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Cross-genus amplification and characterisation of microsatellite loci in the little free tailed bat, *Chaerephon pumilus* s. l. (Molossidae) from South Eastern Africa

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Microsatellite loci for *Chaerephon pumilus* sensu lato from south eastern Africa were cross-amplified using primers developed for the Mexican free-tailed bat, *Tadarida brasiliensis*. Two dinucleotide and four tetranucleotide loci were recovered and genotyped for 74 bats, yielding 9 to 15 alleles per locus. The observed and expected heterozygosities were 0.06 to 0.84 and 0.54 to 0.81 respectively, and the PIC values ranged from 0.51 to 0.80, indicative of considerable variability within the sample. There was no evidence of linkage disequilibrium among pairs of loci, or of deviation from Hardy-Weinberg equilibrium. These six loci were informative in studies of population genetic structure of *C. pumilus* sensu lato.

Key words: Bats, *Chaerephon pumilus*, Chiroptera, microsatellites, Molossidae, cross-genus amplification.

INTRODUCTION

Microsatellite markers have become a powerful tool in investigations of population genetic structure, but can be time-consuming and expensive to develop *ab initio*. It is often more viable to develop markers by cross-amplification using primers published for a related species or genus (Wilson et al., 2004; Zhou et al., 2009), although the number of loci which amplify and are polymorphic tend to decrease with increasing divergence between the taxa in question (Moore et al., 1991; Peakall et al., 1998).

The little free-tailed bat, *Chaerephon pumilus* Cretzchmar, 1830-31 (Chiroptera: Molossidae) has a broad distribution across sub-Saharan Africa, extending to the Arabian Peninsula and islands in the Western Indian Ocean (Peterson et al., 1995; Bouchard, 1998; Simmons, 2005). Goodman et al. (2010) showed that the nominate form from Massawa (Eritrea) was genetically distinct from forms bearing this name found elsewhere on the African continent, referred to here as *C. pumilus* sensu lato (s. l.).

Little has been published about the roosting habits and social structure of these nocturnal insectivorous bats. Taylor et al. (2009) reported four mitochondrial clades of *C. pumilus* s. l. in south eastern Africa separated by intra-specific level cytochrome *b* genetic distances of 0.6 to 0.9% (Baker and Bradley, 2006). It has been hypothesised *inter alia* that these clades are the result of social isolation mechanisms such as philopatry, that they arose through introgression created by past hybridization events, and that they represent speciation in progress. In order to further investigate these issues we decided to assess the population genetic structure of this species

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Table 1. Characteristics of six *C. pumilus* s. l. microsatellite loci cross-amplified using primers developed for *T. brasiliensis* (Russell et al., 2005).

Locus	Repeat motif	Genbank accession number	Ta (°C)	Number of alleles	Allele size (nt)	Number of repeats	PIC	Ho	He	Primer sequence (5'-3')
Tabr A10	TAGA TGGA	KC896691	60	9	178-254	8 - 23 3 - 8	0.69	0.51±0.05	0.69±0.03	F:AAGTGGTTGGGCGTTGTC R:GCGATGCACTGCCTTGAGA
Tabr D10	GATA	KC896693	60	13	331-379	2-14	0.80	0.81±0.07	0.81±0.03	F:CCCCACTCATTTATCCATCCACA R:ATCTCGCAGCTATTGAAGTA
Tabr D15	GATA	KC896692	60	10	148-284	4 - 38	0.51	0.06±0.05	0.54±0.14	F:AGTCCTGGCTCCTATTCTCATTG R:CTATCCGTCTACCTGTCCGTCTAT
Tabr E 9	GA	KC896694	60	15	329-365	6 - 24	0.79	0.84±0.07	0.80±0.03	F: GTTTGTCTTCCCCACTGA R: CTTAGGACAGGAGAAGTCA
Tabr H 6	TAGA	KC896695	60	14	139-318	4 - 49	0.61	0.46±0.04	0.64±0.04	F:ATCTCTCCAGTCCTTACCA R:TTTACCCTCCACAGTCTCA
Tabr A30	GA	KC896690	65	9	240-296	5 - 33	0.61	0.78±0.60	0.64±0.04	F:AGTCGCGGGTTTGATTCCAGTTA R:ACCCCTTCCCTTTGTTCCTTCAG

Locus, name of locus; Ta, PCR annealing temperature; nt, nucleotides; PIC, polymorphism information content; Ho, observed heterozygosity; He, expected heterozygosity; F, forward; R , reverse; Tabr, *Tadarida brasiliensis*.

