenecks - the heterozygosity excess method, mode-shift method and M-ratio test. The heterozygosity excess method as

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implemented in the program Bottleneck v 1.2.02 (Piry *et al.* 1999) was used to assess the possible occurrence of a bottleneck event within the historical, contemporary and combined datasets, using the Wilcoxon signed-rank test method of (Luikart and Cornuet 1998). Given the limited sample size of the historical samples (1870–1946), analysis was performed by placing all individuals in a single group. Two analyses were performed for the contemporary data (1951–2014). One analysis grouping individuals per geographic origin (South, Central and North; see results), and a second analysis with all individuals grouped together. Two mutation models were chosen, the TPM model for microsatellites (Di Rienzo *et al.* 1994) with 90% and 95% stepwise mutations following Garza and Williamson (2001) and Dussex *et al.* (2015) with a variance of 12 as suggested by Piry *et al.* (1999), to encompass the observed range of multistep mutations in natural populations (Di Rienzo *et al.* 1994); and the stepwise mutation model (SMM). The mode-shift test was used as implemented in Bottleneck v1.2.02 to assist in discriminating between recent bottlenecked and stable populations (Luikart *et al.* 1998). The mode-shift distortion caused by a population decline can only be detected within a few dozen generation (Tucker *et al.* 2012), therefore only very recent bottleneck events will be detected by this method. The M-ratio test is more appropriate for detecting bottlenecks that occurred at a more distant time (< 100 generations) than the heterozygote excess method (Williamson-Natesan 2005, Peery *et al.* 2012). The M-ratio analysis was performed using M-P-Val and Critical_M (Garza and Williamson 2001). Pre-bottleneck *Ne* values were set at 100, 1,000 and 2,000 individuals, with a constant mutation rate of 5×10^{-4}. Two TPM mutation models were followed. The first parameter set is more widely used with single step mutations of $p_s = 0.88$ and the number of steps for multi-step mutations at $\Delta g = 2.8$. A second more conservative parameter set, with $p_s = 0.9$ and $\Delta g = 3.5$ was also tested (Garza and Williamson 2001).

A coalescent-based Bayesian method was used to estimate the change in effective population size through time, using the program MSVAR 1.3 (Beaumont 1999, Storz and Beaumont 2002). Eight simulations were run for each heterochronous dataset (historical, contemporary and combined). The coalescent-based method used in MSVAR is known to be affected by strong population structure (Radespiel and Bruford 2014). For this reason, analyses were also performed on the three regional groupings (South, Central and North) independently. An age of first breeding of five years was chosen (Wirminghaus *et al.* 2001a). Five simulations for each contemporary regional dataset (South, Central and North) was performed. Each simulation consisted of 2×10^8 iterations with output values recorded every 10^4 steps per simulation. Prior distribution settings used are provided in Table S2. The first 50% of the output from each chain was removed as burn-in. Convergence of MCMC chains was assessed using Tracer v1.5 (Rambaut and Drummond 2007). The simulations for each dataset were then combined, giving 8×10^4 sampling points for the heterochronous datasets and 5×10^4 for each contemporary subpopulation. The mean and 95% highest posterior densities (HPD) were estimated in Tracer. The effective population sizes for the contemporary and historical datasets were additionally assessed using the program NeEstimator version 2 (Do *et al.* 2014).

Contemporary vs historical gene flow

Gene flow among Cape Parrot subpopulations in the historical and contemporary datasets was estimated using BayesAss v1.3 (Wilson and Rannala 2003). Analyses were performed to test for gene flow among the three regional groupings (South, Central and North) for the historical and contemporary datasets. A third gene flow analysis was performed among four groupings for the contemporary dataset, using the three regional groupings as suggested by STRUCTURE but placing Lusikisiki and EC unknown in a separate group as these specimens showed signs of admixture from the South and Central clusters (Group 1: Alice, King William's Town and East London; Group 2: Lusikisiki and EC unknown; Group 3: Creighton and KZN unknown; Group 4: Tzaneen). Three independent runs were conducted each consisting of 1×10^7 iterations, with a burn-in of 4×10^5 and a sampling frequency of 2,000. The delta values for each parameter were adjusted to

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achieve a 40–60% acceptance rate as recommended in the BayesAss manual. The final delta values used were: delta allele frequency = 0.4, delta migration rate = 0.1 and delta inbreeding coefficient = 0.5. Migration rates below 0.1 indicate that the two populations are demographically independent (Hastings 1993).

