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Genetic diversity of African clawless otters (*Aonyx capensis*) occurring in urbanised areas of Gauteng, South Africa

Genetic diversity is the basis of the evolutionary potential of species to respond to environmental changes. However, restricting the movement of species can result in populations becoming less connected which can reduce gene flow and can subsequently result in a loss of genetic diversity. Urban expansion can lead to the fragmentation of habitats which affects the ability of species to move freely between areas. In this study, the genetic diversity of the African clawless otter (*Aonyx capensis*) in Gauteng (South Africa) was assessed using non-invasive sampling techniques. DNA was extracted from spraint (faecal) samples collected along nine rivers and genotyped using 10 microsatellites to assess population structure and genetic diversity. Samples were grouped based on locality and by catchment to determine whether isolated subpopulations exist. Genetic diversity of *A. capensis* in Gauteng was found to be low (mean observed heterozygosity (*Ho*)=0.309). Analysis of genetic structure provides support for the otter populations being panmictic with high gene flow between populations from different rivers. Results from the study indicate that the movement of *A. capensis* is not affected by physical barriers in urbanised areas. However, because the genetic diversity of the species in the study area is low, these animals may not be able to cope with future environmental changes.

Significance:

- Genetic structure analysis of the sampled Gauteng otter population indicates the population is panmictic; however, a low level of genetic diversity in this population has also been identified and may affect how the population copes with future environmental changes.
- Physical restrictions in urbanised areas do not appear to be affecting movement of the species.

Introduction

Over the past eight decades, urban areas have expanded into surrounding natural environments at a significant rate. This development into previously undisturbed areas has resulted in species being driven out of their habitats in search of suitable environment with less human disturbance. In some cases, species will remain in the urban areas and utilise the novel environment.[1] Many cities include open spaces such as parks and sports fields which provide new habitat for wildlife. However, not all species are suited to living in cities[2,3], and thus a decrease in biotic diversity may occur, whereby only the more resilient (able to recover from adverse conditions) species survive as they possess characteristics allowing them to tolerate the urban setting[1].

Species in urban areas can occur across a broader habitat range which reduces restriction to one specific habitat type, allowing them to move to another area if conditions become unfavourable.[3-6] However, barriers such as roads and fences may prevent movement of some animals between suitable habitats, limiting their movement and reducing their chances of finding shelter, food and mates. A reduction in interactions between unrelated individuals of the same species[7] can lead to reduced genetic diversity and an increase in the level of inbreeding.[8]

Two otter species occur in South Africa: the African clawless otter (*Aonyx capensis*) and the spotted-necked otter (*Hydrictis maculicollis*). The distributions of both species range across most of South Africa and include inland and coastal areas as well as large urbanised areas (such as the current study area), with *A. capensis* having a much greater distribution range than *H. maculicollis*.[9] The IUCN Red List (2016) has categorised both otter species as near-threatened, with habitat degradation posing the highest threat to freshwater environments used by the otters.[10,11] Kubheka et al.[12] demonstrated a decrease in abundance of both otter species along a stretch of the Mooi River in South Africa that has experienced an increase in human activity along its banks in recent years, lending to the urgency to better understand anthropogenic effects on otters. Somers and Nel[13] reported that *A. capensis* has a home range size that varies from 4.9 km to 54.1 km and a core length from 0.2 km to 9.8 km. Their study also indicated that the ranges of male individuals overlapped with those of other male and female individuals, while female otters possibly demonstrated territoriality. However, there is a possibility that the ability of otters to travel great distances in dense urban areas may be hampered by barriers such as buildings, roads, fences and high levels of human activity, which in turn would impact on intraspecific encounters. To date, no studies have focused on the genetic diversity of either of the two otter species, making it impossible to draw conclusions regarding the general genetic health and risks facing these otter species in the future.

The aim of this study was to assess the population genetics of *A. capensis* in a region exposed to varying levels of human disturbance. It was hypothesised that population structuring would reflect division of the local population caused by geographical separation from catchment areas, as well as restriction of movement due to areas of heavy urban development. A study of Hungarian otters demonstrated a level of genetic clustering occurring between to geographically separated river basins.[14] It was also hypothesised that low genetic diversity and inbreeding would be evident as unrelated individuals may not be able to interact and reproduce successfully in urban Gauteng.

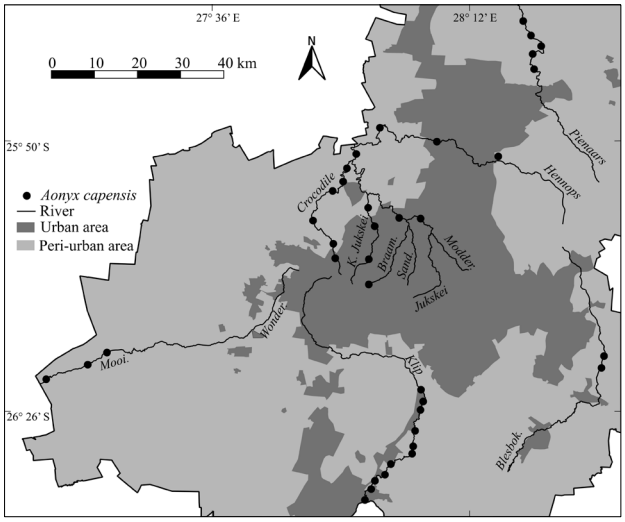
Spraint (faecal) samples were collected and examined from river catchments in the Gauteng Province (South Africa) to determine the level of genetic diversity and structure of the *A. capensis* population, using 10 microsatellite primers developed for *Lutra lutra*, the Eurasian otter). The use of cross-species primers has been conducted

