



# Genetic diversity and origin of captive lion (*Panthera leo*) in South Africa: an assessment and comparison to wild populations

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## Abstract

South Africa has a large captive lion (*Panthera leo*) sector, but detailed knowledge on the origin of individuals and any potential genetic value to conservation targets is lacking. In 2021, the South African government committed to closing the sector and have since appointed a Ministerial Lion Task Team (2022) to initiate this process. Some have suggested that captive lions could be integrated into wild populations as part of the process but information on the genetic origins and diversity of captive lions is critical if this is to be explored further. Both the Biodiversity Management Plan for lions in South Africa (2015) and a High-Level Ministerial report for the South African government (2021) have called for more information on the genetic composition of captive lions. To determine the probable origin of captive lions in South Africa we summarised existing survey responses from captive facilities (collected 2017–2018) and CITES permit data (issued 1991–2019). Survey data suggest that most lions were sourced from within the South African captive sector. However, many CITES permits were also issued for the import of lions from across Africa and beyond, indicating possible mixed origins within the sector. To evaluate genetic relationships between captive and wild lions in South Africa we standardised existing microsatellite marker data from three laboratories and analysed genotypes of captive lions from 31 properties. A comparison of captive and wild lion genotypes revealed that the genetic composition of captive lions is currently comparable to existing wild South African lions. Captive lions cluster with similar probabilities to three of four regional reference populations of wild lions included in the study and no major signatures of inbreeding were identified. However, captive lions are highly genetically interconnected across properties and represent a smaller effective population size compared to Kruger National Park, the largest population of wild lions in South Africa, suggesting some risk of future inbreeding. There were also signatures of genetic drift which should be investigated further as it will likely compromise any potential conservation genetic value of captive lions in the future. The findings of this study should be considered when planning the fate of individuals within South Africa's captive lion sector and within the broader context of African lion conservation.

**Keywords** African lion · Captive breeding · Microsatellites · Ex situ conservation

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## Introduction

Across their range, African lions (*Panthera leo*) have declined by ~75% in the last five decades (Loveridge et al. 2022) mainly due to human-induced habitat fragmentation and prey declines (Riggio et al. 2013; Bauer et al. 2015). While lions were extirpated from much of their historical range in South Africa by the 1900s (Nowell and Jackson 1996), their numbers have recovered following extensive conservation efforts and the current wild population of ~3 500 lions are listed as Least Concern (Miller et al. 2016). Two subspecies of lion described in Bertola et al. (2016) are recognised in the 2016 IUCN Red List assessment (Bauer et al. 2016): *P leo leo* (occurring in West and Central Africa and India) and *P leo melanochaita* (occurring in East and southern Africa. South Africa falls in the range of *P leo melanochaita*.

South Africa has an extensive captive lion sector (not included in the country's Red List assessments) of more than 7 400 individuals on at least 237 properties (Hiller et al. 2022). The sector expanded in the early 1990s with the introduction of the Game Theft Act (Republic of South Africa: The Presidency 1991) allowing for private ownership of wildlife; thus, economic value, in addition to conservation value, was attached to lions and other wild animals (Cousins et al. 2008; Taylor et al. 2016; Schroeder 2018). South Africa's captive lion sector includes a range of properties including larger game ranches where interactions with humans is minimised, wildlife sanctuaries, interactive tourist attractions where petting and walking with lions is a regular occurrence, and captive-breeding farms. These properties keep lions for commercial purposes such as trophy hunting, live sales into the sector, and for interactive tourism through display, petting and "walking with lions" (Lindsey et al. 2012; Williams and 't Sas-Rolfes 2019; Hiller et al. 2022). Additionally, some lion products are traded legally on the domestic market under Threatened or Protected Species (ToPS) permits, and internationally under CITES Appendix II (04/02/1997, but subject to the decision taken at CoP17 in 2016), for traditional medicine, zootherapeutic, decorative and cultural purposes in Africa and Asia (CITES 2016; Williams and Whiting 2016; Williams et al. 2017a, b; Williams and 't Sas-Rolfes 2019; Coals et al. 2022). Any trade in lion products without permits is illegal. International trade has been restricted in recent years with no quota issued for the export of lion bones from South Africa and many countries banning the import of lion trophies and/or requiring proof of conservation value of the source population (e.g. Ares 2022). While it is plausible that captive lions may provide a buffer against exploitation of wild lions, it is also likely that the presence of the sector may be detrimental to

the remaining wild lion populations (Lindsey et al. 2012; Coals et al. 2019b; Williams and 't Sas-Rolfes 2019).

South Africa first developed its Biodiversity Management Plan (BMP) for African Lion in 2015 (Funston and Levendal 2015). At the time, several contrasting views on the potential conservation value of captive lions existed. Critics argued that their presence has no demonstrated conservation value (Hunter et al. 2013). Others claimed that restoration of lion populations using captive-bred animals could contribute to in situ conservation, arguing that it was comparable to established methods using wild-caught founders to establish new prides across the small reserve metapopulation in South Africa (Abell et al. 2013). In response, one of the objectives included in the BMP was to critically evaluate the potential for captive lions to contribute to South Africa's conservation targets for wild lions. More recently, it has been argued that the sector may in fact constitute a threat to wild populations through various channels, including the risk of a legal trade in captive lion parts fuelling an illegal trade in wild lion parts, as well as the persistence of "canned hunts" negatively affecting the reputation of wild lion trophy hunting (Schroeder 2018; Coals et al. 2019a; Everatt et al. 2019; Harvey 2020). Increasing public concern suggests that captive breeding, handling, hunting and trade in lion (and other large mammals) could have important implications for South Africa's conservation and eco-tourism reputation, and led to a high-level Ministerial report exploring these issues (Department of Forestry, Fisheries and the Environment 2020). The High-level Panel (HLP) report questioned the potential conservation value of captive lions, highlighting the risks of inbreeding and hybridization among lion subspecies and, in line with the BMP, recommended research into the genetic status of lions in the sector.

Populations surviving across poorly connected habitats, or in the case of captive lions artificially isolated on properties, are inherently more vulnerable to stochastic events via the loss of genetic diversity and declines in fitness associated with increased inbreeding (Frankham et al. 2017). Even within lion conservation strongholds, demographic decline has been accompanied by significant loss of genetic diversity (Dures et al. 2019). In this study we used microsatellite markers to explore the genetic composition of South Africa's captive lions to (1) determine if they match the expected origin of captive lion populations based on existing interview survey data (Williams and 't Sas-Rolfes 2019) and CITES lion import/export permits, and (2) establish levels of potential inbreeding and general genetic diversity metrics. To do this we first summarised survey results and CITES permit data over time to determine expected geographic origins. We then standardised a set of microsatellite markers for existing captive lion datasets from three independent laboratories and analysed the resulting dataset in comparison to known genetic data for wild populations in South Africa. Data on

the genetic composition and diversity of South Africa's current captive lion populations is critical to aid policy makers and inform the international trade involving existing captive lions and their associated derivatives. We hope these data will be used by those making decisions regarding the fate of lions within the captive lion sector.

## Material and methods

Ethical clearance for the sampling of animals for the standardisation between laboratories was obtained from the South African National Biodiversity Institute (SANBI) Research Ethics and Scientific Committee (ref: P2021/07). The Human Research Ethics Committee (non-medical) of Witwatersrand University approved the questionnaire on the origin of the captive lions (Protocol Number: H17/06/55; VLW). Ethical clearance for the original work on open system and small reserve lions was obtained from Tshwane University of Technology Animal Research Ethics board (AREC2010/11/004; SMM) and the National Zoological Gardens Ethics and Scientific Committee (NZG/P12/04; SMM).

### Origin of lions in captive lion properties

Baseline information on the origin of captive lion populations was extracted from a National Captive Lion Survey of privately-owned properties in South Africa (Williams & 't Sas-Rolfes 2019; relevant questions in File S1), some of which is unpublished. The survey took place from August 2017 to May 2018 and had 117 respondents, 100 of which answered questions on the past and present origins of their lions. Responses relating to lion origins were grouped by year of establishment: 1980s and then four-year intervals from 1990 to 2017 to look for trends in origins over time. Origin of current stock was summarised and any additional information on the introduction of wild lions was reported.

