Genetic rescue of an isolated African lion population

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Compliance with Ethical Standards

This project was approved by Ezemvelo KZN Wildlife (Project number: E/5107/04). Ethical clearance for animal sampling was obtained by R Slotow from the University of Kwazulu-Natal Animal Ethics Committee. Post-translocation samples were all collected as part of a larger monitoring project on Hluhluwe-iMfolozi Park by Ezemvelo KZN Wildlife.

Abstract

Fragmented wildlife populations are challenged by limited gene flow that can lead to significant inbreeding. The lion (*Panthera leo*) population in South Africa's Hluhluwe-iMfolozi Park (HiP) started from a small founder population of one adult male (1958), one adult female, followed by two lionesses and three cubs (two females and one male; 1965; unrelated to the male). A genetic rescue effort was launched after signs of inbreeding were observed in the 1990's. Sixteen lions (13 females and three males) were translocated into the HiP population from Pilanesberg National Park and Madikwe Game Reserve. We assessed the genetic consequences 10 to 15 years post translocation (n = 91), using microsatellite markers. Structure analysis revealed integration of the translocated animals and evidence of a unique post-translocation genetic cluster. Allelic richness increased from 2.26 to 3.88, and heterozygosity from 0.40 to 0.65. However, overall relatedness within (0.19) and among (0.15) existing prides remained higher than in an open system, and both allelic richness and heterozygosity were declining in later post-translocation generations. Thus, although genetic rescue is a valuable tool for the recovery of inbred, isolated populations, genetic augmentation should be performed at regular intervals to ensure continued population viability.

Keywords: fragmented population, genetic rescue, inbreeding, *Panthera leo*

Introduction

Wildlife populations are under increasing pressure due to human activities (Tilman et al. 2017). These occur in increasingly fragmented landscapes with little or no connectivity among populations (exacerbated by increasing human settlement) resulting in decreased gene flow (Frankham et al. 2014). Without proper management, fragmentation can lead to genetic isolation and inbreeding (Frankham et al. 2010, 2017), as has been shown in naturally isolated lion populations (Packer et al. 1991b; Driscoll et al. 2002). The resulting inbreeding depression can lead to an increase in abnormal sperm and may alter testosterone levels in male lions (Wildt et al. 1987), as well as increase susceptibility to diseases such as bovine tuberculosis (bTB) (Trinkel et al. 2011). Whatever the cause

of inbreeding depression, genetic rescue can be used to improve genetic diversity, and, ultimately, the viability of populations (Tallmon et al. 2004; Hedrick and Fredrickson 2010; Whiteley et al. 2015; Frankham 2015, 2016).

Genetic rescue was defined by Tallmon et al. (2004) as "an increase in population fitness owing to immigration of new alleles." Several recent reviews of genetic rescue explain the value, and potential pitfalls, of genetic rescue in the context of small, isolated populations (Hedrick and Fredrickson 2010; Whiteley et al. 2015; Frankham 2015; Hedrick and Garcia-Dorado 2016; Frankham et al. 2017). Some have been cautious about genetic rescue attempts due to concerns about outbreeding depression (reviewed Frankham et al. 2011; Ralls et al. 2018). Outbreeding depression, however, is far less likely than previously thought (Frankham et al. 2011, 2014, 2017; Weeks et al. 2017; Kronenberger et al. 2018), and Frankham et al. (2011) determined that risks are low when populations have been isolated by human activities within the last 500 years. A meta-analysis of genetic rescue attempts has instead revealed large and consistent benefits of gene flow in a wide variety of species (Frankham 2015). Ralls et al. (2018) called for "a paradigm shift" in genetic management of fragmented populations and included a framework to determine when genetic rescue is appropriate (also see Frankham et al. 2017b).

Lions (*Panthera leo*) historically ranged over most of Africa and the Middle East into India (Nowell and Jackson 1996). This range has been extremely fragmented by human activities, and lions had been largely extirpated from South Africa by the early 1900s (Nowell and Jackson 1996). Extensive reintroduction programs have restored South Africa's wildlife (Hayward and Somers 2009). However, many of these reintroductions were into small (<1000 km^2), fenced areas where natural movement patterns are restricted, and their conservation value has been questioned (Hayward et al. 2007; Slotow and Hunter 2009). Hluhluwe-iMfolozi Park (HiP) in KwaZulu-Natal Province, South Africa (between 28°00' and 28°26'S, 31°43' and 32°09'E; Fig. 1) is rich in biodiversity and hosts one of the first reintroduced populations of African lions. The 900 km^2 reserve is completely enclosed by

predator-proof fencing, preventing any natural movement of lions to or from the surrounding countryside.