successfully for amplification of alleles in numerous otter and other animal species in situations in which species-specific primers have not yet been developed.[15-19] Gauteng was selected as it represents a complex landscape comprising urban areas surrounded by less transformed peri-urban areas. There are numerous interconnecting rivers within Gauteng that flow through varying levels of urbanisation (residential suburbs, industrial, mining and commercial areas) and natural environments.

Methods

Study area and sample collection

The Gauteng Province of South Africa has three river catchments: Crocodile River west catchment (A), Olifants River primary catchment (B) and Vaal River primary catchment (C). These catchments contain the headwaters of several major river systems.[20] The study focused on nine rivers in the Province which occur in two of the three catchments: Pienaars, Hennops, Jukskei, Klein Jukskei and Crocodile Rivers in Catchment A and Braamfonteinspruit, Mooi River Loop/Wonderfonteinspruit (henceforth Mooi), Klip River and Blesbokspruit in Catchment C. Sampling was conducted from June 2012 to October 2014 and was restricted to autumn and winter as these seasons have much lower rainfall levels, thus reducing the chance of spraints deteriorating due to rain or being washed away by flooded rivers[21], which is a common occurrence in summer. Google Earth[22] and the Resource Quality Information Services river coverage data for South Africa[23] were used to measure the full length of the chosen rivers (Figure 1) and sampling sites were identified at 5-km intervals along each river. Sites were selected at 5-km intervals as this is the shortest home range length of *A. capensis* found by Somers and Nel[24], but due to possible DNA degradation, individuals could not be identified, preventing the estimation of home ranged based on occurrence of multiple spraints from the same individual. A 400 m by 10 m transect was surveyed once only at each 5-km point along both sides of the river for signs of otter presence (footprints, spraints and sightings of animals).



Mooi., Mooi River Loop; Wonder., Wonderfonteinspruit; K. Jukskei, Klein Jukskei River; Braam., Braamfonteinspruit; Modder., Modderfonteinspruit

Figure 1: Rivers surveyed and collection sites of *Aonyx capensis* spraints in relation to peri-urban and urban areas.

Spraints occurred in various forms: small deposits of anal jelly, a single cigar-shaped faecal deposit, a solitary pile of faeces (comprising three or four cigar-shaped faeces), or a site with numerous piles of faeces. Otter spraints can be easily identified based on a pungent fishy smell that can be detected several metres away, as well as by the characteristic presence of pieces of crab carapace in the spraints[21]). Each spraint sample (anal jelly, single cigar-shaped faeces or solitary pile of faeces) was collected separately in re-sealable plastic bags, and a solitary pile of spraints was considered one sample. At spraint sites, care was taken to select spraint piles (each one collected separately) that were not in contact with neighbouring spraint piles. Multiple spraints were collected separately from spraint sites, as previous studies have shown that multiple individuals[21,25], as well as both otter species (*A. capensis* and *H. maculicollis*)[21], use the same spraint sites on occasion. Spraints were collected regardless of their age (except in the case of extremely weathered spraints that had deteriorated significantly). The Global Positioning System (GPS) coordinates were recorded at every location at which spraints were found using a handheld Garmin eTrex VistaCX GPS device (Garmin, Olathe, USA). Figure 1 shows the locations where positive signs were found. Samples were stored at -10 °C prior to DNA extraction.

DNA extraction and species identification

DNA was extracted from 211 spraint samples using the QIAGEN QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany)[26] according to the manufacturer's instructions for isolation of DNA from stool for human DNA analysis.[27] Species identification was conducted using developed partial *CytB* primers.[28] A homology search was done on all sequences obtained using the BlastN function on the US National Center for Biotechnology Information (NCBI) online database. Control sample DNA for *A. capensis* and *H. maculicollis* was extracted from reference tissue samples obtained from the South African National Zoological Gardens Biomaterials Bank (Biobank). These reference samples were collected from roadkill specimens from various locations across South Africa.