To supplement the survey data, permit data from the CITES Trade Database (CTB) (<https://trade.cites.org/>) on legal trade for live lions imported to South Africa for the purpose of "breeding in captivity" (code B) or "commercial trade" (code T) was summarised. We assessed only the year, exporting countries and number of lions listed on the permits. It must be noted that permits reflect an intention to import which may not always be actioned. Permits were grouped by category (Southern African Wild [W], Captive [C] or Unknown [U]; Africa W or C; International W, C or U) within the same four-year intervals used for the establishment of captive lion properties as above. The total number of lions listed on the permits within each category was evaluated and summed within each period to look for trends over time.

## Standardisation of microsatellite marker analysis

Three laboratories in South Africa have historically generated genotypes for both commercial and research applications in African lion using nuclear microsatellite markers optimised from the domestic cat (*Felis catus*) genome (Menotti-Raymond et al. 1999): the Veterinary Genetics Laboratory, Faculty of Veterinary Science at the University of Pretoria (VGL); the Genetics Services Unit at the South African National Biodiversity Institute (SANBI); and Unistel Laboratories, a commercial human and animal genetic testing facility (Unistel). The markers used at each laboratory are indicated in Table S1 (Online Resource 1). All three laboratories have databases of genotypes for captive origin lions (Table S4). The VGL also has genotypes from wild lion populations and 'metapopulation' lions using microsatellites that have been validated for use in African lion in their database (Miller et al. 2014b, 2014a). 'Metapopulation' lions describes the network of wild lions found in small reserves across South Africa which are referred to as Managed Wild lions in the BMP (Funston and Levensal 2015).

To compare genotypes between the three laboratories, a standardisation exercise was performed since the scoring of microsatellite alleles differ on the different platforms used by the laboratories. This process was similar to standardising genetic profiling of many domestic species across laboratories (ISAG; [www.isag.us](http://www.isag.us)). A set of 39 blood samples were collected by veterinarians in standard EDTA tubes from 10 participating captive lion properties and submitted to the laboratories. Each laboratory analysed 20 selected samples (two individuals from each facility were chosen to prevent sampling related animals and maximise the number of alleles covered) using their current microsatellite markers for lion. The VGL analysed the remaining 19 samples for inclusion in the analysis of existing captive lion genotypes.

DNA was extracted from the blood samples and PCRs performed independently by each laboratory. DNA was extracted at the VGL using the Prepfil® Automated Forensic DNA extraction kit (ThermoFisher Scientific, South Africa) and PCRs performed as in Miller et al. (2014a) except for a few markers as outlined in Table S2. DNA was extracted at SANBI and PCRs performed as outlined in Miller et al. (2020). At Unistel, DNA was extracted using the DNAzol™ reagent following manufacturer's instructions (ThermoFisher Scientific) and their PCR protocol adapted from that used at the VGL.

DNA profiles were compared between the three laboratories to generate correction factors to allow for the combining of existing genotypes held in databases by Unistel and VGL for a retrospective analysis of existing captive lion data in relation to wild lion populations, and to establish a standard system for genotyping in the future. There were not enough

overlapping markers to include the SANBI samples in the current study. VGL was used as the standard for allele calls.

### Genetic origin baseline: confirming resolution with a reduced marker set

Genotypes from 22 microsatellite loci were previously used to discriminate between wild, free-living lions from the Kruger National Park (NP), Kgalagadi Transfrontier Park (TFP), Etosha NP (using Pilanesberg NP samples as a surrogate as they originated from Etosha NP) or Greater Mapungubwe Transfrontier Conservation Area (TFCA) (total of 101 individuals) (Miller et al. 2015a, b). As only 13 of these microsatellite markers were selected following the standardisation exercise above (see results for details; Table S1), we repeated the analysis from Miller et al. (2015a) with these 13 microsatellites to ensure that they were able to discriminate between the four populations. Genotypes with more than one missing data point for the 13 microsatellites were removed ( $n=6$ ) leaving 51 Kruger NP, 10 Kgalagadi, 9 Mapungubwe, and 25 Etosha-origin individuals. Genetic structure of this reduced locus dataset was then re-tested following Miller et al. (2015a, 2015b) using the clustering approach of STRU CTURE v2.3.4. (Pritchard et al. 2000). Tested  $K$ -values ranged from one to six with a burn-in of 100 000 and data collection of 100 000 chains with the no-admixture model (as no mixing between populations was expected), and 10 iterations per  $K$ -value. The Evanno method was applied using Structure Harvester web version 0.6.94 to determine the most likely value of  $K$  (Evanno et al. 2005; Earl and von-Holdt 2012). Data were then combined using CLUMPAK online using default settings (Kopelman et al. 2015).

### Population genetic descriptors of captive lions

A total of 806 captive lion genotypes were available from three sources: 1) 729 located on 30 properties were provided from the Unistel database (generated between 2015 and 2021). No metadata for the location of these individual lions were released due to client confidentiality. All samples were submitted prior to our study and thus sampling was not random, but rather, we used data that were available from existing databases. We acknowledge that this may have led to some bias in our results and have adjusted our interpretations appropriately. The correction factors defined above were applied; 2) 38 genotypes from the VGL database from an additional property (born between 2006 and 2012); and 3) the 39 genotypes from the standardisation exercise above. These captive genotypes were combined with the data for the wild populations from Miller et al. (2015a) for inclusion in some of the analyses. File S2 provides a summary of the number of genotypes available in each category and which datasets were used in the various analyses.

GenAIEx v6.503 (Peakall and Smouse 2012) was used to determine any private alleles in the captive population compared to the wild populations from Miller et al. (2015a). Genotypes with more than one missing data point were then removed. Twenty-two duplicate genotypes identified by GenAIEx were also removed and were assumed to be duplicate sampling of individuals leaving 781 captive lion genotypes for further analyses. Expected and observed heterozygosity, polymorphic information content (PIC) and Hardy–Weinberg Equilibrium (HWE) were calculated for the Kruger NP ( $n=51$ ), metapopulation ( $n=190$ ) and both the full ( $n=781$ ) and reduced ( $n=128$ ; see below for details on how the dataset was reduced) captive genotype data sets using Cervus v 3.0.7 (Kalinowski et al. 2007). Allelic richness and inbreeding ( $F_{is}$ ) were calculated in Fstat v 2.9.3.2 (Goudet 1995). Barlett’s test (base R command) was used to determine if there was any significant difference between the overall observed and expected heterozygosity, the FCA240 locus was removed prior to the calculation as it is a known X-linked microsatellite marker. Allelic richness values were compared using ANOVA and Tukey testing to determine any significant differences between the three datasets in RStudio v2021.09.1 using R version 4.1.2 (R Core Team 2022; RStudio Team 2022). A separate inbreeding coefficient ( $F$ ) was also estimated using the following equation:

$$F = 1 - \frac{H_{Inbred}}{H_{Outbred}}$$

where  $H_{Inbred}$  is the observed heterozygosity of the captive population for which the inbreeding coefficient is being calculated and  $H_{Outbred}$  is the observed heterozygosity of the Kruger NP individuals (Frankham et al. 2010). Individual heterozygosity values were calculated and summarised in a box and whisker plot compared to the Kruger NP and metapopulation. A global Hardy Weinberg Equilibrium (HWE) statistic was calculated for both the Kruger NP and the combined captive lion populations in GenePop using the “heterozygosity deficit” option (Rousset 2008). The X-linked FCA240 marker was excluded.

We determined relatedness between all pairs of captive genotypes ( $n=781$ ) using Wang’s method in Coancestry v 1.0.1.9. The Wang estimator (Wang 2002) was used instead of the more traditional Queller & Goodnight estimator as it is more suited for structured populations (Wang 2011). The Kruger NP genotypes ( $n=51$ ) were included as an outbred population and individuals from the metapopulation ( $n=170$ ) were also included as a separate group (Table S5). A box and whisker plot of the average relatedness between the captive, Kruger NP and metapopulation individuals was created in R. Gephi v 0.9.2 and was used to visualise the relatedness between all individuals among the captive

populations at both 0.25 and 0.50 levels. This was repeated for the metapopulation reserves.