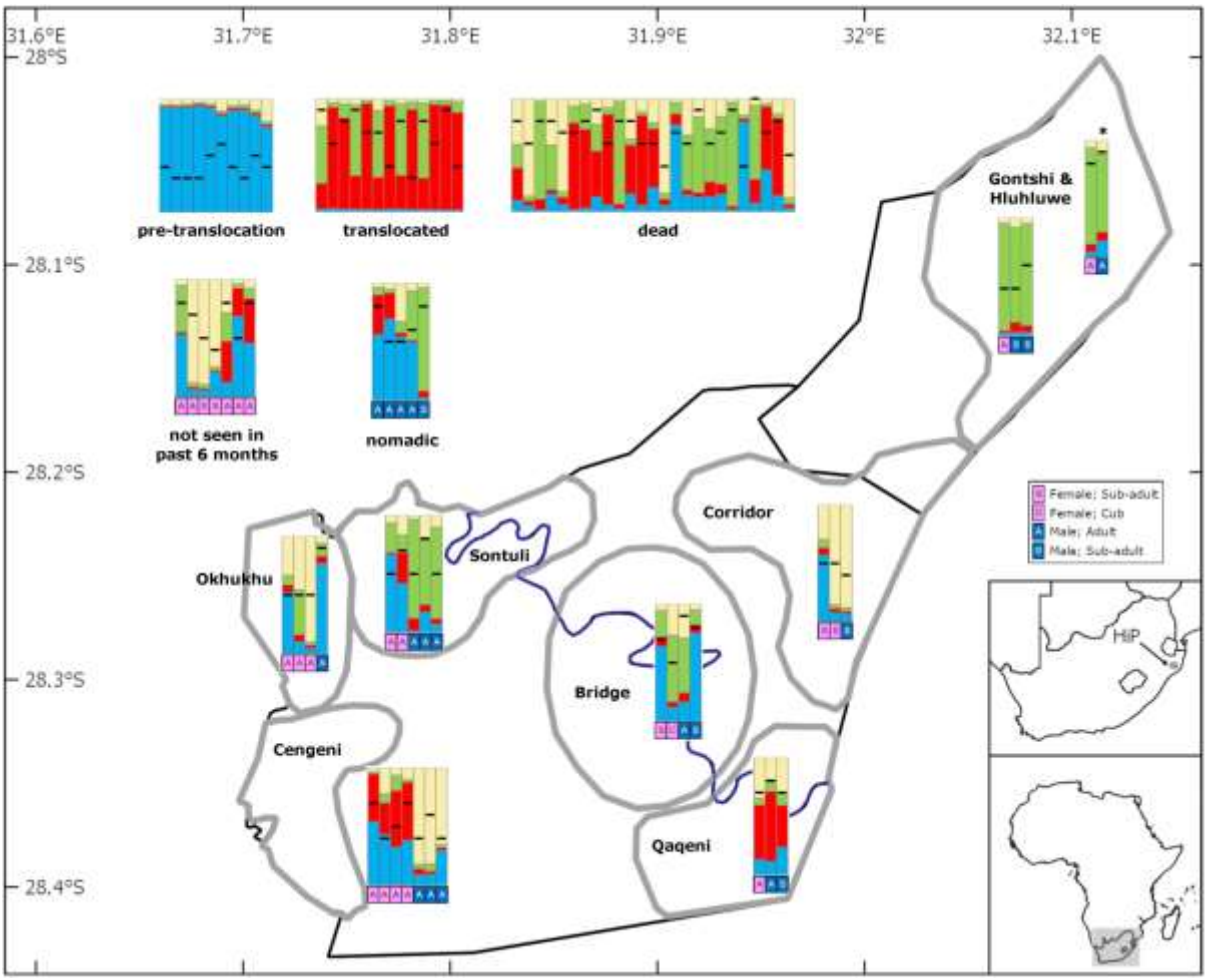

Fig. 1 Inset maps show the location of Hluhluwe-iMfolozi Park (HiP); the main figure shows approximate lion pride locations in 2014 that contained sampled individuals; the Structure *K* = 4 genetic clustering results for all samples are shown at pride locations as well as other groupings. In the Structure bars age is indicated by a letter (A – adult; S – sub-adult; C – cub) and sex by font colour and fill (–see insets); individual heterozygosity values (*n* = 10 microsatellite markers), are indicated by horizontal black bars on the Structure graphs with a scale of 0 to 1. Nomadic individuals are all males that are not currently associated with a pride. Animals not seen in the last six months are included as they may still have been on the reserve.

The founder lion population in HiP consisted of one adult male (natural immigrant, suspected from Mozambique in 1958), one adult female from Kruger National Park (NP) in 1965, and two adult females and three cubs (two females, one male; details of mother(s) unknown) from Timbavati Private Nature Reserve later in 1965 (summarized in Somers et al. 2017). The population grew until the early 1990s, when numbers started declining before all the suitable habitat was occupied (Maddock et al. 1996). The reason for this decline was unclear, however poor sperm quality in a small number of individuals was reported, and cub mortality was high (Maddock et al. 1996). Further study by Stein (1999), reported low heterozygosity levels based on transferrin alleles, and confirmed poor sperm quality (based on decreases in percent motility, progressive motility and altered morphology compared to male lions in Kruger NP), and high cub mortality. In order to combat this decline in genetic diversity which led to suspected inbreeding depression, between 1999 and 2001, sixteen lions (13 females and three males) were translocated into HiP from Pilanesberg NP and Madikwe Game Reserve (GR) (Trinkel et al. 2008). Females were introduced into areas of HiP that did not have resident lions, and successfully established territories in the north of the reserve (Trinkel et al. 2008). Lions were sourced from Pilanesberg NP and Madikwe GR because 1) they were of Etosha NP genetic provenance (Slotow and Hunter 2009), and, hence, believed to be genetically distant from HiP lions; 2) they were free of bTB, and thought to be free of feline immunodeficiency virus (FIV); 3) they were habituated to tourist activities; 4) North West Parks and Tourism Board staff had relevant experience in lion translocations; and 5) there was no monetary value attached to the lions (Trinkel et al. 2008). Lions for translocations were chosen from different pride origins based on studbook data from Pilanesberg NP and Madikwe GR to maximise genetic diversity (Slotow, unpublished data). No formal genetic studies were undertaken, but some of the 16 lions were known to have integrated into existing prides, while others formed new prides (Trinkel et al. 2008), and the descendant population showed increased resistance to bTB (Trinkel et al. 2011).

To study the genetic effects of the translocations, we used microsatellites to analyse samples collected from lions living in HiP before the translocations, the lions translocated in 1999-2001, and lions present in HiP between 2009 and 2014. We have previously shown that, using these same microsatellite markers, we can distinguish between Etosha NP origin lions and other source populations within South Africa's small reserves (Miller et al. 2015). In this study, we determined the extent of the mixing between different source populations within the existing HiP population, and the impact of these translocations on current genetic diversity. We also explored the current levels of relatedness among the existing HiP lions, and recommend management actions to minimize future inbreeding.

Methods

Sample collection

Twenty EDTA blood samples were collected in 2001 from branded individuals known to be alive prior to the 1999-2001 translocations (pre-translocation). EDTA blood samples from the 16 introduced animals were collected during the translocation process (translocation). All of these samples (n = 36) were stored at -20°C and transported to the National Zoological Garden, South African National Biodiversity Institute (NZG-SANBI) for processing. Eighty-six post-translocation samples (EDTA blood or tissue) were collected by the Scientific and Veterinary Services teams at HiP between September 2009 and November 2014; sex and approximate age of individuals at time of sampling were recorded (post-translocation; Supplementary Material). All animals were branded for individual identification, and many had microchips inserted. Samples were stored at -20°C and shipped to the Veterinary Genetics Laboratory (VGL) at the University of Pretoria for processing. Sixty three of these samples were part of an earlier study (Miller et al. 2014a, b).

DNA extraction and genotyping

Genomic DNA extractions at NZG-SANBI were obtained from blood samples of pre-translocation and post-translocated individuals using the Qiagen© DNA isolation kit, according to the manufacturer's protocol. DNA extractions of post-translocation individuals were performed at the VGL as described

in Miller et al. (2014a). PCR amplification of microsatellite loci at NZG-SANBI were prepared using Promega GoTaq® Flexi DNA polymerase (Promega Corporation) in 12.5 µL reactions with final reaction conditions as follows: 1 x PCR buffer, 1 mM $MgCl_2$, 200 µM of each dNTP, 10 pmol of each primer (forward and reverse), 1 unit *Taq* DNA polymerase and 50 ng genomic DNA template. PCR amplification was conducted in a BOECO TC-PRO Thermal Cycler as follows: five minutes at 95°C, 30 cycles for 30 seconds at 95°C, 30 seconds at 50-60°C and 30 seconds at 72°C, followed by extension at 72°C for 40 minutes. PCR amplification of microsatellite loci at the VGL was performed as indicated in Miller et al. (2014a).

Ten microsatellite markers were used by both laboratories: FCA057, FCA075, FCA085, FCA096, FCA097, FCA113, FCA193, FCA224, FCA275 and FCA391. This subset of ten microsatellite markers was evaluated using the cumulative match probability (Probability of Identity and Probability of Sib-identity) statistics in GenAlEx v6.5 (Peakall and Smouse 2006). A total of 16 post-translocation samples were analysed at NZG-SANBI at the 10 microsatellite loci common to both laboratories to allow for calibration of data due to genetic-analyser differences (see Supplementary Material for details).