Amplification and genotyping

Ten microsatellite markers developed for studies of *L. lutra*[29] were used for genotyping analysis: Lut435, Lut453, Lut457, Lut604, Lut615, Lut701, Lut715, Lut782, Lut818 and Lut832. These markers have been shown to be polymorphic in up to six otter species (including *A. capensis*), but not in other carnivores.[30] Optimisation of the primers was conducted at various annealing temperatures (Ta) to ensure amplification of the correct fragment, with the subsequent temperature (Ta) deemed the most effective based on number of successful amplifications of the correct fragment size. Amplification was carried out using a 15 μL reaction volume containing 7.5 μL of Platinum master mix (1X), 3 μL of forward and reverse primer (10 pmol), 2.5 μL of double distilled water and 2 μL template DNA (~20 ng). The cycling conditions for polymerase chain reaction (PCR) amplification were: 5 min at 95 °C initial denaturation, 38 cycles for 30 s at 95 °C, 30 s at 45–52 °C and 30 s at 72 °C, followed by extension at 72 °C for 20 min. The PCR was carried out in the Bio-Rad T100 Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, USA). PCR products were run against GenescanTM 500 LIZ™ (Applied Biosystems, Inc., ABI, Foster City, USA) internal size standard on an ABI 3130 Genetic Analyzer. Samples were genotyped using GeneMapper v.4.0.[30] For each sample, PCR reactions were conducted once and then repeated four times, resulting in each sample being amplified five separate times. Genotyping was conducted using a comparative method in which alleles obtained for each sample were compared, and the most frequently observed alleles for each locus were selected for each sample. Allelic peaks were scored based on height and occurrence in prescribed binning areas based on the range of each marker. In cases presenting multiple allelic peaks, the highest peak was chosen as the first allele. The second allele was selected if it was no less than half the height of the first allele, fell into a prescribed bin, and was of a reasonable distance of base pairs apart from the first allele selected (see Supplementary figures 1 and 2 for visual representation of genotype scoring).

Microsatellite analysis

In order to exclude possible errors, MICROCHECKER version 2.2.3[31] and FreeNA[32] was used to detect the presence/absence of null alleles and allelic dropout. GenAlEx 6.5[33,34] was used to test for deviations from Hardy–Weinberg equilibrium. Linkage disequilibrium was determined using the online version of Genepop 4.2 (Genepop on the web[35]). Duplicate samples of individuals were identified using the probability of identity in GenAlEx 6.5.[33,34] Matching profiles indicating duplicate sampling of the same individual were excluded from further analysis to prevent redundancy. However, genetic profiles are dependent on the quality of DNA extracted, which can be compromised in non-invasive samples. Some samples were older than others, which may have impacted the quality of DNA extracted. If an individual defecated several times along a study river, the presence of null alleles due to low-quality DNA would

greatly affect the genetic profile obtained for that individual. A single locus difference will render a genetic profile unique, requiring multiple repeats to be conducted to increase the accuracy of allele detection. Genetic diversity was assessed using GenAlEx 6.5[33,34] to determine the number of alleles (Na), expected heterozygosity (*He*) and observed heterozygosity (*Ho*).

Population structure

Due to areas of heavy urbanisation, and large distances between study rivers restricting movement of otters, each river was defined as a potential population, resulting in nine theoretical populations. As the study rivers occur in two catchment areas, these were considered individual populations for the catchment analysis portion of this study. GenAlEx 6.5[33,34] was used to determine population differentiation (F_{ST}) and for analysis of molecular variance (AMOVA). In addition, F_{ST} was calculated following balancing for null alleles with FreeNA.[32] In order to assess the genetic partitioning across Gauteng river otter populations, two different approaches were used based on multilocus genotypes. A Bayesian clustering analysis was conducted using the statistical program STRUCTURE version 2.3.4[36] for the assignment of individuals to groups based on genetic similarity. STRUCTURE was run with and without LOCPRIOR using 100 replications at each value for *K* (*K*=1-12) for the 'per river' analysis and for the catchment analysis (*K*=1-4). The values used for *K* for 'per river' analysis took into account the possible nine populations designated to each study river, and an extra three populations in case more than nine independent populations occurred. If 12 populations were detected, the value for *K* would be increased further to accurately detect the possible number of populations. The *K* value for catchment analysis included the two actual catchments and two extra potential populations as the large areas of the catchments may be occupied by more than two populations. The runs were conducted with a burn-in period of 100 000 repeats followed by 1 000 000 repeats of the Markov chain Monte Carlo. The result files from each run (with LOCPRIOR and without LOCPRIOR) were uploaded to the web-based STRUCTURE-HARVESTER[37] program which uses likelihood methods to assume the correct number of genetic clusters (*K*). In addition, the genetic distance was calculated and a principal coordinates analysis (PCoA) was conducted for the data using GenAlEx version 6.5.[33,34] Nei's genetic distance was also compared to geographical distance between populations using the computer program IBD for Windows (version 1.52).[38] IBD (running 5000 randomisations) was used to calculate Mantel tests to highlight any significant relationships between genetic and geographical distance and estimate regression values.

Relatedness analysis

Pairwise relatedness was calculated between individuals per river, with each river considered as a separate population, using GenAlEx version 6.5.[33,34] Results were obtained for three different relatedness tests: Ritland[39] estimator, Lynch and Ritland[40] estimator, and Queller and Goodnight[41] estimator. The mean of the three results obtained for each pairwise comparison was used to create a box-and-whisker plot for each river. This analysis was restricted to the Blesbokspruit, Crocodile, Jukskei, Klein Jukskei, Klip and Pienaars Rivers as we did not obtain sufficient numbers of samples for an adequate analysis from the Braamfonteinspruit, Mooi and Hennops River. The same procedure was conducted for the comparison between catchments, with Catchment A comprising samples from the Pienaars, Jukskei, Klein Jukskei and Crocodile Rivers, while Catchment C included the Blesbokspruit and Klip River. If the median occurs at or below zero, individuals within the population are not highly related, whereas if the median is above zero, individuals in the population are considered related.