Friends&Family v22 was used to determine which individuals were “friends” – unrelated at the 0.25 level based on relatedness analysis above (de Jager et al. 2017). These unrelated individuals ( $n = 128$ ) were used for STRUCTURE analysis (STRUCTURE v 2.3.4; Pritchard et al. 2000) rather than the whole dataset of captive animals to reduce the bias that could result if a lot of related individuals were included. K-values from one to six with a burn-in of 100 000, data collection of 100 000 chains using the Admixture model, with 20 iterations per K-value were run. As above, the Evanno method was applied using Structure Harvester to determine the most likely value of K and the data were combined using CLUMPAK online using default settings.

Principal component analysis (PCA) was performed in RStudio using the *adeigenet* package in R (Jombart 2008) to assess the clustering of captive genotypes in relation to Kruger NP, Kgalagadi TP, Etosha NP and Mapungubwe GTFCA.

We used the “Assignment” function in GenAIEx to assign the captive lions to one of the four wild populations based on the wild origin individuals. This test uses the algorithm developed by Paetkau et al. (2004). The captive lions were treated as the “last population unknown” to be compared to the individuals from the four known wild populations with the “leave one out option”.

Effective population sizes ( $N_e$ ) were calculated for both the Kruger NP ( $n = 51$ ) and combined captive lion populations ( $n = 781$ ). The Linkage Disequilibrium model (Waples and Do 2008) was implemented in NeEstimator v 2.1 with “random mating” and critical value of 0.05 (Do et al. 2014)

## Results

### Reported origin of lions in captive lion properties: survey results

The majority of lions originally sourced for the captive lion properties analysed in this study were reported as sourced from within the South African captive lion sector (Fig. 1a, ‘South Africa C’). Only 13% of properties did not list South African captive-bred lions as an original source (Fig. 1a) In recent years (2014–2017), only one property sourced lions from outside the South African captive lion sector, and these were from lion breeders “elsewhere in Africa” (no further details provided). Prior to 2014, this practice was more common with some original stock sourced from captive lion breeders outside of South Africa (including Namibia, Botswana, and Zimbabwe), and a small number of wild South African lions, from circuses, zoos, other or unknown (Fig. 1a). Anecdotal evidence also suggests that

wild lions from Botswana and Zimbabwe were imported on rare occasions into the captive lion populations (pers. comms. V. Williams, C. Hiller), however wild lions from other African countries were not reported as sources for imported individuals (Williams and ‘t Sas-Rolfes 2019). Data are summarised in Table S3.

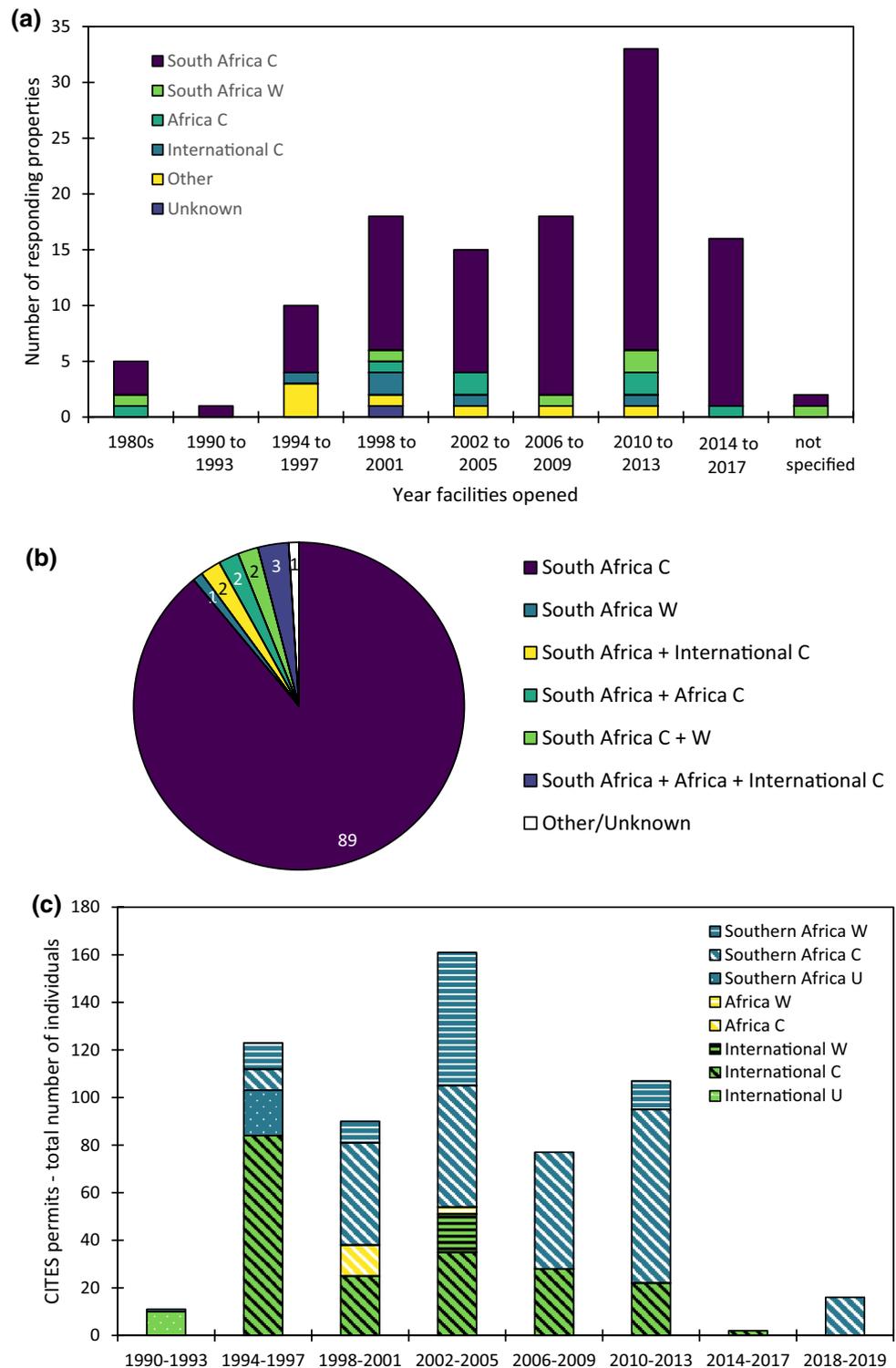
Survey respondents indicated that most of their properties currently include stock (as compared to the original stock) sourced from within the South African captive lion sector, either sourced from another facility or bred in their own facilities (usually a second farm) as compared to the original stock (97/100; Fig. 1b, Table S3). Only 11 respondents indicated the continued presence of lions that originated from outside the South African captive lion sector. Of these, seven included lions of captive origin from either the rest of Africa or elsewhere, three included wild sourced lions from South Africa, and one respondent did not know the origin of any of their current stock.

Analysis of CITES permits issued between 1991 and 2019 for the import/export of captive lions (source code: C, F, R) into South Africa with the intention of commercial trade (code T) or breeding in captivity (code B) revealed multiple source regions: Botswana, Egypt, Eswatini, Malawi, Namibia, Senegal, Zimbabwe, Europe, Middle East, East Asia, North America, and South America. Similarly, CITES permits for wild lion (source codes: W) translocations into South Africa were issued for: Botswana, Eswatini, Liberia, Namibia, Mozambique, Somalia, and Zimbabwe (Table S4). The total number of lions listed on CITES permits for import into South Africa as code B or T peaked between 1994 and 2013 with 587 lions listed issued permits (average of 31 lions per year), in contrast only 11 lions were listed on permits issued between 1990 and 1993 and 18 lions on permits issued between 2014 and 2019 (Fig. 1c).