The VGL used an additional 11 microsatellite markers: F42, FCA001, FCA026, FCA031, FCA126, FCA230, FCA240, FCA272, FCA453, FCA506 and FCA628. A zinc finger marker was used to sex the animals (Pilgrim et al. 2005). All 21 of these microsatellite loci and the sex marker were previously validated for use on lions by Miller et al. (2014a). The subset of ten microsatellite markers was used for all analyses, except for the relatedness analyses, where the eleven additional microsatellite markers were included and FCA085 was excluded as it was shown to be linked to FCA453 (Miller et al. 2014a; Supplementary Material). This set of 20 microsatellite markers was also evaluated using the cumulative match probability (Probability of Identity and Probability of Sib-identity) statistics in GenAlEx.

Measuring effects of genetic rescue

Samples were categorized as pre-translocation, translocated or post-translocated. The following basic statistics for each locus were calculated in GenAlEx: number of alleles, minimum and maximum sizes of alleles, observed (H_O) and expected (H_E) heterozygosity and Hardy Weinberg Equilibrium (HWE). The following basic population statistics were calculated for each category: allelic richness (A_R) using rarefaction to account for unequal sample sizes, implemented using the *allel.rich* command in the R package PopGenReport (Adamack and Gruber 2014) using R v2.5.1 (R Core Team 2018) in RStudio (RStudio Team 2016), H_O, H_E and individual heterozygosity levels were calculated in GenAlEx. Diversity indices within the HiP groupings were compared with South Africa's largest unmanaged lion population, the Kruger NP population, using ten random samplings of 13 individuals from 46 complete genotypes reported in Miller et al. (2014a, b). Ten random samplings of 13 individuals from the post-translocation group were also averaged to account for sample size differences. Inbreeding coefficients (*F*) were estimated using the following equation (Frankham et al. 2010):

$$1 - \frac{HInbred}{HOutbred}$$

where *HInbred* is the observed heterozygosity of the population for which the inbreeding coefficient is being calculated and *HOutbred* is the observed heterozygosity of 13 Kruger NP individuals randomly sampled 10 times from the dataset of 46 individuals.

Principal Component Analysis (PCA) was performed in RStudio using the *adegenet* package (Jombart 2008) to assess the clustering of genotypes within and between the pre-translocation, translocated, post-translocation and Kruger NP populations. The Kruger NP population was represented by 49 previously published genotypes (Miller et al. 2014a, b). STRUCTURE was used to determine population structure and to probabilistically assign individuals (with assignment probability *Q*) to inferred clusters (*K*) (Pritchard et al. 2000). Post-translocated individuals were roughly divided by half-generation based on field notes; generation time was estimated at three years. A burn-in of 100 000 was used, followed

by 200 000 generations of collection for *K*=2 to *K*=7. Each *K* value was repeated 20 times. STRUCTURE Harvester was used to determine the most likely value of *K* (Earl and vonHoldt 2012). The CLUMPAK web service (http://clumpak.tau.ac.il/index.html) was used to summarize the output (Kopelman et al. 2015). Values for *K*=4 were averaged and sorted by category, including post-translocation dead individuals and post-translocation prides. Sorting by half-generation was also performed.

Determining genetic status of population in 2014

Frankham et al. (2017) suggested that mean kinship is the most useful metric for managing genetic variation within populations. We therefore determined the relatedness values between all pairs of post-translocation individuals using 20 microsatellite markers in Coancestry v1.0.1.9 (Wang 2011a). As the number of markers was double that of the other analyses, missing data were relaxed to allow one missing value. Forty nine Kruger NP genotypes (Miller et al. 2014a, b) were included as an open system control population. These Kruger NP genotypes represented 30 prides across the park, and thus were expected to have an average relatedness value at or below zero (Wang 2017). The Wang estimator (Wang et al. 2002) was used instead of the more traditional Queller & Goodnight as it is more suited for structured populations (Wang 2011b). The *boxplot* function in R v3.5.1 was used in RStudio to summarize relatedness between males and females, both within and among existing prides in HiP (based on pride structure at the end of 2014 as shown in Fig. 1). Relatedness values between male and female individuals in Kruger NP were also plotted and represent among-pride values from prides sampled across the whole of Kruger NP (Miller et al. 2015).

Predicting the need for future genetic augmentation

To estimate trends in genetic parameters over time, the half-generation groupings were used to calculate average observed heterozygosity, allelic richness, relatedness and average cluster allocation (average of *Q* values per cluster from *K* = 4) over time in comparison to the pre-translocation and translocated groups. Relatedness values were calculated as above, however the smaller dataset of genotypes for 10 microsatellite markers were used to allow for comparisons between all groups.

Results

Full genotypes at 10 microsatellite loci were generated from ten pre-translocation (NZG-SANBI), 13 translocated (NZG-SANBI), and 68 post-translocation (68 at VGL; 16 duplicated at NZG-SANBI) samples and were used for determining the success of the translocations (deposited in ZivaHub, doi:10.25375/uct.9505409). Genotypes from NZG-SANBI were adjusted to match those at VGL due to genetic analyser differences (Supplementary Material). The match probability for related individuals was 3.0×10^{-8} and the match probability for sib-identity was 6.5×10^{-4}. Basic summary statistics for each subset of samples are given in Table 1 and basic locus statistics are in the Supplementary Material. The pre-translocation individuals had lower allelic richness compared to post-translocation individuals, lower observed and expected heterozygosities compared to post-translocation individuals and a higher inbreeding coefficient (Table 1). The post-translocation animals had similar values to translocated animals (Table 1). The randomly sampled post-translocation animals had a slightly lower allelic richness than the randomly sampled Kruger NP animals but similar observed and expected heterozygosity values (Table 1).

Table 1 Summary statistics comparing pre-translocation (Pre), translocated (Trans), post-translocation (Post), an average of 13 post-translocation individuals randomly sampled ten times (Post 13), and an average of 13 Kruger NP genotypes randomly sampled ten times (Kruger13), based on genotypes at ten polymorphic microsatellite loci

	Pre	Trans	Post	Post13	Kruger13
N	10	13	68	13	13
A_R	2.26	3.42	3.88	3.82	4.45
H_O	0.40	0.68	0.65	0.65	0.68
H_E	0.39	0.58	0.62	0.60	0.66
F	0.41	0.01	0.05	0.05	–

N number of samples, A_R allelic richness, H_O observed heterozygosity, H_E expected heterozygosity, F inbreeding coefficient

The first two axes from the PCA explained almost all of the variation in the data (54.6% for the first and 43.5% for the second axis; Fig. 2). It positioned the post-translocation population intermediate to the pre-translocation and translocated populations on the first axis (Fig. 2). All HiP populations were separated from the Kruger NP population on the second axis (Fig. 2).