Results

Of the total 211 samples collected, 171 spraint samples were identified as being from *A. capensis* (except for two samples, relatedness analysis indicated that these were all unique individuals), while 8 were identified to be from *H. maculicollis*. A total of 32 samples remained unidentified to species, possibly because sample DNA was too degraded for successful amplification or was not present in a sufficient quantity in the sample. Due to the low number of samples identified as *H. maculicollis*, population genetic assessments were conducted only for *A. capensis* in the study presented here.

Marker assessment

Aonyx capensis samples from two catchments in Gauteng (South Africa) were genetically analysed using 10 microsatellite markers. Sample distribution included 14 samples from Blesbokspruit, 2 samples from Braamfonteinspruit, 48 samples from Crocodile River, 3 samples from Hennops River, 16 samples from Jukskei River, 15 samples from Klein Jukskei River, 37 samples from Klip River, 6 samples from Mooi and 30 samples from Pienaars River. The sample collection comprised the first genetic analysis of *A. capensis* in South Africa. Summary statistics calculated using GenAlEx 6.5[33,34] indicated that all loci were polymorphic in each population (Supplementary table 1). All loci were affected by null alleles in all populations following analysis in GenAlEx 6.5[33,34] and FreeNA.[32] Genotypes obtained were corrected using MICROCHECKER and three markers (Lut604, Lut782 and Lut818) showed a high presence of null alleles (mean >0.45) and were thus excluded from further analysis. Using probability of identity, no matching profiles were identified, consequently, the home range of individuals could not be estimated. All markers appeared to be significantly linked based on Genepop analysis, which may be due to the presence of non-amplified alleles (null alleles). Markers from Blesbokspruit, Crocodile, Jukskei, Klein Jukskei, Klip and Pienaars Rivers deviated from the Hardy–Weinberg equilibrium. The observed deviations may be from null alleles, low levels of observed heterozygosity at all loci and/or differences in sample sizes between rivers.

Genetic analysis: Populations defined by river

Genetic assessments were then carried out for each river referred to here as 'per river' analyses. All loci were polymorphic with the number of alleles ranging from 4 to 27 and averaging 9 alleles per locus. Genetic diversity estimates by observed and expected heterozygosity and the number of alleles within each river were moderate to high. The mean expected heterozygosity (*He*) was 0.730 with a mean observed heterozygosity (*Ho*) of 0.344 (Table 1).

Table 1: Genetic variation estimates (mean±s.e.) for (a) 'per river' analysis and (b) catchment analysis

a)

River	*Ho*	*He*	*Na*	*Ne*
Blesbokspruit (*n*=14)	0.406 ± 0.044	0.753 ± 0.027	6.9 ± 0.526	4.47 ± 0.449
Crocodile (*n*=48)	0.312 ± 0.051	0.879 ± 0.026	17.4 ± 1.796	10.388 ± 1.279
Jukskei (*n*=16)	0.289 ± 0.044	0.805 ± 0.028	8.6 ± 0.806	6.107 ± 0.805
Klein Jukskei (*n*=15)	0.319 ± 0.05	0.854 ± 0.013	10.0 ± 0.715	7.460 ± 0.767
Klip (*n*=37)	0.266 ± 0.033	0.879 ± 0.015	14.4 ± 1.185	8.989 ± 0.702
Pienaars (*n*=30)	0.292 ± 0.042	0.888 ± 0.019	16.3 ± 2.05	11.205 ± 1.614
Mean ± s.e.	0.314 ± 0.044	0.843 ± 0.021	12.267 ± 1.18	8.103 ± 0.936

b)

Catchment	*Ho*	*He*	*Na*	*Ne*
A (*n*=114)	0.311 ± 0.033	0.905 ± 0.016	25.5 ± 2.684	12.938 ± 1.792
C (*n*=57)	0.308 ± 0.026	0.873 ± 0.014	16.8 ± 1.191	8.65 ± 0.824

Ho, mean observed heterozygosity; He, mean expected heterozygosity; Na, mean number of alleles per locus: Na = sum of all alleles detected per river/number of loci; Ne, mean number of effective alleles: Ne = 1/(sum of squared population allele frequencies)

All analyses were conducted in GenAlEx version 6.5.[33,34]

Braamfonteinspruit, Mooi and Hennops results have been excluded due to small sample size.

In all instances, *Ho* was lower than *He* and values for *Ho* varied per river with Klip River being the lowest (*He*=0.266) and Blesbokspruit being the highest (*Ho*=0.406). Upon using each river as a potential population, STRUCTURE HARVESTER identified *K*=3 (Figure 2) as the most likely number of subpopulations, although no significant population structure was observed. A low mean genetic differentiation (F_{ST}=0.037) between all rivers is shown in Table 2a. The F_{ST} values were lower (F_{ST}=0.01) but similar when applying the Excluding Null Alleles (ENA) method in FreeNA (Table 2b). Populations along the Crocodile and Pienaars Rivers show the lowest differentiation (F_{ST}=0.014 and F_{ST}=0.001). Populations from all rivers displayed private alleles at all loci, with the Crocodile and Pienaars River populations showing the highest number with eight private alleles each. No significant relationship was observed between genetic and geographical distance (Table 3) at the river population level through Mantel tests (r^2=0.267; p=0.8732).