### Standardisation of microsatellite markers for genotyping

Nine markers overlapped between the three laboratories, 22 overlapped between the VGL and Unistel and 12 overlapped between the VGL and SANBI (Table S1). Genotypes from 20 lions were generated by all three laboratories. Microsatellite markers with alleles that did not correspond between the three laboratories were assessed and a consensus to use 13 of the markers for the retrospective analysis was reached and correction factors were determined for Unistel and SANBI (Table S1): FCA057, FCA275, FCA097, FCA224, FCA391, FCA453, FCA026, FCA240, FCA272, FCA506, FCA628, F42, FCA031. Two further markers were identified that may be useful to include in a standard panel going forward: FCA105 and FCA075. FCA105 was not used by the VGL at the time due to linkage with FCA113 and thus not included in

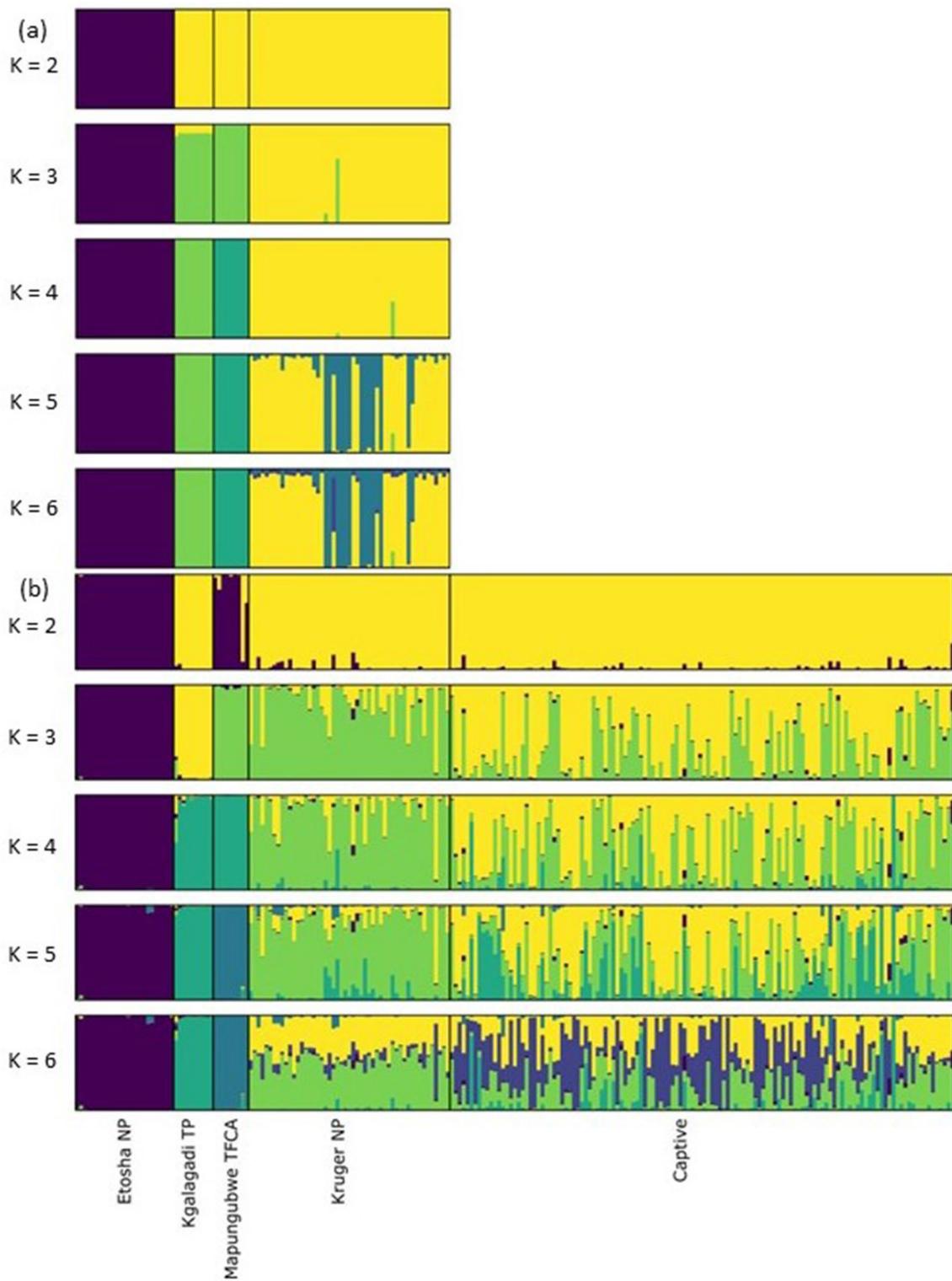
**Fig. 1** Sources of captive lions (C) on captive properties in South Africa. **a** Responses to survey—sources of original stock over time; **b** Responses to survey—current stock origins (percentages indicated); **c** Lions listed on CITES permits issued over time (number of individual lions listed for import into South Africa for captive breeding (code B) or commercial trade (code T). C captive lion, W wild lion, U unknown source code



the standardisation exercise, but it could be useful since FCA113 was not recommended for the standard panel (Miller et al. 2014a). FCA075 could also be included but unfortunately, the quality of the peaks in the historic Unistel data was poor for this marker (oversized and sheared

off in many cases, Fig. S1) making scoring inaccurate. Thus, the historic data for FCA075 could not be used in this study.

The 13 microsatellite markers successfully discriminated between 95 lions from the wild populations (Fig. 2a). The



**Fig. 2** Hierarchical population structure based on STRUCTURE analysis. Each bar chart represents a different value of K. **a** 95 wild lion genotypes based on 13 microsatellite markers chosen following the standardization exercise. **b** 95 open system lion genotypes and 128 unrelated captive lion genotypes based on 13 microsatellite markers

chosen following the standardization exercise. The black lines divide the individuals based on geographical origin of the samples: “Etosha” (Pilanesberg NP as surrogate; n = 25), Kruger NP (n = 51), Kgalagadi TP (n = 10), Mapungubwe GTFCA (n = 9) and in (b) captive population (n = 179)

Evanno method supported a  $K=2$ , however the mean of the estimated Ln of the probability of data had not levelled out and thus  $K=4$  was also likely (Fig. S2a) and supported by the known geographical separation of the groups.

### Captive lion population genetic descriptors

The final dataset combined for analysis represented 729 genotypes from Unistel, 38 from the VGL, the 39 genotypes from captive individuals generated in this study, 120 from SANBI (limited analysis due to low coverage of 13 chosen microsatellites) and 101 wild individuals from Miller et al. (2014b; Table S5). Out of a total 134 possible alleles, 25 private alleles were identified in the captive population and two in the wild populations. Genotypes with more than one missing data point and duplicate genotypes were removed leaving a total of 781 genotypes from captive lions and 95 genotypes from wild populations (Table S6).

Descriptive statistics for both Kruger NP and the unrelated captive lion population were calculated, including allelic richness, observed and expected heterozygosity, PIC, HWE and Fis (Table 1). Overall observed and expected heterozygosity values ( $H_o=0.68$ ,  $H_e=0.71$ ) for the Kruger NP population were not significantly different (Barlett's  $K$ -squared=0.78,  $df=1$ ,  $p$ -value=0.37). The allelic richness within the combined captive population was higher than that in Kruger NP for many alleles (Table 1), however there was no statistically significant difference between the average allelic richness values for the captive lion dataset and either the Kruger NP or metapopulation populations ( $p=0.385$ , Fig. 3). The average inbreeding based on the  $F$  coefficient was 0.04 for the reduced, unrelated captive dataset and 0.03 for the full captive dataset. Individual heterozygosity distribution was similar between Kruger NP lions, metapopulation and captive lions (Fig. 4).

Relatedness testing of the captive lion genotypes followed by an assessment of the individuals related at or below 0.25 revealed a high level of relatedness within the captive lion populations with only 128 “friends” or unrelated individuals observed among the 781 captive lions in the study. Interconnectivity between properties was high (Fig. 5). However, the average pairwise relatedness within the captive properties compared to Kruger NP and the metapopulation reserves was similar (Fig. 6).

STRUCTURE analysis of the 128 “friends” and the 95 wild lions suggested  $K=3$  was most supported by the Evanno method, however, the mean estimated Ln of the probability of data had not levelled out, and we therefore provide the results at higher  $K$  values as well (Fig. 2b, Fig. S3b). At  $K=3$  suggested the captive lions were most closely related to Kruger NP and/or Kgalagadi TFP lions (Fig. 2b). The captive population had a disproportionately high representation of the less common Kruger NP signature (Fig. 2b).