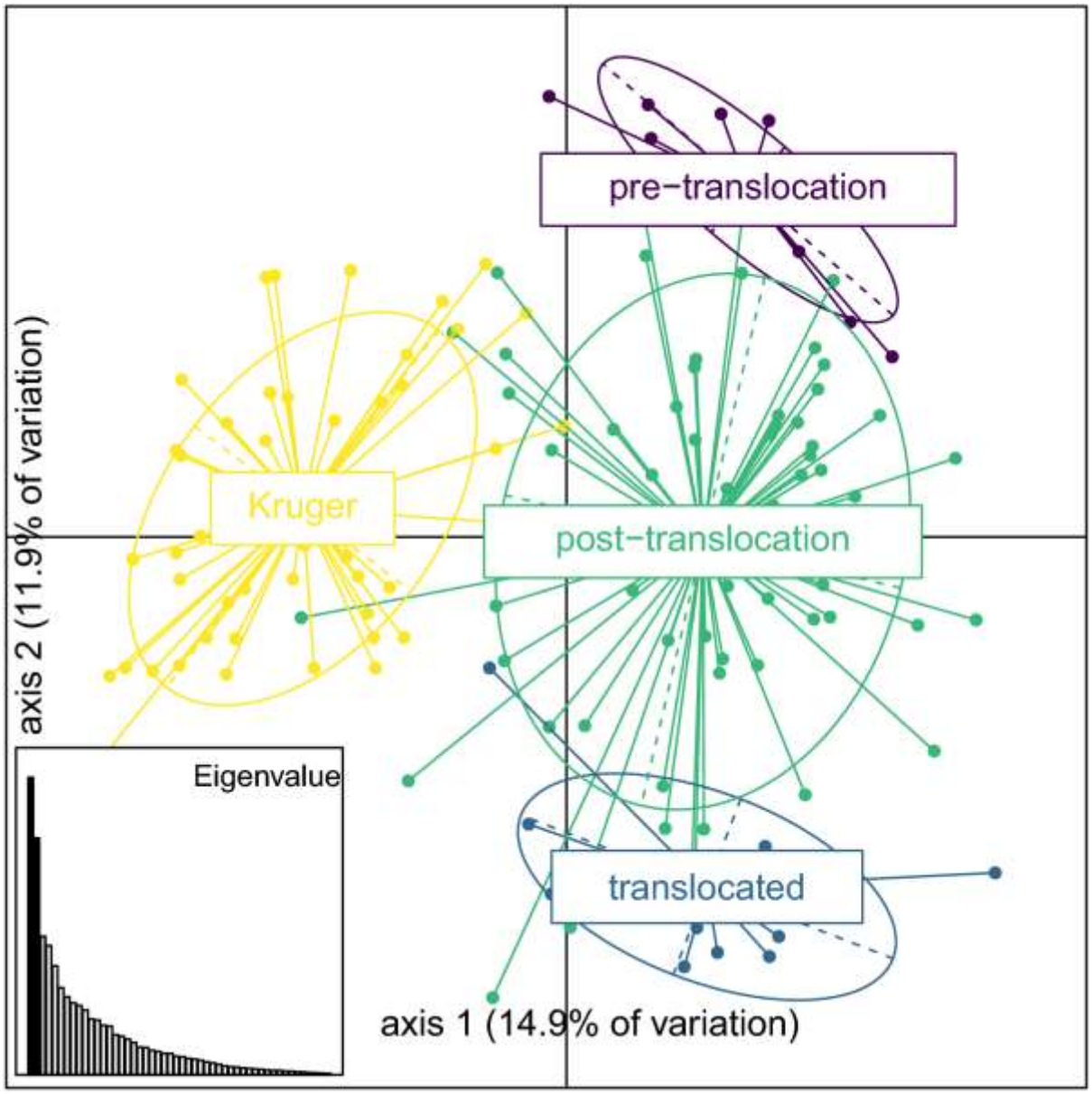

Fig 2. Principal Component Analysis of lion microsatellite genotypes (plot of first two axes explaining 98.1 % of the variation; inset shows corresponding Eigenvalues). Dots indicate individuals from different (lines) population categories compared in the present study (rectangular labels, with ellipses indicating x% insertia). The colours indicate the four categories.

Based on Structure, the pre-translocation and translocated animals separated into two distinct clusters (Fig. 3). Increasing values of *K* resulted in splitting within the translocated and post-translocation animals, with the pre-translocation animals remaining in a separate group. The *K* = 4 cluster was most supported by the STRUCTURE Harvester results (Supplementary Material). At *K* = 4, a new cluster is evident; this cluster was virtually unsampled or absent in both the pre-translocation and translocated populations (Fig. 4).