Results

Genetic diversity

For this study a total of 114 individuals (historical = 29; contemporary = 85) were genotyped using 16 microsatellite loci. Deviations from Hardy-Weinberg equilibrium (HWE) estimates showed that within the historical data seven loci (*Prob01*, *Prob15*, *Prob17*, *Prob23*, *Prob26*, *Prob34* and *Prob36*, p-value < 0.05) are deviating from equilibrium. Only two loci (*Prob15* and *Prob17*; p-value < 0.05) deviated significantly from Hardy-Weinberg equilibrium within the contemporary data. No signs of linkage disequilibrium were detected in either the historical or contemporary datasets. DNA quality of archival samples is expected to be lower than fresh samples (Wandeler *et al.* 2007), it is unsurprising that the historical dataset contained the highest amount of missing data, with 15.5% missing data across all loci. A minimal amount of missing data was included in the contemporary dataset, with only 0.7% missing data across all loci. The mean null allele frequency over all loci and samples was 4.7% (Na; Table S3). As expected, the historical dataset had the highest level of null alleles (Na = 18%), with a null allele frequency of only 3.1% observed for the contemporary dataset. These observed null allele values are, however, well below values reported in other studies (Dakin and Avise 2004). No significant difference was observed between ENA corrected and uncorrected F_{ST} values (P-value > 0.003) and all loci were used for the subsequent analyses.

Private alleles were detected in the historical (n = 9) and contemporary datasets (n = 39; Table 1). All genetic diversity estimates, except uH_E, showed significant differences between the historical and contemporary datasets (P-value > 0.003). The average number of alleles, Ar and H_O estimates were highest for the contemporary dataset (Table 1). The F_{IS} values indicated significantly lower levels of heterozygotes within the historical samples (F_{IS} = 0.31), with low levels of inbreeding observed within the contemporary samples (F_{IS} = 0.04). The uH_E values were only slightly higher for the contemporary samples (Table 1).

The genetic diversity for each regional grouping using the historical and contemporary data were also assessed. The average number of alleles and allelic richness were highest for the contemporary South group and Central group, where the highest average number of alleles for the historical dataset was observed in the South and North groups (Table 1). The North group showed the highest observed heterozygosity in the historical dataset, with the highest estimate in the contemporary dataset calculated for the Central group. Low levels of inbreeding were observed in the contemporary South group (F_{IS} = 0.04), with slightly negative values for the Central and North groups. Much higher F_{IS} values were observed for the historical groups (Table 1). The South and Central groups contain the highest levels of genetic diversity within the contemporary dataset (H_E = 0.63, SE = 0.05 and H_E = 0.64, SE = 0.05; Table 1).

Historical vs contemporary population structure

Using the historical data, the Bayesian clustering analysis recovered two clusters as the most probable number of genetic clusters (K = 2, mean LnP(K) = -985.14). These clusters were geographically correlated (Figure 2). The results of this analysis group the specimens from the EC distribution of Cape Parrots into one cluster (Alice, Q = 0.869; Frankfort, Q = 83; King William's Town, Q = 0.551; Figure 2). The remaining samples (EC Unknown, Q = 0.97; KZN unknown, Q = 0.856; Piet Retief, Q = 0.603; Modimolle, Q = 0.957; Tzaneen, Q = 0.94) belong to the second genetic cluster. There is some evidence of admixture as one of the Piet Retief samples (LH 10)

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Table 1. Sample details and genetic diversity for each *Poicephalus robustus* population analyzed in the current study. Standard error values for the mean number of alleles, observed heterozygosity, unbiased expected heterozygosity and allelic richness are provided in parentheses. Deviation from Hardy-Weinberg equilibrium (HWE) is also provided for each population per dataset.

Region/Time period:	Number of samples:	Average number of alleles (N_A):	Observed Heterozygosity (H_O):	Unbiased Expected Heterozygosity (uH_E):	Deviation from Hardy-Weinberg Equilibrium (HWE) probability	Allelic richness (Ar):	Inbreeding coefficient (F_{IS}):	Private alleles (P_A):
Historical (1870-1946)	29	3.688 (0292)	0.445 (0.041)	0.604 (0.035)		4.302 (0.314)	0.314	9
South	15	4.188 (0.579)	0.382 (0.058)	0.604 (0.051)	High. sign.	2.792 (0.222)	0.384	15
Central	4	2.688 (0.373)	0.443 (0.093)	0.563 (0.084)	0.558	NC**	0.258	2
North	10	4.188 (0.476)	0.509 (0.057)	0.645 (0.044)	0.018	2.985 (0.224)	0.224	14
Contemporary (1951-2014)	85	4.917 (0.561)	0.617 (0.033)	0.613 (0.030)		7.239 (0.039)	0.039	39
South	60	6.563 (1.252)	0.605 (0.055)	0.632 (0.053)	High. sign.	3.791 (0.400)	0.042	23
Central	20	5.313 (0.898)	0.647 (0.058)	0.635 (0.050)	0.085	3.708 (0.386)	-0.02	6
North	5	2.875 (0.340)	0.600 (0.063)	0.572 (0.052)	0.905	2.875 (0.340)	-0.055	5
All specimens:	114	5.854 (0.599)	0.581 (0.030)	0.633 (0.028)		7.680 (0.095)	0.095	-
South	75	6.563 (1.252)	0.574 (0.053)	0.632 (0.053)	High. sign.	4.792 (0.651)	0.093	22
Central	24	5.313 (0.898)	0.625 (0.059)	0.632 (0.053)	0.0029	4.718 (0.667)	0.011	6
North	15	2.875 (0.340)	0.544 (0.044)	0.634 (0.043)	High. sign.	4.725 (0.638)	0.146	8