As we did not have any metadata regarding the time of the sampling of the captive individuals, it was not possible to determine if this is the result of genetic drift, a chance founder effect or something else.

PCA analysis of all captive and wild population genotypes did not show any separate clustering for the captive lions (Fig. 7). Lions from one captive facility (number 22) clustered mid-way between Kruger NP/Kgalagadi TFP and Etosha NP origin lions (Fig. 7).

Population assignment analysis did not distinguish between Kruger NP, Kgalagadi TP or Mapungubwe TFCA. Etosha NP origin individuals were separated by the analysis. Captive lions were assigned to the Kruger NP, Kgalagadi TP and Mapungubwe TFCA cluster with similar probabilities (Fig. 8).

The effective population size ( $N_e$ ) of the Kruger NP sample was 151.1 (95%  $CI$  78.3–769.9). The  $N_e$  of the combined captive population was 87.6 (95%  $CI$  78.0–98.3).

## Discussion

A detailed understanding of the genetic composition and possible conservation value of South Africa's large captive lion sector is currently lacking. Using a standardised panel of 13 robust microsatellite markers we found that the genetic composition and diversity of the captive lions analysed are representative of that found in existing wild lion populations in South Africa. While no major signatures of inbreeding were identified, our analysis does indicate that captive lions are highly genetically interconnected across properties and represent a smaller effective population size compared to Kruger NP lions, suggesting that they are at some risk of future inbreeding. There were also signatures of genetic drift which should be investigated further as it is likely to compromise the genetic integrity and any future conservation value of captive lions.

### Standardisation of microsatellite markers

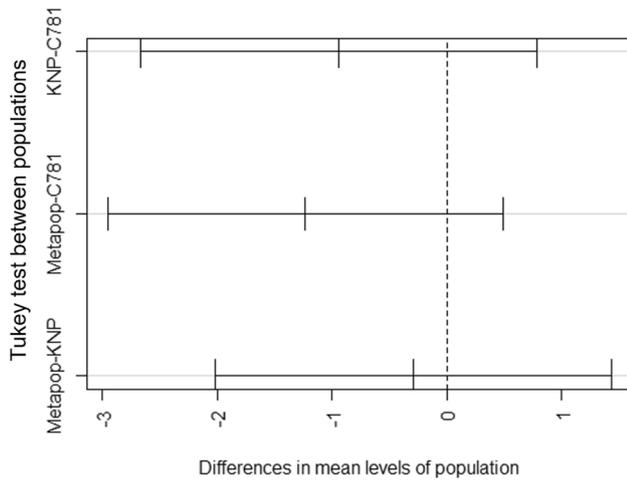
Three laboratories in South Africa use a panel of microsatellite markers to genotype lions for both commercial and research purposes. To combine these datasets for both the analysis presented here and for future analysis of captive and wild lion comparisons, we successfully performed a standardisation exercise that resulted in 13 standardised microsatellite markers with two additional promising markers. This standard panel was able to discriminate between genetic origins and provided useful statistics for assessing genetic diversity of lion populations. Such a comparison is not common among research groups using microsatellites studying wild species despite the obvious benefits of combined datasets. We encourage researchers to actively engage

**Table 1** Summary statistics for Kruger NP lions, captive lions (unrelated dataset and full dataset)

Locus	Kruger NP							Captive Full							Captive Unrelated							Captive SANBI						
	N	k	Ho	PIC	AR	Fis	Ho	N	k	Ho	AR	Fis	Ho	N	k	Ho	AR	Fis	Ho	N	k	Ho	AR	Fis	Ho			
	FCA026	51	7	0.71	0.71	7.00	0.07	0.66	781	8	0.66	6.75	0.08	0.62	128	8	0.62	7.21	0.17	0.57	120	8	0.57					
FCA031	51	6	0.65	0.56	6.00	-0.08	0.63**	781	8	0.63**	7.44	0.09	0.67	128	8	0.67	7.81	0.09	-	-	-	-	-					
F42	51	5	0.69	0.64	4.96	0.02	0.67	777	6	0.67	4.77	0.07	0.62	126	6	0.62	5.26	0.17	-	-	-	-	-					
FCA057	51	7	0.71	0.77	7.00	0.12	0.74***	775	9	0.74***	7.15	0.02	0.69	128	8	0.69	7.66	0.12	0.76	120	10	0.76						
FCA097	50	6	0.68	0.60	5.98	-0.03	0.75	781	11	0.75	7.96	0.05	0.68	128	10	0.68	8.52	0.16	0.54**	106	9	0.54**						
FCA224	51	8	0.82	0.74	7.96	-0.06	0.74*	781	10	0.74*	8.51	0.02	0.71*	128	10	0.71*	9.53	0.13	0.76	117	11	0.76						
FCA272	49	4	0.57	0.58	4.00	0.12	0.61	778	4	0.61	3.92	0.01	0.56	128	4	0.56	4.00	0.17	-	-	-	-						
FCA275	51	4	0.53	0.57	4.00	0.15	0.66	781	7	0.66	4.77	-0.02	0.60	128	6	0.60	5.29	0.12	0.58	120	6	0.58						
FCA391	51	6	0.74	0.68	5.96	-0.01	0.75**	781	9	0.75**	7.99	0.06	0.66	128	9	0.66	8.37	0.20	0.71	119	12	0.71						
FCA453	51	5	0.78	0.64	4.96	-0.11	0.71	772	4	0.71	4.00	-0.02	0.62*	127	4	0.62*	4.00	0.13	-	-	-	-						
FCA506	51	8	0.63	0.72	8.00	0.16	0.66**	778	17	0.66**	12.35	0.07	0.69	128	16	0.69	13.98	0.16	-	-	-	-						
FCA628	51	6	0.72	0.70	5.96	0.02	0.68***	779	9	0.68***	7.44	0.05	0.73	128	9	0.73	8.54	0.02	-	-	-	-						
FCA240*	51	7	0.35***	0.66	7.00	0.50	0.41***	778	10	0.41***	7.99	0.41	0.41***	128	10	0.41***	9.19	0.47	-	-	-	-						
Average ± SE			0.66 ± 0.03	0.66 ± 0.02	6.06 ± 0.37	0.07 ± 0.04	0.67 ± 0.025	7.00 ± 0.63	0.06 ± 0.03	0.64 ± 0.02	7.64 ± 0.74	0.16 ± 0.03																

\*FCA240 is x-linked, and was not included in the average Ho calculation

N number of samples, k number of alleles, Ho observed heterozygosity, PIC polymorphic information constant, AR allelic richness, Fis inbreeding



**Fig. 3** Tukey test result for average allelic richness comparison between Kruger NP (KNP;  $n=51$ ), captive lions (C781;  $n=781$ ) and metapopulation lions (Metapop;  $n=190$ ). ANOVA indicated no significant difference between groups ( $p=0.385$ ); Tukey test confirmed that confidence intervals of all comparisons overlap 0, indicating no differences in overall allelic richness averages between groups

with each other when studying the same species and perform similar standardisation exercises. Regular standardisation exercises as organised for domestic species by ISAG should be performed to ensure continued compatibility between laboratories ([www.isag.us](http://www.isag.us)).