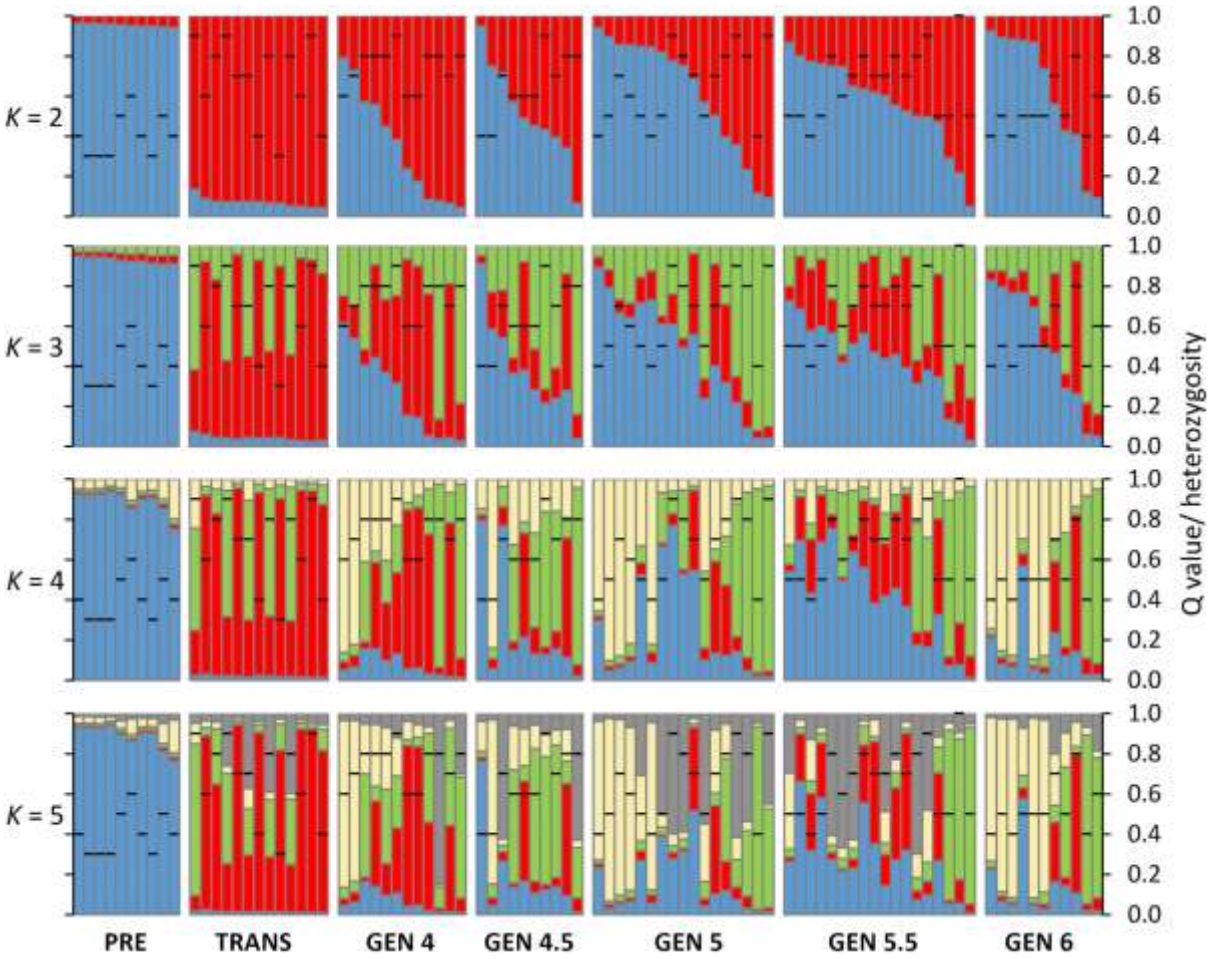

Fig. 3 STRUCTURE plots of *K*=2 to *K*=5 for pre-translocation (PRE), translocated (TRANS) and post-translocation (subdivided by approximate half-generation: GEN 4 - 6) individuals, based on genetic variation at 10 polymorphic microsatellite loci. Along the X-axis each vertical bar represents one individual and the Y-axis is the probability of individual assignment to *K* clusters (*Q*). Individual heterozygosity values are plotted as horizontal black bars on all plots (–).

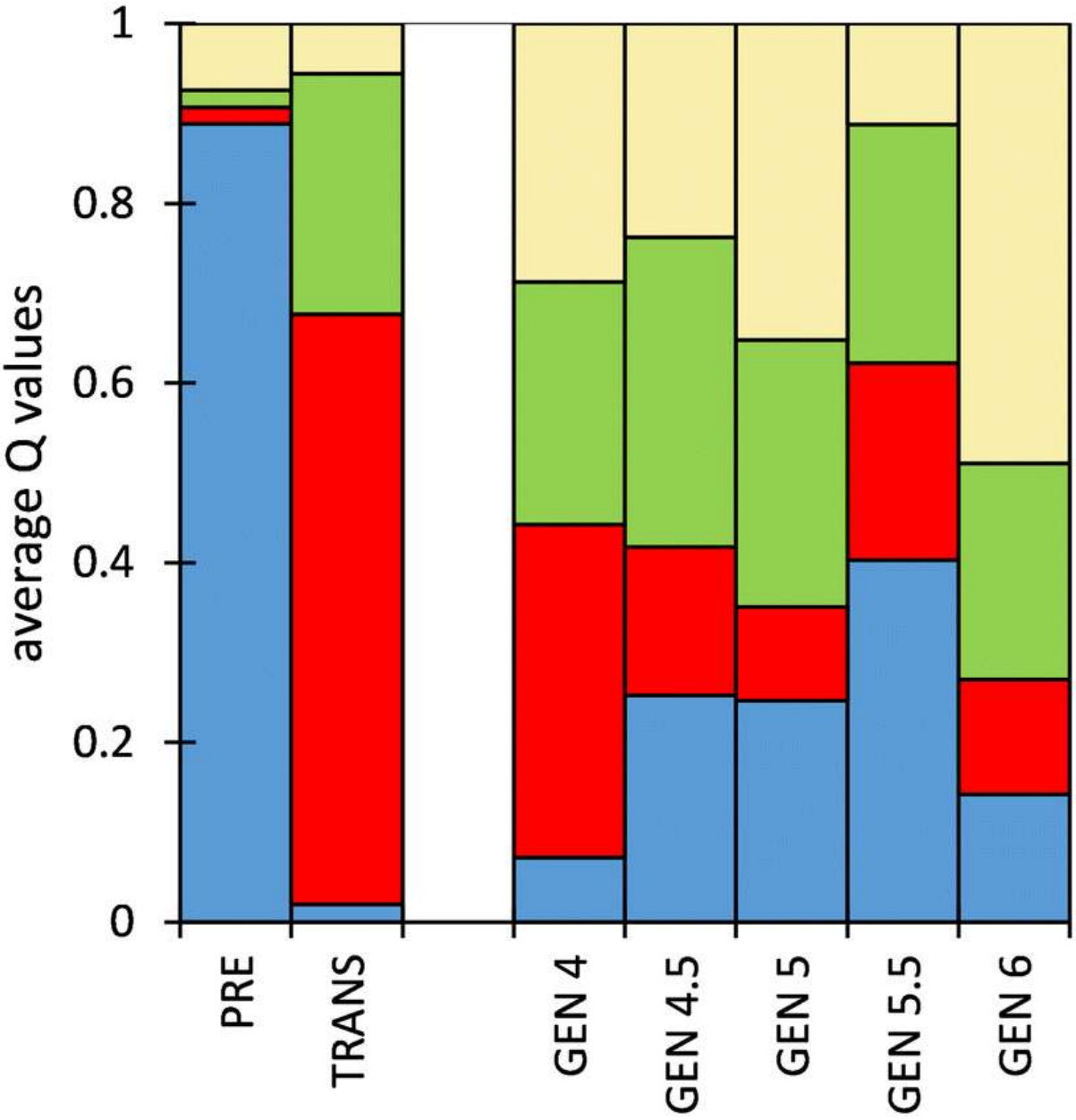

Fig. 4 Average *Q* values for *K*=4 cluster for pre-translocation (PRE), translocated (TRANS) and post-translocation (subdivided by approximate half-generation: GEN 4 - 6) groups.