*Negative values were converted to zero

**Value could not be calculated due to missing data

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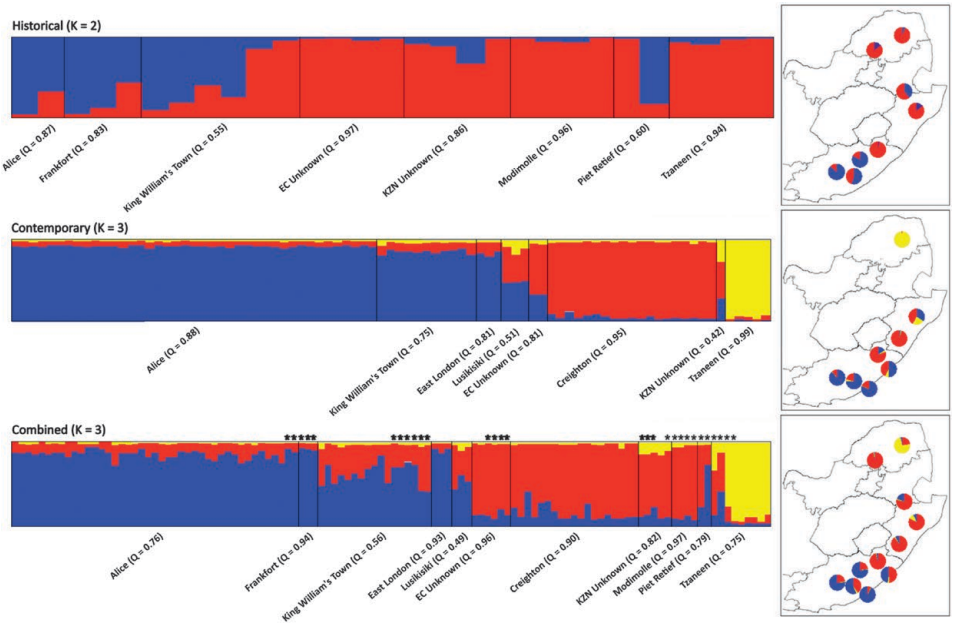

Figure 2. The estimated population genetic structure of *Poicephalus robustus* in South Africa using historical, contemporary and combined datasets (left). Each vertical line in the bar plot represents an individual and is coloured according to every individual's estimated membership coefficient (Q) values. Asterisks indicate historical samples. The mean Q-value of each cluster is provided. Maps showing the mean Bayesian assignment probabilities per locality for historical, contemporary and combined datasets (right). Each colour indicates the mean proportion of an individual's genotypes assigned to a particular lineage in each locality.

clustered strongly with the southern EC cluster (Q = 0.833). In this analysis of historical samples, there is no evidence of genetic isolation of the northern Limpopo population. In contrast, Bayesian analysis of the contemporary data recovered three genetic clusters using the Evanno method (K = 3, mean LnP(K) = -3683.365). Running the analyses without LOCPRIOR and examining alternative STRUCTURE graphs (K = 2 or K = 4) did not change the overall clustering pattern (results not shown). The EC and KZN Cape Parrot populations group separately, although individuals collected from Lusikisiki (Q = 0.51 for the South cluster) and EC Unknown (Q = 0.81 for the Central cluster) do show admixture. Lusikisiki is located on the EC and KZN boundary and this population could represent the transitional zone separating the South cluster from the Central cluster. A striking result to emerge from the analysis of the contemporary data is the genetic distinctiveness of Limpopo population (Q = 0.99). The STRUCTURE analysis of the Alice and King William's Town datasets showed no signs of substructuring within the southern region which could have been obscured by skewed sampling (Figure S1).