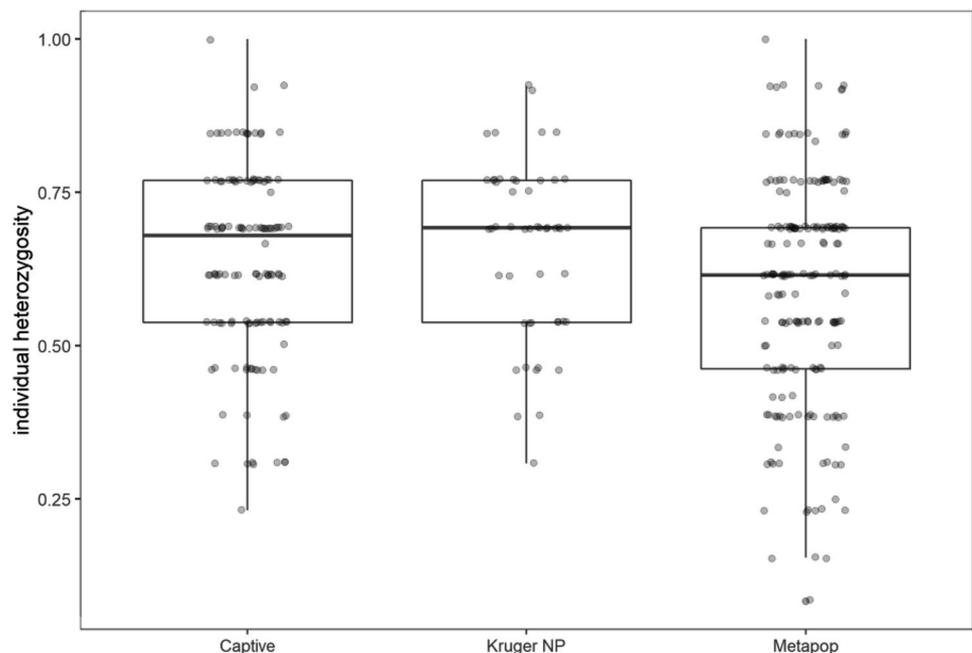
During the standardisation process of the microsatellite panel, we found some challenges that are worth highlighting when considering a similar exercise. Several microsatellite markers were discarded due to anomalies in the allele calls

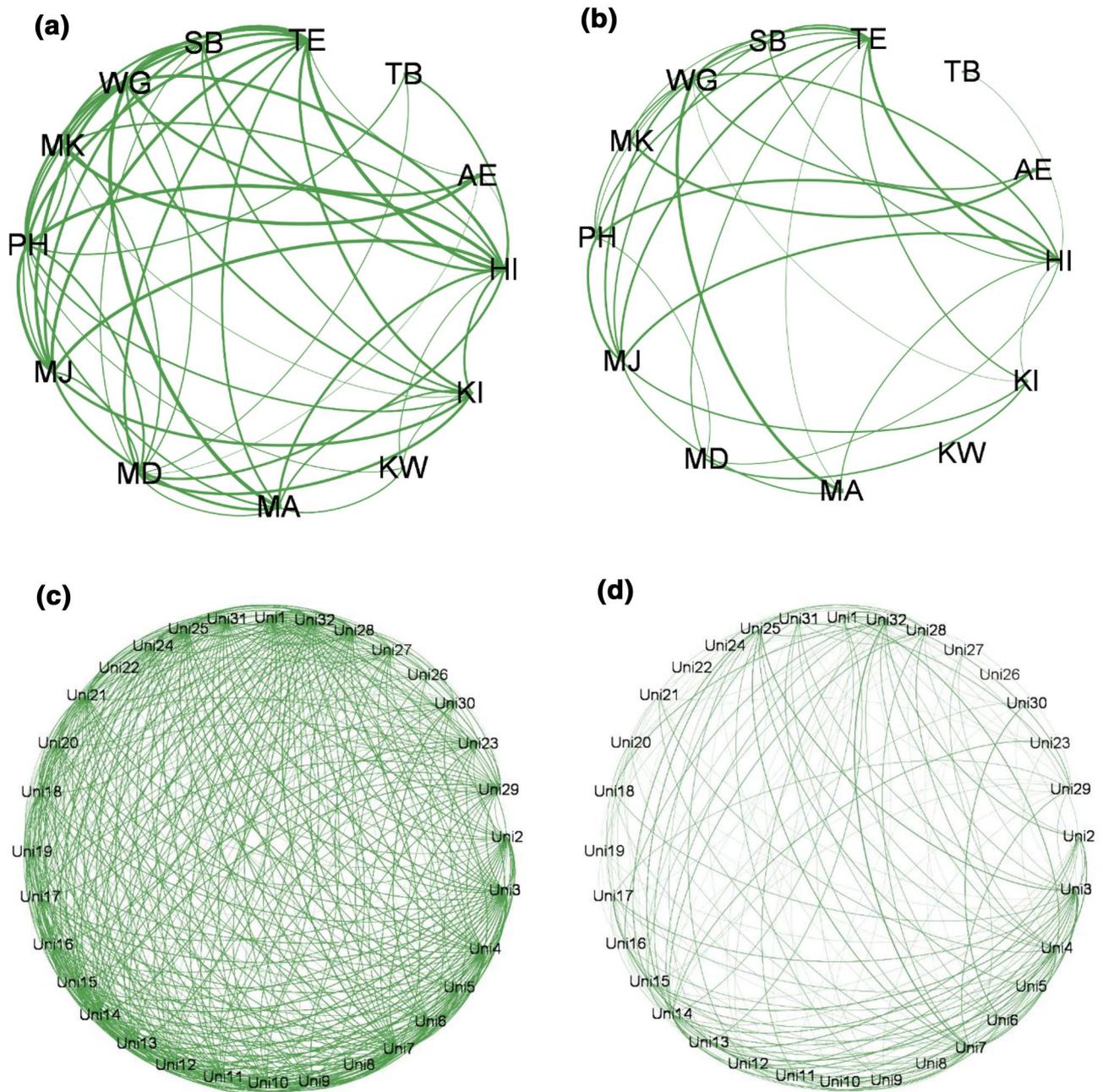
between the laboratories that made them very difficult to standardise. For instance, FCA113 appeared to have single base pair repeats making it impossible for laboratories to round to a standardised allele call. Microsatellite markers with larger allele ranges caused a size-shift at the extremities of the locus ranges making them difficult to score consistently, (e.g. FCA126 which was excluded). Another challenge was discovered in the historic data for FCA075. At one of the laboratories, the peaks were large and had sheared off, making it very difficult to distinguish between homozygotes and heterozygotes for alleles two base pairs apart. The laboratory has since corrected this and the individuals used in the standardisation exercise did not have excessively large peaks, allowing us to note the potential of this marker going forward as high levels of variation were observed in the VGL and SANBI datasets (data not shown).

### Origin of captive lions in South Africa

Several scientific papers have assumed that the origin of the captive lions is either not South African or is of mixed origin (Hunter et al. 2013; Schroeder 2018; Green et al. 2021). Prior to this study, only two peer-reviewed publications referred to the origin of South African captive lions: a scientific survey based on owner-responses which indicated that the majority were sourced from within South Africa, with ‘*some lions originating from outside South Africa*’ (Williams and ‘t Sas-Rolfes 2019) and a study which included CITES permit data indicating a possible wide range of source countries (Bertola et al. 2022). Neither of these studies included any genetic results. We re-examined the

**Fig. 4** Individual heterozygosity of captive ( $n=781$ ) compared to Kruger NP ( $n=51$ ) and metapopulation (Metapop) lions ( $n=170$ )



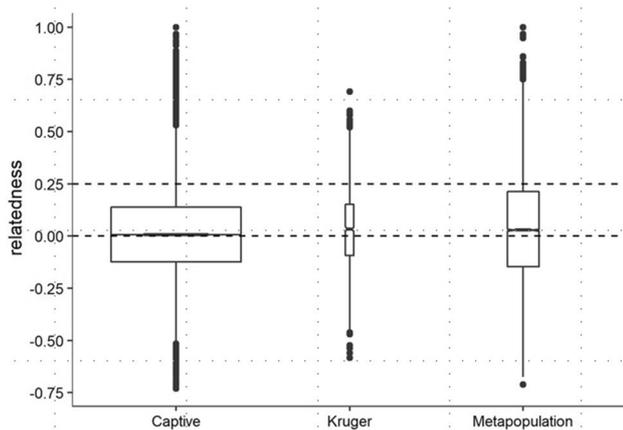


**Fig. 5** Pairwise relatedness between individuals based on Wang estimator to show connectivity between populations. **a** 0.25 level metapopulation individuals **b** 0.5 level metapopulation individuals **c** 0.25

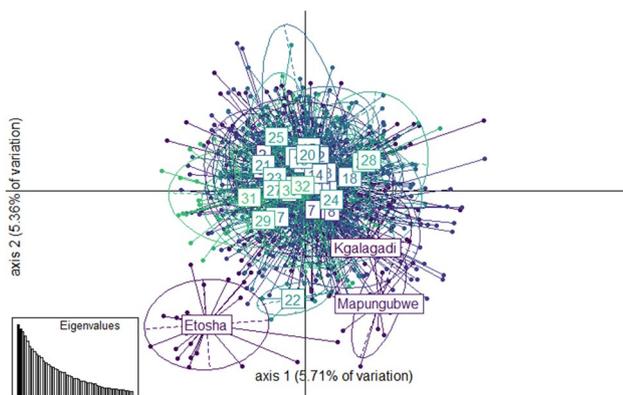
level captive individuals **d** 0.5 level captive individuals. Each label represents a different reserve within the metapopulation (a and b) or a captive facility (c and d)

data from Williams and ‘t Sas-Rolfes (2019) survey and the CITES permit data over time, within the context of the current captive lion population of South Africa. CITES data indicate that permits were issued between 1990 and 2017 to import substantial numbers of lions (n = 587 individuals listed on permits) from facilities from both other parts of Africa and outside of Africa. Survey responses also indicated that some lions were sourced outside of South Africa.