Table 2: Genetic differentiation between otter populations grouped according to the river along which spraint samples were collected, (a) including null alleles using Weir and Cockham analysis[60] and (b) excluding null alleles using FreeNA[32]

a)

	Blesbokspruit	Crocodile	Jukskei	Klein Jukskei	Klip
Crocodile	0.038				
Jukskei	0.063	0.043			
Klein Jukskei	0.051	0.028	0.058		
Klip	0.039	0.015	0.047	0.032	
Pienaars	0.043	0.014	0.041	0.029	0.020

b)

	Blesbokspruit	Crocodile	Jukskei	Klein Jukskei	Klip
Crocodile	0.027				
Jukskei	0.023	0.009			
Klein Jukskei	0.020	0.005	0.011		
Klip	0.026	0.002	0.011	0.009	
Pienaars	0.025	0.001	0.004	0.005	0.004

Table 3: Nei's genetic distance (measured in GenAlEx version 6.5[33,34]) and geographical distance (km) between river populations. Geographical distance is displayed above the diagonal, genetic distance below the diagonal.

	Blesbokspruit	Crocodile	Jukskei	Klein Jukskei	Klip	Pienaars
Blesbokspruit	–	77	61	66	50	83
Crocodile	0.365	–	17	14	66	60
Jukskei	0.613	0.562	–	6	61	50
Klein Jukskei	0.551	0.463	0.880	–	60	56
Klip	0.371	0.246	0.682	0.550	–	108
Pienaars	0.437	0.231	0.559	0.513	0.362	–

Relatedness: Populations defined by river and catchment

Pairwise relatedness comparisons between individuals within each river population indicated that the mean relatedness for each river is low as the median of the box-and-whisker plots for each river falls below zero. However, the Bleskbokspruit had two maximum outliers, which may be the result of two spraints collected from the same individual. Overall relatedness of individuals in both Catchments A and C was low based on the box-and-whisker plot (Figure 3).

Genetic analysis: Populations defined by catchment area

Due to sample size differences (small sample size of Braamfonteinspruit, Mooi, and Hennops River), which resulted in limitations, some analyses could not be performed in the 'per river' analyses. These rivers were subsequently clustered depending on the water catchment to which they belong (Catchment A – Pienaars, Hennops, Jukskei, Klein Jukskei, Crocodile Rivers; Catchment C – Braamfonteinspruit, Mooi, Klip River and Blesbokspruit) and genetic assessments for each of the two catchments (A and C) were conducted. When separated into catchment areas, all loci were polymorphic, with the number of alleles ranging from 9 to 39 and averaging 21 alleles per locus. Expected heterozygosity within all groups (*He*) was 0.889 and observed heterozygosity (*Ho*) was 0.309; this difference may be due to the use of non-species-specific markers or genotypic error. There was a significant deviation from the Hardy–Weinberg equilibrium ($p<0.001$) for all markers, which may indicate genotyping error and resulting underestimation of heterozygosity. However, genetic differentiation (F_{ST}=0.01) between the two catchments (A and C) was low and non-significant ($p\geq0.05$).

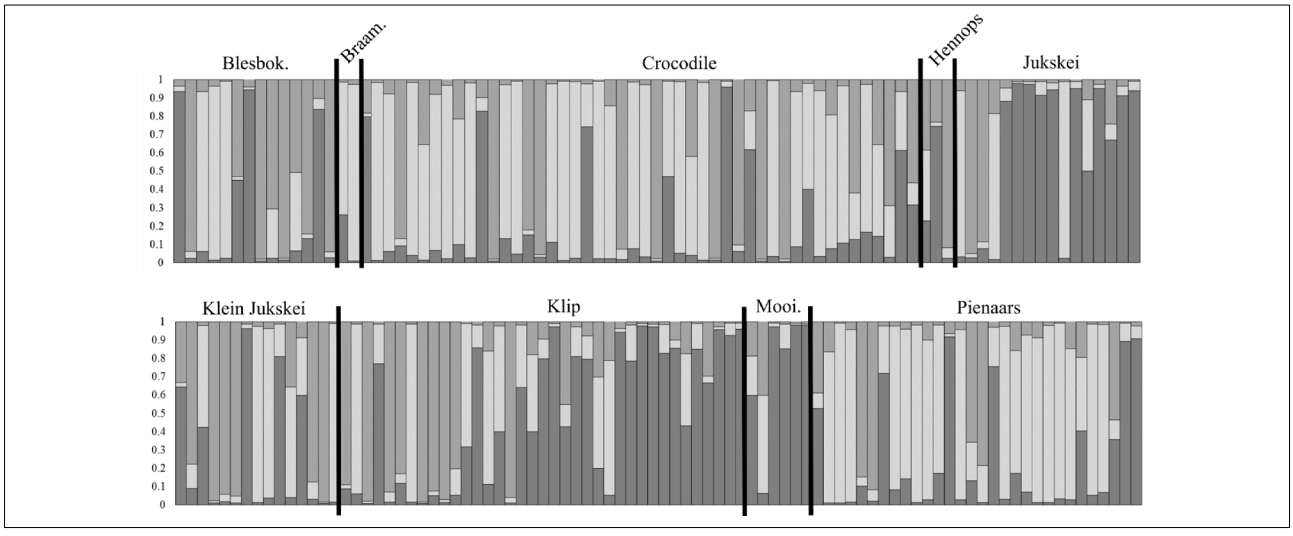


Figure 2: Plots of assignment probabilities from STRUCTURE for the 'per river' analysis showing the posterior probability of assigning each individual to each of the inferred clusters. Each individual is represented by a vertical bar and each shade refers to a different cluster. Average cluster membership for $K = 3$.