The TESS results differed slightly from the STRUCTURE results in that there appear to be two genetic clusters for both the contemporary and historical datasets (Figures S2 and S3). Although the DIC values for both analyses indicated more than two clusters, one could clearly see from the figures that only two clusters are present in both cases. The TESS population structuring observed for the historical dataset was similar to that observed for the STUCTURE analysis. The TESS analysis for the contemporary dataset grouped the northern Cape Parrots in one genetic cluster, but grouped all the remaining individuals (South and Central specimens) into one genetic cluster.

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The TESS population structure analyses were supported by pairwise F_{ST} PCoA analysis. The historical dataset showed significant genetic partitioning between the South and North groupings (F_{ST} = 0.067; *P*-value = 0.001). There was no support for the differentiation between isolated northern populations of Modimolle, Piet Retief and Tzaneen from the central Cape Parrot population (F_{ST} = 0.025; *P*-value = 0.148) or between the southern and central populations (F_{ST} = 0.014; *P*-value = 0.225; Figure S4). The contemporary dataset showed significant genetic differentiation between the two genetic groupings (South/Central and North) identified in the TESS analysis (Table 2; Figure S5).

The temporal difference in population structure was supported by the Mantel tests (Table S4). While both the contemporary and historical data show significant positive correlation between genetic diversity and difference in age of sample (contemporary: r = 0.328, *P* = 0.02; historical: r = 0.254, *P* = 0.001), only the historical data showed a significant correlation between geographic distance and genetic diversity (r = 0.431, *P* = 0.001). These results suggest that while isolation-by-distance could explain the distribution of genetic diversity in historical samples, during contemporary times genetic diversity and geographic distance were not significantly correlated.

Demographic history

Bottleneck analysis using the heterozygote excess method, implementing TPM models with 90% and 95% stepwise mutations, only showed signs of a recent (early 1900s) bottleneck in the South and North regional groupings of contemporary Cape Parrot populations (Table 3). The more conservative SSM model, however, showed no signs of a bottleneck event. Only the contemporary North group showed significant deviation from the normal L-shaped distribution of allele frequencies expected from a population in mutation-drift equilibrium, providing evidence of a possible recent decline in this population. The sample size of this group is low (n = 5) and this result should be interpreted with caution. The *M*-ratio analyses showed no signs of bottlenecks in any of the datasets, with *M*-ratio values ranging from 0.645 to 0.826.

The ancient effective population sizes for the three main datasets were large (historical N_A = 236,047.82, contemporary N_A = 269,153.48 and combined N_A = 278,612.12), with much smaller recent effective population sizes (historical N_R = 104.71, contemporary N_R = 305.49 and combined N_R = 495.45), indicating a severe bottleneck. The ratio of the contemporary and historical effective population sizes ($r = N_R/N_A$) for each dataset where used to assess the direction of demographic change, with $r < 1$ indicating a population decline and $r > 1$ a population expansion. All r values were smaller than 0.0021 (Table 4). This indicates that the recent effective populations size (N_R) is less than 1% of the ancient effective population size (N_A). There is clear support for a population decline long before European settlement of South Africa in the 17th century, with the average time since the decline estimated at 2649.289 years BP. Estimates for the three contemporary regional clusters also supported a drastic population decline with the time since decline ranging from 1,807.174 to 3,026.913 years BP (Table 4). The N_R value for the contemporary data was three times larger than a century ago, as the N_R recovered from the historical data is three times lower. There is some overlap between the 95% HPD values for the contemporary and historical datasets,

Table 2. The pairwise F_{ST} estimates for the combined dataset and the historical and contemporary datasets. Comparisons were made between the three sampling regions; South, Central and North. The pairwise F_{ST} values are below the diagonal, with *P*-values above the diagonal. The significance threshold was adjusted for multiple tests: *P*-value = 0.003.

Historical dataset:				Contemporary dataset:			Combined dataset:		
	South	Central	North	South	Central	North	South	Central	North
South	*	0.225	0.001	*	0.007	0.001	*	0.004	0.001
Central	0.014	*	0.148	0.014	*	0.001	0.013	*	0.003
North	0.067	0.025	*	0.091	0.078	*	0.043	0.031	*

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Table 3. Bottleneck results obtained from a Wilcoxon signed-rank test for heterozygous excess (one tail) using two mutation models (*P*-values), the Mode-shift test and the *M*-ratio method for bottleneck detection. The two-phase mutation (TPM) and single-step mutation (SSM) models were used for the heterozygous excess tests. The Bonferroni correction was applied to all *P*-values (*P*-value = 0.003).