There are also anecdotal reports of wild lions being acquired from Zimbabwe (Hiller et al. 2022) and an unknown country in North Africa (V.L. Williams, pers. obs., 2018). The North African lions were apparently sourced to introduce darker manes in the captive population. The few CITES permits issued for importing wild individuals were mostly from other Southern African countries (Namibia, Botswana, and



**Fig. 6** Box plots of average pairwise relatedness values for captive (Captive), Kruger NP (Kruger) and lions in the metapopulation reserves (Metapopulation)



**Fig. 7** Principal Component Analysis of lion microsatellite genotypes from captive facilities (represented by numbers) and open systems (Kruger—not visible, under the captive facilities; Kgalagadi, Mapungubwe, Etosha). Plot of the first two axes explaining 11% of the variation; inset shows corresponding Eigenvalues; ellipses indicate the distribution of the individuals from different groups

Zimbabwe), thus supporting the Botswana and Zimbabwe origins, but not the North African anecdote.

If the CITES permits were all actioned (i.e. that many lions were all imported) and the survey results are representative of the sector, we would expect to see some genetic signature of non-South African lions within the South African captive population sector (note, this percentage would probably be lower if the lions were from neighbouring countries as they are from the same genetic clade as South African lions (Bertola et al. 2022)).

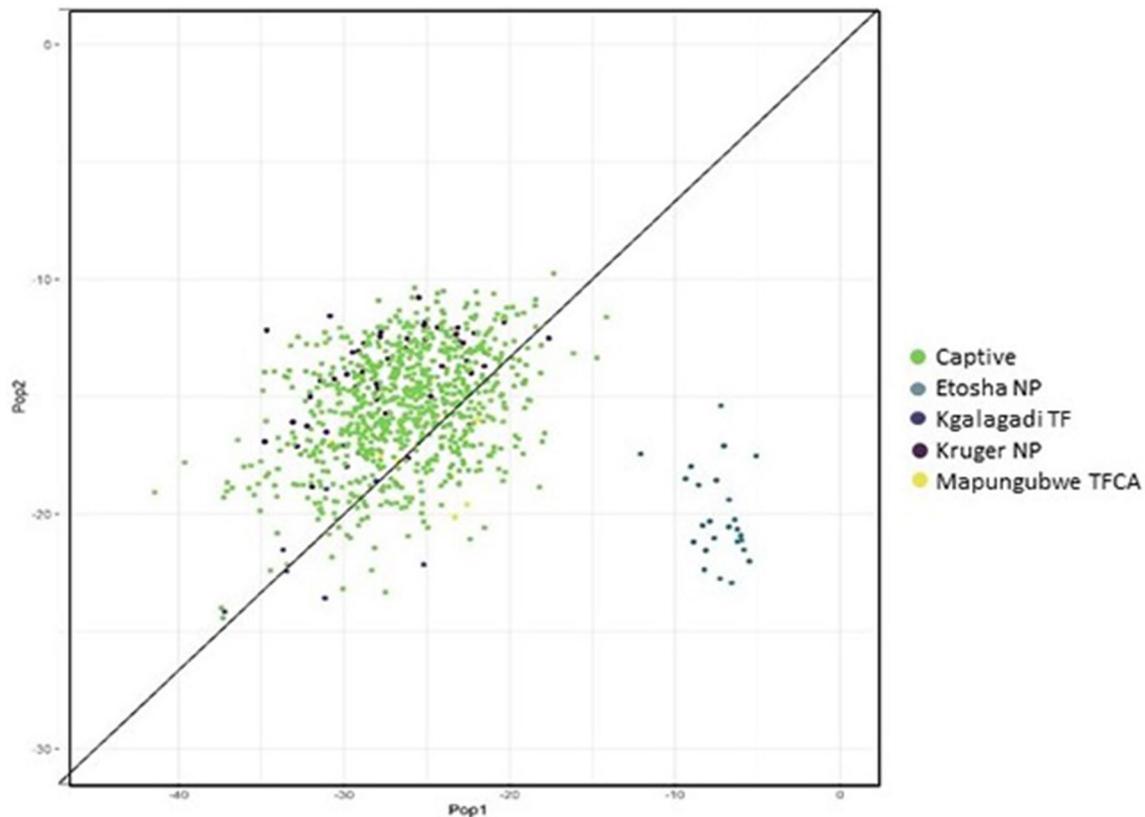
However, this signature would likely be diluted due to the high percentage of captive populations founded using other captive lions from within South Africa and the non-random sampling of captive lion properties may not have included

these properties. Thus, unless a property with the majority non-South African lion stock served as a major source for other populations, they would not be detected in our study. Indeed, we did not detect any genetic signatures apart from that expected in southern Africa and thus we conclude that there was no genetic signature of North African lions in the populations sampled. Sampling would have to have included these individuals for them to be detected in our study.

Any differences between the expected and actual genetic origins of captive lions in this study could be the result of unintentional bias introduced by survey respondents (only 100 out of several hundred properties responded), the fact that CITES permits are not always actioned, the inability to distinguish between lions from South Africa and neighbouring countries (see below), the sampling bias of the genetic samples or a combination of all four. Despite these challenges, our results are still highly relevant as they indicate that many captive properties have lions of South African origin. Further testing would be required to determine if this is the case of the entire sector.

The results of our various genetic analyses suggest that the captive lions assessed in this study are highly similar to wild South African lions from the Kruger NP and Kgalagadi TFP regions. Lions from populations in southeastern Zimbabwe have previously been shown to cluster with Kruger NP (e.g. Savé Valley Conservancy; Miller et al. 2015a) and lions from parts of southwestern Botswana would be expected to cluster with South African Kgalagadi TFP lions as this population is contiguous (Bauer et al. 2016). Thus, lions originating from areas in close geographical proximity to South Africa would be expected to have similar genetic clustering, making it very difficult to differentiate individuals from a ‘South African only’ origin. Therefore, we can only confirm that the captive lion samples analysed here are from the same genetic cluster as represented by Kruger NP and Kgalagadi TP and not that they are of South African origin. This generally supports the expected origins drawn from the interviewees discussed above and the original import of lions from Botswana and/or Zimbabwe (provided they were from the southern regions of these countries) would not be genetically detectable.

The inability to genetically differentiate captive from wild southern African lions has important implications for regulating commercial trade in captive lions and their body parts and confounds the use of a forensic DNA tool in this regard. Relying on DNA for distinguishing captive from wild populations is not ideal as a well-managed ex situ population, where warranted, would ideally not be distinguishable from a wild population as demonstrated in the Tsushima leopard cat (*Prionailurus bengalensis euptilurus*) (Ito et al. 2022). Both stable isotope analysis (Hutchinson and Roberts 2020) and Direct Analysis in Real Time (DART) mass spectrometry have shown promise as alternative methods



**Fig. 8** Assignment test results placing captive individuals into populations of individuals from known origins: Kruger NP, Etosha NP, Kgalagadi TP and Mapungubwe TFCA. The axes represent the log likelihood of assignment from a particular population

to distinguish between captive and wild lion bones (Coals et al. 2021).