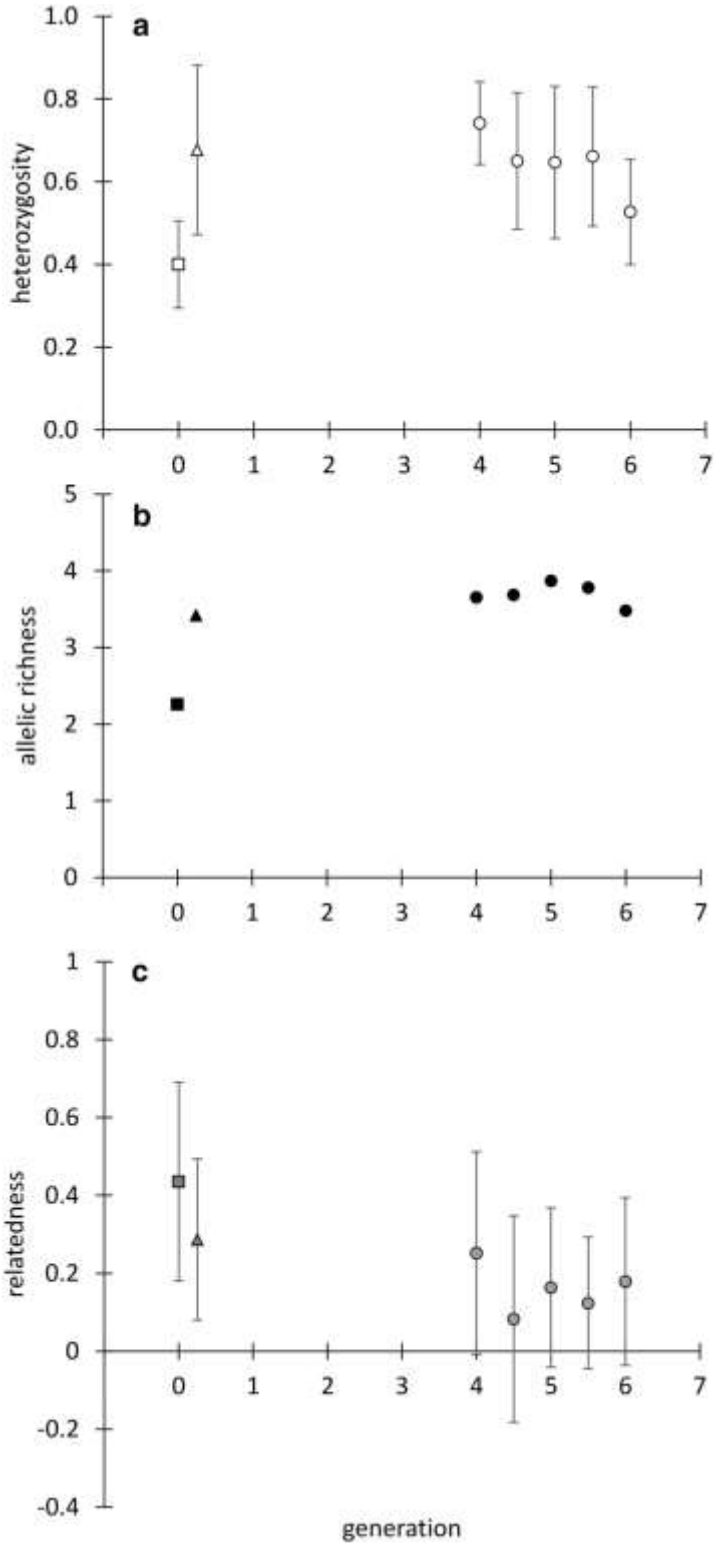

Fig. 5 Observed heterozygosity (a), allelic richness (b), and relatedness (c), over time within the HiP population. Pre-translocation averages are represented by squares (□), translocated averages by triangles (△), and post-translocation averages by circles (○). Standard error is plotted for observed heterozygosity and relatedness values.

Individual heterozygosity values were all at or below 0.6 in the pre-translocation individuals (Fig. 3). Translocated and post-translocation individuals had a wide range of individual heterozygosity levels (Fig. 3). Average heterozygosity was higher in post-translocation individuals compared to pre-translocation lions, however, there appears to be a decline in later generations (Fig. 5a). Allelic richness values were higher in post-translocation lions compared to pre-translocation lions, with a peak in generation 5 (Fig. 5b). Relatedness among prides (between males and females) had decreased compared to the pre-translocation individuals, however no clear trend was apparent through time (Fig. 5c).

Within the post-translocation population the lions in the north of the reserve (Gonshi & Hluhluwe prides) were still dominated by a subset of the translocated cluster (Fig. 1). The remaining individuals were variously assigned to multiple genetic clusters, with several individuals having a high probability of assignment to the unique cluster that was not present in either the pre-translocation or translocated populations (Fig. 1 and 3).

Sixty eight post-translocation samples amplified at least 19 of the 20 loci used at VGL. The match probability for related individuals was 1.2×10^{-15} and the match probability for sib-identity was 3.5×10^{-7}. Basic locus statistics are in the Supplementary Material. These genotypes were used to evaluate the relatedness among the post-translocation individuals. The relatedness within and among prides, , compared to among prides in Kruger NP is summarized in Fig. 6. Only male/female relatedness estimates were used (a relatedness table between all pairs of living individuals can be found in the Supplementary Material). The relatedness between males and females was higher within prides (mean = 0.19) than among prides (mean = 0.15) (Fig. 6). The average relatedness between males and females in Kruger NP was below zero (Fig. 6). Non-overlapping notches indicate a significant difference (95 percentile) in median values suggesting that all the groups depicted in Fig. 6 are significantly different from one another.