Test	Parameter		Historical ($n = 29$)	South ($n = 60$)	Central ($n = 20$)	North ($n = 5$)	Contemporary ($n = 85$)	All samples ($n = 114$)
H*e* excess	TPM (90% SMM)		0.391	0.003	0.004	0.0001	0.334	0.57
	TPM (95% SMM)		0.53	0.003	0.005	0.0001	0.353	0.812
	SMM		0.666	0.59	0.334	0.008	0.666	0.884
Mode shift			NO	NO	NO	YES	NO	NO
M-Ratio	$ps = 0.88$	$\Delta g = 2.8$						
	$\theta = 0.2$; $Ne = 100$	Mc	0.532	0.532	0.531	0.528	0.534	0.533
	$\theta = 4$; $Ne = 2000$	Mc	0.542	0.586	0.517	0.415	0.605	0.618
	$\theta = 10$; $Ne = 5000$	Mc	0.543	0.517	0.507	0.281	0.632	0.652
		M-Ratio	0.78	0.826	0.82	0.645	0.817	0.772
	$ps = 0.9$	$\Delta g = 3.5$						
	$\theta = 0.2$; $Ne = 100$	Mc	0.456	0.457	0.457	0.455	0.458	0.456
	$\theta = 4$; $Ne = 2000$	Mc	0.453	0.493	0.429	0.34	0.511	0.527
	$\theta = 10$; $Ne = 5000$	Mc	0.455	0.606	0.42	0.349	0.544	0.563
		M-Ratio	0.78	0.826	0.82	0.645	0.817	0.772

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Table 4. Estimates of effective population size changes over time as calculated in MSVAR using the exponential model. The 95% highest posterior density for each estimate is provided in parentheses.

	Mean ancestral effective population size (N_A)	Mean recent effective population size (N_r)	Time (in years) since decline	r = N_C/N_H
Historical	236047.823	104.713	2437.811	0.0004
(*n* = 29)	(67142.885 - 824138.115)	(25.351 - 436.516)	(645.654 - 8933.055)	(0.0004 - 0.0005)
Contemporary	269153.48	305.492	2355.049	0.0011
(*n* = 85)	(83176.377 - 847227.414)	(69.984 -1462.177)	(568.853 - 8810.489)	(0.0008 - 0.0017)
South	272270.131	275.423	3026.913	0.001
(*n* = 60)	(86696.188 - 843334.758)	(69.823 - 1150.800)	(874.984 - 9931.160)	(0.0008 - 0.0014)
Central	198609.492	69.502	1807.174	0.0003
(*n* = 20)	(56234.133 - 685488.226)	(17.458 - 286.418)	(454.988 - 7311.391)	(0.0003 - 0.0004)
North	169433.78	40.272	1815.516	0.0002
(*n* = 5)	(44771.330 -656145.266)	(10.447 - 154.882)	(473.151 - 7379.042)	(0.0002 - 0.0002)
Combined	278612.117	495.45	3155.005	0.0018
(*n* = 114)	(85506.671 - 897428.795)	(127.938 - 1896.706	(824.138 - 12941.958)	(0.0015 - 0.0021)

and this should be interpreted with care. This result, however, still suggests that although the Cape Parrot population experienced a drastic population decline c.2,600 years BP, contemporary populations have increased during the last century. The *Ne* values calculated for the contemporary and historical datasets with NeEstimator support the N_R results obtained from MSVAR. The CI values for the N_e estimates do, however, also overlap and should be observed with caution (contemporary N_e = 395, CI = 289.1-600.4; historical N_e = 140.7, CI = 74.5-664.3).

Gene flow

Migration analysis of the historical data indicates that there was gene flow between populations in the South and Central geographical regions (*m* = 0.12, % confidence interval (CI) = 0.012–0.282) and between the Central and North regions (*m* = 0.119, 95% CI = 0.002–0.258; Table S5). This is unsurprising given the population partitioning seen in the STRUCTURE analysis. A similar pattern of gene flow was observed in the contemporary data. Analysis indicates elevated migration rates between contemporary populations when the data was grouped by region (South-Central, *m* = 0.278, 95% CI = 0.182–0.327; Central-North, *m* = 0.199, 95% CI = 0.001–0.286) and by sampling locality (Alice/King William's Town-Lusikisiki, *m* = 0.222, 95% CI = 0.097–0.311; Alice/King William's Town - Creighton, *m* = 0.267, 95% CI = 0.176–0.325; Creighton-Tzaneen, *m* = 0.181, 95% CI = 0.001–0.307; Figure 3). The only non-overlapping CI values were observed for migration estimates between the contemporary southern and central populations, providing clear evidence for a south to north migration pattern between these regions. This analysis suggests that the southern populations may act as a source for the central Cape Parrot populations. In contrast to the STRUCTURE results which indicated that the contemporary Limpopo population is isolated from the rest of the Cape Parrot distribution, the migration analysis indicates some gene flow between the contemporary Central and North regions (*m* = 0.199, 95% CI = 0.001–0.286), The CI values of this migration pair did, however, overlap.