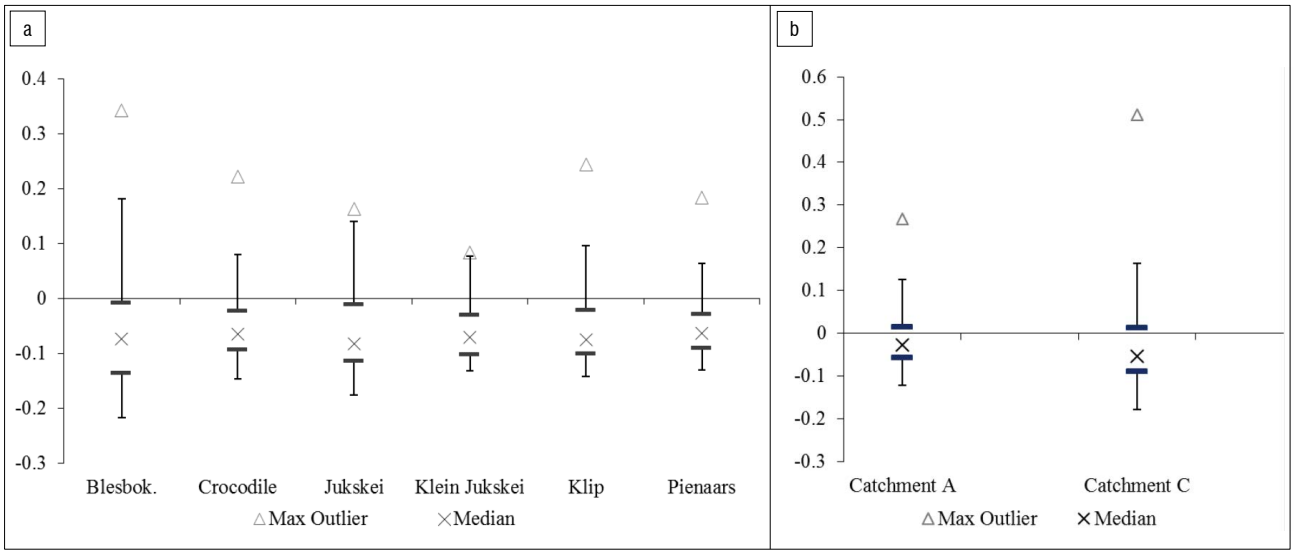


Figure 3: Box-and-whisker plots depicting the relatedness within each (a) river population and (b) catchment population. Overall relatedness is low for all populations, indicating that interbreeding is not considerably high in the populations.

Results from STRUCTURE Harvester identified $K=2$ as the most likely number of genetic clusters for the catchment analysis (Figure 4a,b). No significant population sub-structuring could be observed, with allele frequencies being somewhat similar, although more frequent in other rivers. This finding could be attributed to high gene flow but also shows that some rivers may be more favoured than others. Although STRUCTURE analysis supports the presence of two subpopulations, this was not observed in the PCoA, which clearly illustrates no significant clustering occurring (Figure 5). Private alleles were observed in all 10 loci for Catchment A, with 100 private alleles occurring in the Catchment A population. Of the 10 loci, 7 had private alleles, totalling 13 alleles for the Catchment C population.