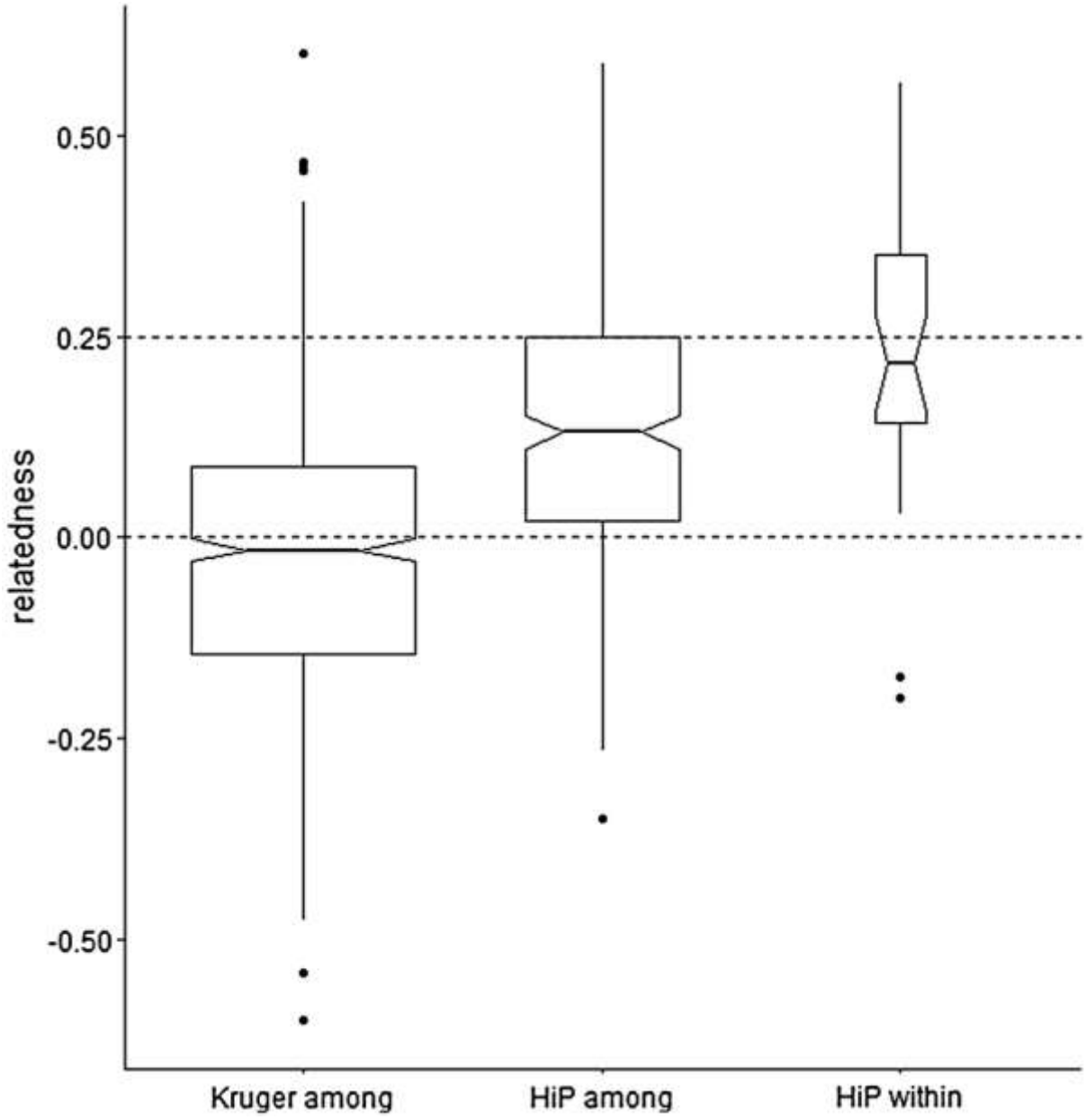

Fig. 6 A Tukey boxplot showing relatedness of male to female lions among prides (Kruger NP and HiP) and within prides (HiP only). Box width reflects sample size. Non-overlapping notches indicates strong evidence (95% confidence) that medians differ. The average relatedness value for first order relatives is expected to be 0.50, for second order relatives 0.25, and for unrelated individuals 0.00.

Discussion

The genetic rescue effort of the HiP lion population was initially successful as evidenced by an increase in overall allelic richness and heterozygosity levels, and a decrease in the inbreeding coefficient of the post-translocation population. Successful genetic rescue requires that the translocated animals

integrate into the existing population. Observations in the mid-2000s indicated that some of the original HiP lions had survived (20 out of 84) without mixing with the translocated individuals (Trinkel et al. 2008). Our post-translocation STRUCTURE analysis of individuals from 2009-2014 indicated that there were no longer any lions of pure pre-translocation genetic origin, and only a handful assigned to the translocated genetic cluster in the northern part of the reserve (Hluhluwe). In fact, the frequency of a unique genetic cluster was high in the southern half of the reserve (iMfolozi), indicating a truly mixed population. Similar clustering patterns were evident following the Florida panther (*Puma concolor*) genetic rescue effort, where, ten years post-translocation, most of the individuals were hybrids, and a unique genetic cluster became apparent (Johnson et al. 2010).

While not the primary concern when sourcing animals for a genetic rescue attempt, genetic origin should be considered. However, maintenance of the genetic variation of smaller, isolated populations can be detrimental to the overall conservation success of the species, and should not take precedence over reducing the detrimental effects of inbreeding (Reed and Frankham 2003; Weeks et al. 2016; Ralls et al. 2018; Fenster et al. 2018). Prior to the genetic rescue effort, the HiP lion population was thought to be closely related to the Kruger NP population (Stein 1999). However, subsequently Dubach et al. (2005) found that a limited sample of HiP cytochrome *b* sequences were either the same as those found in Kruger NP, or very closely related. Dubach et al. (2013) subsequently, using microsatellite markers, determined that the pre-translocation HiP population formed a unique genetic cluster when compared to lions across Africa. This cluster had no known historical basis, and was likely the result of small founder size and genetic drift over time, and, so despite possibly being descended from Kruger NP lions, the apparently unique genetic make-up of HiP was not deemed of special conservation concern (Miller et al. 2015), and a healthy, genetically diverse lion population was deemed a better goal.

Other genetic factors, such as individual heterozygosity and relatedness to the existing population, as well as disease, availability, habituation, cost, and social structure, become more important when

sourcing animals for genetic rescue efforts. In HiP, high inbreeding levels had resulted in immunocompromised animals that were highly susceptible to bTB, which was present in the population (Trinkel et al. 2011). Bovine tuberculosis also occurred in Kruger NP, and, thus, that lion population was not considered as a source population. Bovine tuberculosis-free lions that were thought to be FIV-free were available from Pilanesberg NP and Madikwe GR (Trinkel et al. 2008). The Etosha NP origin of these lions would ensure that they were unlikely to be related to the existing population, and, thus, would have maximal impact on genetic diversity if they bred successfully.

Genetic rescue has been documented in one other social carnivore, the wolf (*Canis lupus*) (reviewed Frankham 2015b). Three separate subspecies of wolf had artificial or natural genetic rescue events: Mexican wolves (*C. l. baileyi*), where isolated, inbred captive populations were successfully crossed and reintroduced into the wild (Fredrickson et al. 2007); Scandinavian grey wolves (*C. l. lupus*), where natural immigration of a single migrant "saved" an isolated pack that was severely bottlenecked (Vilà et al. 2003); and, the wolf population in Isle Royale National Park (*C. l. lupus*), a naturally isolated island population, where one male immigrant "rescued" the population (Hedrick et al. 2014). Unfortunately, as there was no further immigration, the impact only lasted for a few generations (Hedrick et al. 2014). Further investigation suggested that this population decline following the genetic rescue event may have been exacerbated by an increase in deleterious variation from the introduction of individuals from a large population of mainland wolves (Kyriazis et al. 2019). Using animals sourced from smaller populations should, theoretically, reduce extinction risk, as strongly deleterious variants will be have been purged from these populations (Kyriazis et al. 2019).

The Scandinavian and Isle Royale wolf examples provide a cautionary tale, that, if a population remains isolated, continuous immigration (either natural or artificial) will be required to maintain genetic diversity. This is referred to as "genetic augmentation", for which guidelines have been developed (Frankham 2009). Even the lion stronghold in the Serengeti NP is showing signs of reduced gene flow from a decline in incoming males, presumably due to increased anthropogenic pressure in the

surrounding area (Borrego et al. 2018), and may eventually need human-assisted translocations to maintain genetic diversity. A closed population, such as HiP, will always require artificial genetic augmentation to reduce inbreeding, and maintain genetic diversity (Miller et al. 2015). Without this, the population would, inevitably, require future major genetic rescue events. Thus, while the genetic rescue effort was deemed a success, assessment of the post-translocation individuals was needed to assess the existing situation, and advise on future genetic augmentation efforts.

The mean kinship relatedness values within and among prides in the HiP population 15 years post genetic rescue were higher than expected in an open population. In Kruger NP (this study), the Serengeti NP (Packer et al. 1991a) and Selous NP in Tanzania (Spong et al. 2002), pride males were typically unrelated to pride females, while the relationships between males and females among prides in HiP were at the level of third order relatives. This is similar to what was reported in other isolated populations, Savé Valley Conservancy and Gorongoza NP, and slightly lower than in Bubye Valley Conservancy, where lions were related at the level of second order relatives (Tensen et al. 2018).