Based on nuclear microsatellite markers. Our approach was to cross-amplify hypervariable microsatellites reported for another molossid genus, the South American free-tailed bat, *Tadarida brasiliensis* (Russell et al., 2005), in order to identify markers which were appropriately variable in our sample of the little free-tailed bat, *C. pumilus* s. l.

This strategy, if successful, was aimed at producing markers useful in the analyses of population genetic structure, kinship and colony structure of populations of this bat in south eastern Africa and possibly also other regions of Africa.

MATERIALS AND METHODS

Analyses were carried out on 74 samples of *C. pumilus* s. l. from South Eastern Africa (Table 2). TheDNeasy® blood and tissue Kit (QIAGEN Inc., QiagenStraße 1,40724 Hilden, Germany) was used to isolate genomic DNA. Polymerase chain reaction (PCR) amplifications were performed in 25 µl volumes containing: 9 µl DNA (3 ngμl^{-1}), 0.8 µl sterile water, 2.5 µl 10 X reaction buffer (Super-Therm), 4 µl 25 mM $MgCl_2$ (Super-Therm), 0.5 µl 10 mM deoxynucleoside-triphosphate mixture (dNTPs) (Fermentas), 0.2 µl *Taq* polymerase (5 u/µl) (Super-Therm) and 4 µl of each primer (6 µM) (forward and reverse) per reaction.

The thermal cycling parameters were: 95°C for 1 min, followed by 39 cycles of 95°C for 30 s, annealing temperature for 30 s, 72°C for 2 min, followed by 72°C for 10 min. The optimal annealing temperature for each primer pair was standardised using gradient PCR (Table 1). The reaction mix comprised 1 µl of PCR product labelled with the dyes 5' 6-FAM or 5' HEX, and 0.5 µl of a ROX500 size standard, brought to 15 µl with Hi-Di Formamide (Applied Biosystems, agents: LifeTechnologies, 200 Smit Street, Fairland, Johannesburg). STRs were genotyped on an ABI 3500 genetic analyzer (Applied Biosystems) at the South African Sugar Research Institute, Mount Edgecombe, South Africa. Raw allelic peak data were analysed using STR and v. 2.2.30 (Locke et al., 2000).

Genalex (Peakall and Smouse, 2006) was used to calculate the observed and expected heterozygosities (Ho and He). The polymorphism information content (PIC) was calculated using a web-based PIC calculator (Kemp, 2002).

Table 2. Details of specimens used in this study.

Field number	Locality in South Eastern Africa	Latitude	Longitud E
C. pumilus			
UWWW1CP1	Umbilo Waste Water	29.846 S	30.890 E
UWWW1CP3	Umbilo Waste Water	29.846 S	30.890 E
UWWW1CP4	Umbilo Waste Water	29.846 S	30.890 E
UWWW1CP5	Umbilo Waste Water	29.846 S	30.890 E
UWWW1CP6	Umbilo Waste Water	29.846 S	30.890 E
URPV1CP1	Paradise Valley	29.831 S	30.892 E
URPV1CP2	Paradise Valley	29.831 S	30.892 E
URPV1CP3	Paradise Valley	29.831 S	30.892 E
URPV1CP4	Paradise Valley	29.831 S	30.892 E
URPV1CP5	Paradise Valley	29.831 S	30.892 E
URPV2CP6	Paradise Valley	29.831 S	30.892 E
URPV2CP7	Paradise Valley	29.831 S	30.892 E
URPV2CP8	Paradise Valley	29.831 S	30.892 E
PNT1	Pinetown	29.828 S	30.866 E
PNT2	Pinetown	29.828 S	30.866 E
PH1	Phinda: Swilles	27.695 S	32.356 E
PH2	Phinda: Swilles	27.695 S	32.356 E
PH3	Phinda: Swilles	27.695 S	32.356 E
PH4	Phinda: Swilles	27.695 S	32.356 E
PH5	Phinda: Swilles	27.695 S	32.356 E
PH6	Phinda: Swilles	27.695 S	32.356 E
PH7	Phinda: Swilles	27.695 S	32.356 E
PH8	Phinda: Swilles	27.695 S	32.356 E
PH9	Phinda: Swilles	27.695 S	32.356 E
PH11	Phinda: Swilles	27.695 S	32.356 E
EH1	Effingham Heights	29.769 S	31.010 E
EH2	Effingham Heights	29.769 S	31.010 E
EH3	Effingham Heights	29.769 S	31.010 E
EH4	Effingham Heights	29.769 S	31.010 E
EH5	Effingham Heights	29.769 S	31.010 E
EH6	Effingham Heights	29.769 S	31.010 E
EH7	Effingham Heights	29.769 S	31.010 E
EH8	Effingham Heights	29.769 S	31.010 E
EH9	Effingham Heights	29.769 S	31.010 E
EH10	Effingham Heights	29.769 S	31.010 E
EH11	Effingham Heights	29.769 S	31.010 E
EH12	Effingham Heights	29.769 S	31.010 E
EH13	Effingham Heights	29.769 S	31.010 E
EH14	Effingham Heights	29.769 S	31.010 E
EH15	Effingham Heights	29.769 S	31.010 E
EH16	Effingham Heights	29.769 S	31.010 E
EH17	Effingham Heights	29.769 S	31.010 E
CH1	Chatsworth	29.930 S	30.925 E
D1	Durban Int. Airport	29.967 S	30.942 E
D2	Hell’s Gate	28.067 S	32.421 E
D4	Hell’s Gate	28.067 S	32.421 E
D5	Hell’s Gate	28.067 S	32.421 E
D6	Hell’s Gate	28.067 S	32.421 E
D7	Hell’s Gate	28.067 S	32.421 E
D8	uMkhuze Game Reserve	27.583 S	32.217 E