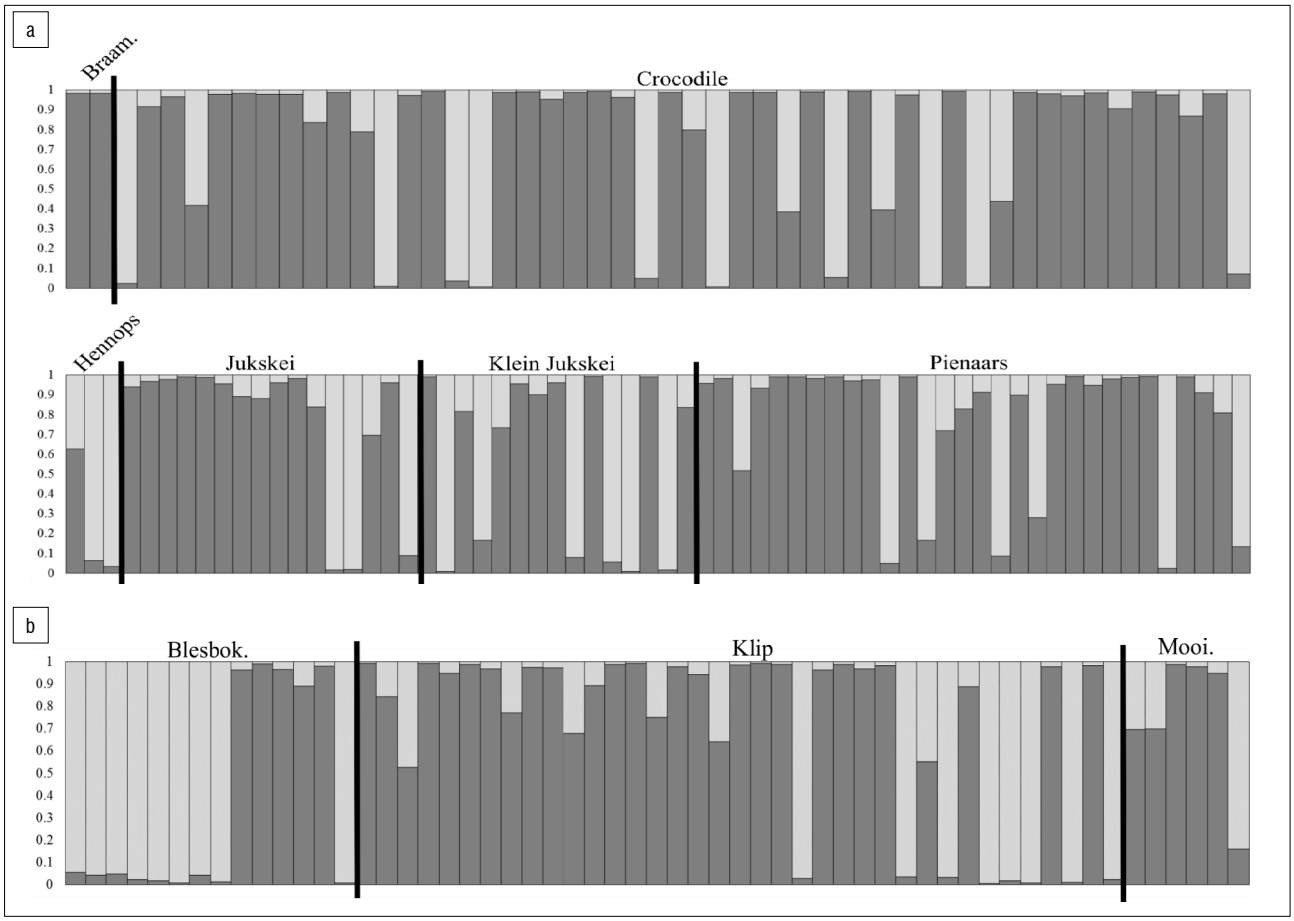


Figure 4: Bayesian assignment probabilities for (a) Catchment A samples at $K=2$ and (b) Catchment C at $K=2$. Each vertical bar represents an individual, which is divided into K shades representing estimated membership fractions in K clusters. The black vertical lines separate individuals into the nine rivers along which samples were found. No definite structure appears to occur within the overall provincial population.

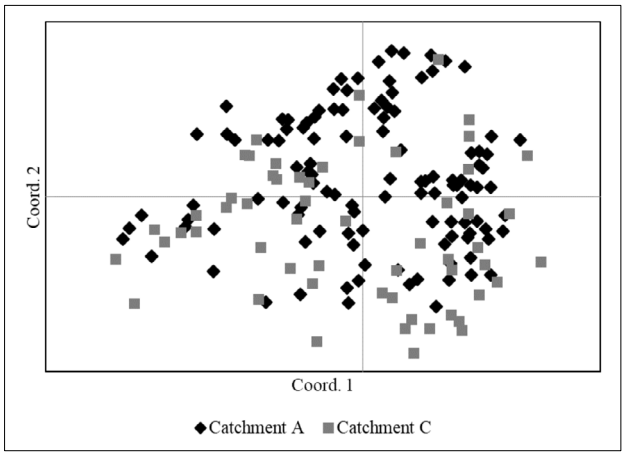


Figure 5: Principal coordinates analysis (PCoA) for the two water catchment areas assessed in Gauteng, South Africa.

Discussion

A relatively high level of genetic diversity is considered fundamental for species survival.[42] To achieve this diversity, high levels of gene flow within a population is required, but can be difficult where the landscape presents barriers such as cities, mountain ranges, valleys and large rivers.[43] Thus, human-mediated activities may have had an effect on patterns of genetic structure and diversity in *A. capensis* samples from two catchments in the Gauteng Province (South Africa). The genetic diversity across all the sampled river populations was low for observed heterozygosity (*Ho*=0.309), with a high expected heterozygosity (*He*=0.889). Historical data for the otter populations in Gauteng are, however, not available for direct comparison to assess whether genetic diversity has increased or decreased. Low genetic diversity introduces several negative effects for a population such as inbreeding, susceptibility to diseases, and reduced genetic fitness. All these factors combined can eventually lead to population decline. Previous studies have shown an increase in diversity after species were reintroduced into the areas, or from repatriation or ingress of species from adjacent areas, following initial declines.[44-46] Thus, the observed low genetic diversity in the Gauteng *A. capensis* population may be because of a history of extirpation and recolonisation, as has been identified in other mustelid species.[47,48] This answer is the most logical as there is no evidence in the literature indicating reintroduction of *A. capensis* to the area. It has been reported that genetic diversity decreases along a path of range expansion.[49,50] A similar pattern has been observed in the Minnesota river otter population from Central North America for which a decrease in heterozygosity was observed from the core population.[52]