Discussion

Many studies have used DNA from contemporary populations to infer past demographic changes in populations of South African species (Eick *et al.* 2005, Herron *et al.* 2005; Tolley *et al.* 2006). This approach, although useful, may not accurately reflect changes in population structure over time. For example, low levels of genetic variation in contemporary populations could be the result of recent anthropogenic-driven changes or could represent an ancestral state (Wandeler *et al.* 2007).

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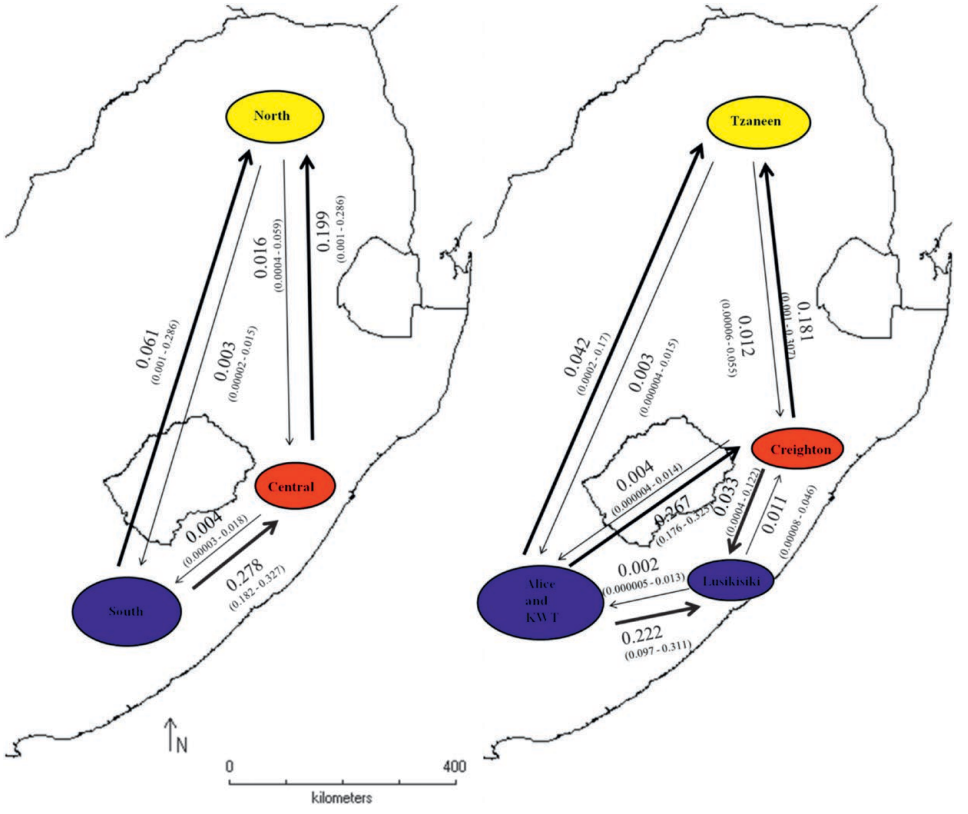

Figure 3. Migration rates (*m*), estimated from the contemporary data using BayesAss. Samples were grouped according to (left) the three sampling regions and (right) individual sampling locality sites. Higher migration rates are indicated by thicker lines. The 95% confidence interval is provided in parentheses.