The proportion of males related to females among prides in the HiP post-translocation animals indicated that, even if there are natural pride takeovers, mating events could easily result in inbreeding. This was not surprising given that it has been estimated that a minimum of 50 prides are required for a self-sustaining lion population (Bjorklund 2003). Furthermore, the translocation efforts re-established lions in the north of the park with translocated animals (no original individuals were present at the time) (Trinkel et al. 2008), and these lions were still predominantly composed of the translocated genetic cluster. Two of the individuals sampled in this area were sub-adult males with low individual heterozygosity. This suggests that there has been minimal natural movement of lions into the north of the park. This was not entirely unexpected due to the topography of HiP, with limited access from the south into the north. However, there is anecdotal evidence that males can move from south to north: in 2017, two males from the south moved into the north of the park, killed the dominant male and set up a territory. The two young sons of the dominant male headed south and

established a territory in the south (pers. obs. authors). This, we presume, was a rare event based on the genetic makeup in 2014. In addition to concerns regarding relatedness, both allelic richness and observed heterozygosity values appeared to be declining in the later generations of post-translocation animals.

Therefore, this is concrete evidence that more translocations are, and will always be, required to prevent another potential inbreeding crisis. Frankham (2009) outlines some general guidelines for genetic augmentation, suggesting that artificial immigration be encouraged in every generation and that it will be needed more frequently in smaller populations compared to larger ones. Ideally translocations will mimic the natural social system of lions, that includes frequent pride take-overs by nomadic males from neighbouring areas (Borrego et al. 2018), as proposed in Miller *et al*. (2013; 2015a). Two genetic augmentation events (translocations from other small reserves) using male lions occurred in 2016/7. The hope is that these males will naturally take over prides from existing pride males. Adding males to a reserve with an existing population does not always result in pride takeovers, as evidenced by an attempt in Addo Elephant NP where two young males were introduced with the hopes that they would replace the older pride male (Tambling et al. 2013). Unfortunately, in this case, one male joined up with the existing pride male and no takeover occurred. HiP is a much larger and more complex system than Addo Elephant NP, and, so, it is hoped that these males will act as nomadic males initially, and then succeed in taking over a pride (or two), and reproducing. If this plan does not succeed, more directed efforts may be necessary, such as removal of existing pride males to promote pride male turnover (Kettles and Slotow 2009). The results of these translocations will be reported on in a separate paper, where we will also explore the complexities of lion social structure, and how it can impact genetic augmentation efforts.

The HiP lions are part of a larger, fragmented lion population of “managed wild lions” in South Africa, which was recognized in the latest regional Red List assessment (Miller et al. 2016). Managed wild lions include lions reintroduced into small fenced reserves, where managers actively manipulate some

of their vital rates and demographics to limit population growth, and maintain genetic diversity (Funston and Levendal 2015). Many *ad hoc* translocations between reserves have been ongoing for years to mimic natural systems, such as immigration of young males and pride takeovers, which indirectly prevent inbreeding and assist with lion population management (Funston 2008; Slotow and Hunter 2009; Kettles and Slotow 2009; Miller et al. 2013, 2015).

While the HiP population had declined prior to the genetic rescue effort, the current population is at or near carrying capacity (Packer et al. 2013; pers. obs. authors). Therefore, any genetic augmentation efforts should not be focussed on increasing numbers or establishing new prides, but rather on mimicking natural processes such as pride takeovers; other approaches may be necessary to control population growth. HiP is not alone in this regard, as lion populations in small fenced reserves are prone to overpopulation (Miller and Funston 2014). Historically much of this population control was done through translocation of "excess" lions to new reserves – mimicking the movement of young individuals – however, with a decrease in the number of new reserves and an increase in the number of reserves with excess individuals, managers have increasingly relied on various methods of contraception to assist with population control (Miller et al. 2013; McEvoy et al. 2019). South Africa is working towards a "managed metapopulation" plan for all managed wild lions as outlined in the Biodiversity Management Plan (BMP) for South African lions (Funston and Levendal 2015), which should improve their conservation value (Miller et al. 2013, 2015), and, hopefully, prevent the need for future genetic rescue efforts.

Periodic testing of the populations within the metapopulation to ensure that management activities are having the desired effect is recommended (Funston and Levendal 2015). Microsatellite markers, that were used here, could be employed, or more modern genomic approaches, such as Single Nucleotide Polymorphisms, could be applied, which would allow for broader comparisons among laboratories without the need to calibrate for machine difference, and allow researchers to assess loci linked with inbreeding depression, disease resistance, and other adaptive traits (Pardo-Diaz et al.

2015). Criteria to determine when genetic diversity is "good enough" are lacking. Due to the complex social structure of lions, this is not a simple task, and currently managers must consult with geneticists to evaluate their particular population. High relatedness values between nomadic males and non-natal prides, and between prides, are indications that overall genetic diversity has decreased to a point where new genetic variants or bloodlines are required. Ideally genetic augmentation will occur before this point, as it can take some time for new individuals to be introduced and integrate into the population (Trinkel et al. 2008). We are aiming to develop a model that will assist with determining how frequently genetic augmentation events are, in theory, required to prevent a loss of genetic diversity within a given lion population.

The BMP is complicated by the presence of bTB in the lion population (Michel 2002; Michel et al. 2006), which precludes surplus lions from HiP (or other areas that are positive for bTB) being translocated to areas that are negative for bTB (Miller et al. 2013). Currently, bTB has been confirmed in the buffalo *Syncerus caffer* populations of Kruger NP and Madikwe GR (Michel et al. 2009; Hlokwe et al. 2016); lion within Kruger NP were confirmed as bTB positive (Keet et al. 2000). The long-term impact of bTB on lion populations is not fully understood, however, there is some evidence of disrupted social structure within prides, reducing life expectancy and cub survival in areas of Kruger NP where bTB infection of lions is prevalent (reviewed Viljoen et al. 2015). Trinkel et al. (2011) clearly demonstrated that the pre-translocation population on HiP were much more susceptible to bTB than the post-translocation lions, which they linked to inbreeding depression. Wherever possible, the spread of bTB should be contained to prevent infection of more populations. Bovine TB has recently been detected in warthogs (*Phacochoerus africanus*), kudus (*Tragelaphus strepsiceros*) and baboons (*Papio ursinus*) on several reserves within the KwaZulu-Natal Province (pers. obs. authors, November 2018), further complicating the movement of lions within the country. The BMP for lions will have to be adapted to accommodate the changing bTB landscape across the country.

Conservation implications and future direction

This genetic rescue success adds to the growing body of evidence that genetic rescue can successfully improve the reproductive fitness of isolated, inbred populations. Without increased connectivity, natural gene flow declines, and isolated populations will need regular genetic augmentation. Disease can complicate genetic augmentation efforts, and more research is needed into the role of bTB in lion management and movement. The issues raised here for lion are also relevant to other threatened social species, and attention should focus on genetic augmentation to prevent the need for genetic rescue events.

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