The assessment of the population genetic structure of the otters occurring in Gauteng revealed no sub-structuring between the two populations/groups sampled within the two catchments as supported by a non-significant genetic differentiation (F_{ST}=0.01). These results provide evidence of high levels of gene flow between groups sampled in Gauteng which is further supported by the low relatedness coefficient value (0.048). Although STRUCTURE identified three genetic clusters for the populations defined by river analysis and two genetic clusters for the populations defined by catchment analysis, this does not seem to be the case when considering the genetic distance between individuals within the two catchments (Figure 5). Cluster analysis programs such as STRUCTURE tend to introduce uncertainty to results obtained in situations in which the study groups present low levels of divergence.[52] The minimum number of genetic clusters that can be assigned by STRUCTURE is two, thus resulting in one homogenous population being labelled as *K*=2, or two different groups. Lack of sub-structuring between the populations/groups was supported by the PCoA. Although F_{ST} values were moderate for the populations defined by river analysis (F_{ST}=0.13), the value was reduced when the ENA method was applied (F_{ST}=0.001), providing support that this value may be overestimated due to the presence of null alleles.

The presence of null alleles may have influenced the overall outcome of the study and could be attributed to the use of primers designed for different species and possible degraded DNA from faecal samples. Null alleles refer to alleles at any given locus that constantly fail to amplify and as such cannot be detected by PCR or subsequent analysis. They usually occur due to mutations in the flanking regions where the primers anneal for amplification, resulting in poor or no amplification at the affected locus.[53] The presence of null alleles does not necessarily impact the outcomes of population genetic analyses – their presence tends not to have significant consequences in analyses that use average probabilities (as opposed to individual parentage analyses), but they may cause overestimation of F_{ST} and genetic distances, as well as underestimation of observed heterozygosity, and may slightly lower the power of assignment tests (such as STRUCTURE).[13,53,54]

The overall low genetic diversity of the Gauteng otter population is possibly linked to the rapid expansion of urbanised areas outward into previously undisturbed environment at an exponential rate due to a human population increase. The rapid expansion would have affected the established riparian habitats scattered throughout the province, driving species outward to less disturbed habitats, or possibly resulting in the extirpation of more sensitive species.[55] The emigration of species from the area would result in more resources becoming available for opportunistic species able to adapt to the novel urban environment, which could lead to conflict over resources with native species remaining in delineated areas.[56-58]

Another explanation for the low genetic diversity could be related to the home range of otters, which can be extensive, ranging from 4.9 km to 54.1 km^2[4], and may be even larger. Coyotes in developed areas have been found to possess home ranges double that of individuals in less developed areas as well as having dens in less developed forested areas,[3] and it is possible that otters in urban areas may also be increasing the size of their home range to improve chances of finding food and mates. *A. capensis* present in Gauteng may have core ranges (areas with increased frequency of activity, usually where refugia are located) outside of the province from which the animals venture into Gauteng to forage. This practice is seen in urban mammals that can navigate and utilise matrix habitats like those seen in urban areas (discussed in Baker and Harris[59]). The lack of holts (otter refuges) observed during surveys is possible evidence of this being the situation with *A. capensis* in Gauteng. A larger breeding population may occur further north along the Crocodile River, which may have undergone range expansion into the Hennops River and subsequent tributaries with headwaters occurring in the city of Johannesburg.[51] This range expansion could explain the lower levels of genetic diversity in the tributary rivers (Jukskei River and Klein Jukskei). The low genetic differentiation between samples from Pienaars River and Crocodile River is interesting as the rivers are a considerable distance apart in Gauteng, but they share a confluence to the north of Gauteng. This may be considered further evidence for a larger breeding population further north along the Crocodile River which has divided and moved into Gauteng. Otter movement does not seem to be hindered by physical barriers as there was no evidence of sub-structuring occurring, relatedness was low, and there was evidence of high gene flow. These results suggest that urbanisation has not led to fragmentation of the population due to disruption of gene flow, which may indicate the otter population in Gauteng is successful (surviving and reproducing viable offspring). However, further sampling must be conducted to confirm that their genetic health is improving.

This analysis represents the first genetic analysis of a South African otter species to date, and additional studies in the future would be required to assess changes in genetic diversity and differentiation. In addition, future studies should be conducted throughout the otters' distribution range. This is imperative to assist in the assessment of the otter population and the effect urbanisation has had on the ecology of the otters. Future studies could provide evidence of a recovering population with good genetic health, which would support the hypothesis that otters can adapt to urbanisation and associated human activity.

Authors' contributions

D.W.P: Research design; fieldwork and sample collection; analysis of samples in laboratory; data analysis; and writing. M.T.M.: Analysis

of samples in laboratory; statistical analysis of samples; writing and reviewing article drafts. U.S.: Research supervision, writing and reviewing article drafts. D.L.D.: Analysis of samples in laboratory; statistical analysis of samples; writing and reviewing article drafts.

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