The allelic composition of captive lions included almost all the alleles found previously in wild populations in South Africa with a few additional alleles (see Miller et al. 2015a for details). We also found several private alleles at very low frequency in the captive population that have not been reported in wild South African samples to date. These could have come from individuals originating outside of South Africa and which have now been incorporated into the general captive lion population gene pool, but a more detailed comparison with a larger geographic spread of African lions is required to fully interpret the significance of this finding. Additionally, mitochondrial DNA sequencing could also be used to further explore the genetic origin of South Africa's captive lions, allowing them to be placed within the current phylogeographic framework for lions (see Bertola et al. 2016).

Our initial study also dispels general perceptions that ‘all captive lions are inbred’ (Hunter et al. 2013; Schroeder 2018; Green et al. 2021). This perception was not based on any published scientific studies that established the genetic origin or levels of inbreeding within the captive

lion population in South Africa. Schroeder (2018) refers to a study of lions introduced to Hluhluwe-Umfolozi Park in KwaZulu–Natal, South Africa, as an example of how inbreeding can happen in a closed system with a small number of founders, however no scientific paper is referenced. This most likely refers to the study of Trinkel et al. (2011) on the original lions of Hluhluwe-Umfolozi, a wild population constrained by fences and is therefore not a specific example from the captive lion sector, but rather a cautionary tale of inbreeding following a small founder population (Frankham et al. 2017).

While our study did not identify any major genetic signatures of inbreeding, our analysis did reveal relatively high connectivity among the captive properties (using pairwise  $r=0.25$  and  $0.5$ ; Fig. 4) and a lower effective population size ( $N_e$ ) where  $N_e$  across the 31 captive properties analysed was approximately 58% of the  $N_e$  of the Kruger NP population. But we also note that our study represents only 31 out of a minimum 237 properties and, depending on general husbandry across properties, it is likely that some unsampled properties may have inbred lions. Further sampling is required to determine the genetic origin and diversity of lions on properties not included in this study. Successful

breeding programs that optimize  $N_e$  while minimizing kinship is a key goal of ex situ conservation and requires standard pedigree-informed management of mating events. In the absence of this approach, these highly connected properties will likely experience the negative effects of inbreeding in the future. Our conclusion is further supported by the survey response of captive lion owners which indicated that 87/100 properties sourced their original lions from existing captive properties (Williams and 't Sas-Rolfes 2019), that most captive lions were sourced in South Africa from other breeders (Hiller et al. 2022), and the decline in number of lions listed on CITES import permits since 2014. While provincial ToPS permits are required for the movement of lions between provinces and captive properties within South Africa, there is no digital database of these permits, thereby making analysis of these movements virtually impossible. However, anecdotal evidence supports regular buying and selling between captive properties (V. Williams' observation).

Despite high allelic richness and apparent lack of inbreeding there were signs that captive lions are experiencing enhanced genetic drift, with the STRUCTURE analysis revealing the emergence of a "captive cluster". This is likely the result of a genetic founder effect in the captive population which is managed in isolation from the larger wild population and, while relatively large, shares high levels of genetic connectivity across properties. Species that generally outbreed are particularly susceptible to enhanced genetic drift via small population size and local inbreeding, and both in situ and ex situ management of wild species, together with reintroduction programs, must be cognizant of this risk. For example, the Florida panther (*Felis concolor*) population which was isolated for many years from the main panther population in the American Southwest (Johnson et al. 2010) and the African lions of Hluhluwe-iMfolozi Park in South Africa that were a result of a very small founder population (Johnson et al. 2010; Miller et al. 2020) both demonstrate the predicted effects of small population size on genetic variation and fitness. Accelerated drift has also been observed in farmed forest musk deer (*Moschus berezovskii*) populations in China, where directional breeding of males for "superior traits" is likely to have enhanced the effects of drift (Fan et al. 2019). In addition to enhanced drift under captive breeding conditions, genetic adaptations to captivity can also occur over time, even if inbreeding is avoided, and the reintroduction of these individuals into wild populations can have a detrimental impact, even if only small numbers are introduced (Willoughby and Christie 2019). Potential negative impacts of ex situ breeding can be mitigated against with carefully monitored management that maximises and maintains the conservation value of individuals (Witzenberger and Hochkirch 2011; Willoughby and Christie 2019). The High-Level Panel report (Department of Forestry, Fisheries

and the Environment 2020) raised similar concerns surrounding genetic composition change:

changes in natural genetic composition, evolutionary trajectory and adaptive potential of wild populations through the introgression of captive population genetics wherein genetic changes in the captive population may lead to an altering genetic composition and/or evolutionary trajectory and/or adaptive potential of wild populations through deliberate and accidental introductions.

Thus, information on the genetic composition and structure of captive lions will be useful to inform the fate of individual lions within this sector. If a decision is reached to maintain some animals in captivity or use them for re-wilding projects, genetic testing of these individuals should be performed to ensure they are preserving/contributing genetic material that is relevant within clearly defined goals.

In summary, our results demonstrate that the genetic composition of captive lions analysed here is comparable to that of wild South African lion populations. Thus, arguments for a potential role in re-wilding for population supplementation cannot be excluded based on genetic characteristics alone. If a captive lion population is to be maintained in South Africa, its management should be committed to, and informed by, (i) the very clear scientific guidelines that inform ex situ breeding programs for species conservation, and (ii) integrated with in situ management plans, rather than merely continue as an ad hoc sector (McGowan et al. 2017). Currently, however, there are, no significant conservation concerns for South Africa's wild lions; this is evident in the regional Red List assessment listing lions as "Least Concern" and the global IUCN Red List assessment highlighting the resilience of southern African lion populations (Bauer et al. 2016; Miller et al. 2016). One of the strengths of South Africa's wild lion population is that lions are maintained in both multiple populations within the open systems (Kruger NP, Kgalagadi TFP and Mapungubwe TFCA with approximately 2400 lions combined), and across some 60 small reserves which together support approximately 800 lions that capture the genetic diversity of the open systems, thereby increasing broader resilience for the species in South Africa (Miller et al. 2015a, Selier et al. in review). There is also currently an excess of wild lions on small reserves in South Africa (Miller et al. 2013; Miller and Funston 2014) and thus any new reserves can source wild lions for their initial population without resorting to the complex, expensive process of re-wilding captive animals. Nevertheless, a recent PhD study reports on the reintroduction of captive-bred lions into a private wildlife reserve in South Africa (Booyens 2021). While the reintroduction was deemed successful by the criteria established at the outset of the study (Booyens 2021), there is still much to learn about the long-term impacts of captive breeding on lions as captivity can impact a wide range of aspects of lion

biology that were not included in the PhD study (Crates et al. 2022). If further re-wilding does occur, the movement of lions for wild population establishment or supplementation must consider the broader genetic make-up of both the individuals being moved and the receiving facility, thus ensuring that conservation management of South Africa's lions is in line with current recommended guidelines (Bertola et al. 2022). More recently, following the High-Level Panel report, a process was initiated to shut down captive lion activities (Department of Forestry, Fisheries and the Environment, May 2021), and a Ministerial Lion Task Team was appointed in December 2022 to identify and recommend voluntary exit options and pathways from the sector (Department of Forestry, Fisheries and the Environment, Dec 2022). The findings presented here should be considered in any development of an exit strategy and addressed in the broader context of lion conservation targets in South Africa.

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**Author contributions** SMM, AK, CKH, DLD and SAJS contributed to the initial study conception and design. Laboratory work at SANBI and VGL was supervised by AK and CKH. Laboratory work at SANBI was performed by MdB. Laboratory work at Unistel was performed by CR and JS. Genetic data analysis was performed by SMM with initial input from AK, CKH, DLD, CR, JS and MdB; JMB provided extensive additional input into data analysis. VLW collated survey and CITES data and provided expert insight into this aspect of the study. The first draft of the manuscript was written by SMM with input from VLW, AK and SAJS. All authors commented on previous versions of the manuscript with extensive editorial input from JMB. All authors read and approved the final manuscript.

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**Data availability** All data used in this manuscript are included in the supplementary materials. The supplementary materials are archived here: <https://doi.org/10.25375/uct.23174729>.

## Declarations

**Competing interests** The authors have no relevant financial or non-financial interests to disclose.

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