Advances in molecular biology now allow historic and even ancient genotypes to be added to phylogeographic studies (Bouzat *et al.* 1998, Groombridge *et al.* 2000, Edwards *et al.* 2012, Welch *et al.* 2012, Dussex *et al.* 2015) allowing for temporal changes in allele frequencies and effective population size to be directly measured. This is the first study in South Africa to use historical archival genotypes to measure changes in the phylogeographic pattern of a range-restricted species over time. Genetic diversity estimates showed some significant differences between the historical and contemporary datasets, with the contemporary dataset generally containing higher levels of genetic diversity and lower levels of inbreeding compared to the historical dataset. Although this may be an artifact of limited number of archival specimens available, the heterozygosity estimates calculated for the contemporary Cape Parrot population are not as low as would be expected from a species which has experienced a pronounced bottleneck but are within the range of genetic diversity estimates observed in three other old world parrot species. Taylor and Parkin (2010) observed moderate to high values for H_O and H_E for the Echo Parakeet *Psittacula eques* ($H_O = 0.53$, $H_E = 0.56$), the Ring-necked Parakeet *Psittacula krameri* ($H_O = 0.56$, $H_E = 0.7$) and the African Grey Parrot *Psittacus erithacus* ($H_O = 0.77$, $H_E = 0.83$) using four microsatellite loci. These values are also consistent with H_O and H_E estimates for new world parrots, for example *Amazona auropalliata* ($H_O = 0.59$, $H_E = 0.62$; Wright *et al.* 2005) and *Ara militaris* ($H_O = 0.58$, $H_E = 0.62$; Rivera-Ortíz *et al.* 2017).

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High levels of spatial genetic structuring were recovered within the study species. Analyses provide evidence for at least three independently evolving distinct groups within the species. The South cluster is distributed within the EC mistbelt forests, the Central cluster is found within the KZN forests and the relict North cluster is found in the Limpopo province.

Historical vs contemporary population structure

Bayesian structure analysis of historical and contemporary data recovered strikingly different patterns of phylogeographic structure (Figure 2). In particular, the historical and contemporary data do not agree on the genetic affinity of the Tzaneen lineage. This lineage represents the most northern distribution boundary of this species. In the historical data, there is no evidence to suggest the genetic distinctiveness of the Tzaneen lineage with individuals genotyped from this region clustering with populations from Mpumalanga, and KZN. In contrast, genotypes from Tzaneen collected after 1950 formed a separate well-supported cluster with both the STRUCTURE and TESS analyses, indicating the genetic isolation of this population. The temporal sampling used suggests that the isolation of the Tzaneen lineage has occurred within the last century, which highlights anthropogenic influence as the main driver of differentiation of this population. The distribution of historical records provides additional evidence of habitat loss during the last century with six historical specimens included in the study collected in areas (Modimolle, Limpopo and Piet Retief, Mpumalanga) where neither Cape Parrots nor suitable habitat are currently found.

The role of gene flow and genetic drift in the demographic history of these parrots has also changed over time. Migration played a greater role in historical populations, probably because forest fragmentation was likely less extensive a century ago. In contrast, the contemporary Limpopo population is affected by genetic drift, highlighting the genetic isolation of this population. The low levels of gene flow that was detected between the northern and central regions can be attributed to the retention of ancestral alleles within the small northern population and given time these alleles will be removed from the population by genetic drift and inbreeding. There is evidence to suggest that contemporary KZN and EC population are still genetically connected. Conservation of these mistbelt forests is therefore crucial to maintain the genetic diversity of the species.

Recent population decline

There is no doubt that the extensive logging of indigenous forests during the colonial period has influenced Cape Parrot population sizes. The genetic signatures of population decline are often subtle and difficult to detect in very recent bottleneck events (Cornuet and Luikart 1996, Luikart *et al.* 1998). The detection of a recent population bottleneck can be difficult if the genetic diversity at the time of the bottleneck is already low due to the population experiencing a previous, more ancient population decline (Cornuet and Luikart 1996), as is the case here. Although the heterozygote excess method, was able to detect bottleneck events in the southern and northern groups, all other methods did not find strong evidence of a recent bottleneck.

Ancient population decline

Although human-mediated habitat loss has certainly had a profound effect on indigenous Cape Parrot populations, the role of long-term climatic change cannot be discounted. This study provides strong support for a dramatic population decline ~1,815 years before present (BP; North population), ~1807 years BP (Central population) and up to ~3,026 years BP (South population). Southern African climates are known to have undergone significant regional fluctuations during the Holocene (Scott and Vogel 2000, Lee-Thorp *et al.* 2001, Chase *et al.* 2009, Metwally *et al.* 2014).

The rapid decrease in *Podocarpus* containing forests in eastern South Africa (Neumann *et al.* 2010) from c.3,500–3,600 years BP, falls within the 95% HPD of the time since population decline estimate for the central Cape Parrot population (1807.174 years BP, 95% HPD: 454.988–7311.391). Mazus (2000) reported a similar decrease in *Podocarpus* containing forests c.3,100 years BP in KZN strongly linked to the dry period during the late Holocene (Neumann *et al.* 2010). During this period a shift from C_3 to C_4 plants was also observed in the EC area (Fisher *et al.* 2013). This corresponds to the Cape Parrot population decline observed for the southern lineage (3,026.913 years BP, 95% HPD: 874.984–9,931.160).

Cool, dry conditions were recorded at Wonderkrater and Tswaing Crater, Limpopo (Scott 1999, Scott *et al.* 2003, Metwally *et al.* 2014) at around 2,000 years BP. It was also noted that *Podocarpus* forests in the Zoutpansberg region, Limpopo, declined at c.1,500 years BP (Scott 1987), coinciding with the arrival of early Iron Age people in Limpopo around 1,700 years BP (Klapwijk 1974, Evers 1975, Maggs 1984). These early settlers might have cleared forests for settlements and pastures (Bruton *et al.* 1980, Neumann *et al.* 2008). This time period overlaps with the 95% HPD for the time since the Cape Parrot population decline estimate for the northern region (1,815.516 years BP, 95% HPD: 473.151–7,379.042).

These decreases in *Podocarpus* forests would have deprived ancient Cape Parrot populations of food and nesting sources and probably led to the population decline observed in Cape Parrots during the mid-Holocene. Both the climate-driven habitat fragmentation and the more recent anthropogenic habitat contraction have played important roles in shaping the current Cape Parrot population structure.

Population recovery

There was a significant difference in allelic richness between the historical and contemporary data. The contrasting allelic richness and inbreeding values observed between the historical and contemporary datasets, suggest that the population had started to recover from the ancient climate-change driven population contraction. One mechanism responsible for an increase in allelic richness following a bottleneck is gene flow (Lacy 1987, Greenbaum *et al.* 2014) with new migrants introducing new alleles to the sink population. The end of extensive commercial logging practices in South Africa during the early 1900s most probably allowed the Cape Parrot populations, especially in the Eastern Cape and KZN regions, to grow. The observed three-fold increase from the estimated historical *Ne* to the contemporary *Ne* further supports this notion of population growth following the end of intense commercial logging in yellowwood forests. The ability of bird populations to respond rapidly post-bottleneck has been reported previously in the literature. For example, an increase in genetic variation, over a relatively short time (< 30 years), has been recorded in the Great Reed Warbler *Acrocephalus arundinaceus* which was founded by only a few individuals in 1978 (Hansson *et al.* 2000). It has been suggested that the way bird species react to large-scale population decline is largely associated with resource specialization (Shultz *et al.* 2005). Species which are more generalist and have the ability to exploit other available food resources tend to respond quicker after a bottleneck event. The Cape Parrot's ability to utilise food sources other than *Podocarpus*/*Afrocarpus* kernels (Wirminghaus *et al.* 2002) could certainly have assisted these birds to recover from a population decline following severe habitat loss.

Conservation implications

The mid-Holocene population contraction led to a severe decrease in the effective population size of the Cape Parrot, which in turn led to decreased levels of allelic diversity. This population then suffered an additional decrease in population size through anthropogenic habitat destruction in the 1900s (Downs *et al.* 2014). Estimates from the contemporary data suggests that the current effective population size of the total wild Cape Parrot population is ~ 395 individuals. This value is realistic given the 2015 population census data (Cape Parrot Newsletter, Number 17, November 2015),

in which 1,508 individuals were recorded in the wild (459 in KZN, 427 in the former Transkei, 554 in EC and 68 in Limpopo Province). The genetic data from this study do suggest tentative signs of a more recent bottleneck, but it was observed that the Cape Parrot populations are either in the process of recovering or have already recovered from the population decline. It is, however, important to monitor the Cape Parrot populations in spite of the observed signs of population recovery. A more recent population contraction event led to the isolation of the northern Cape Parrot population from the remaining populations. The northern Cape Parrot population is clearly a distinct isolated population, and it is therefore important to monitor the genetic health of this population to prevent possible inbreeding.

The highest level of genetic diversity was observed within the southern populations, highlighting the significance of these populations for future conservation management. These populations play an important role as source populations for migration to the KZN populations. These populations can be considered as source populations for possible future translocations. Translocation of wild parrot populations into areas that has been extirpated of a species (Oritz-Catedral *et al.* 2010, Oehler *et al.* 2001) or which shows low genetic diversity has previously been shown to be an effective conservation management tool following sufficient planning prior to releases (Collazo *et al.* 2013).

Further habitat contraction would be highly detrimental to Cape Parrot populations. It is therefore crucial to monitor climatic trends in South Africa and to preserve the local mistbelt forests. The establishment of forest corridors could also help reduce the detrimental effects of fragmentation and could improve gene flow to disjunct populations, such as the northern population. The Cape Parrot populations should be regularly monitored to ensure the survival of these birds.

Supplementary Material

To view supplementary material for this article, please visit https://doi.org/10.1017/S0959270919000315

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