Table 2. Contd.

D9	uMkhuze Game Reserve	27.583 S	32.217 E
D10	Amanzimtoti	30.05 S	30.883 E
D11	Amanzimtoti	30.05 S	30.883 E
D12	Morningside	29.833 S	31.00 E
D13	CROW	Unknown	
D14	Hell's Gate	28.067 S	32.421 E
D15	Hell's Gate	28.067 S	32.421 E
D16	CROW rehab	Unknown	
D17	Hell's Gate	28.067 S	32.421 E
D18	Bluff	29.933 S	31.017 E
D19	Ballito	29.533 S	31.217 E
D20	Bluff	29.933 S	31.017 E
D22	Amanzimtoti	30.05 S	30.883 E
D26	Umbilo	29.833 S	31.00 E
D27	Athlone Park	30.016 S	30.917 E
D29	Pinetown	29.817 S	30.85 E
D30	Illovo	30.1 S	30.833 E
D34	Park Rynie	30.317 S	30.733 E
D35	SZ: Mlawula	26.192 S	32.005 E
D36	SZ: Wylesdale	25.819 S	31.292 E
D37	Durban City Hall	29.858 S	31.025 E
D39	Durban	29.867 S	31.00 E
D40	Yellowwood Park	29.917 S	30.933 E
D43	Durban	Unknown	

RESULTS AND DISCUSSION

Three of the nine loci initially tested were discarded, as it was either not possible to amplify them across all samples, or because the banding pattern was too ambiguous to score. The data were checked for errors in scoring due to stuttering, large allele dropout or null alleles using Micro-checker (van Oosterhout et al., 2004). Individuals with missing data at more than two loci were discarded.

All individuals were genotyped for the loci TabrA10, TabrD10, TabrD15, TabrE9, TabrH6 and TabrA30 (Russell et al., 2005). There was no evidence of linkage disequilibrium among pairs of microsatellite loci after standard Bonferroni correction, and none of the 6 loci showed significant deviation from Hardy Weinberg equilibrium ($p>0.05$). The genotyped loci were all polymorphic, yielding 9 to 15 (mean 11.67) alleles per locus. This is considerably lower than the 15 to 55 (mean 36.7) alleles per locus reported by Russell et al. (2005) for the confamilial South American bat, *T. brasiliensis*. A finding of lower levels of polymorphism in microsatellites cross-amplified from another genus is likely to be related to the degree of divergence between the genera in question (Moore et al., 1991; Peakall et al., 1998). The smaller sampling range used in this study may also be reflected in the lower number of alleles recovered; we sampled bats over a north/ south distance of less than 1000 km, whereas Russell et al. (2005) compared bat populations from Texas and Argentina, which are separated by a much greater distance and are therefore more likely to be divergent. Nonetheless, the expected (He) and observed (Ho) heterozygosities over all samples ranged from 0.54 to 0.81 and 0.06 to 0.84, respectively, and the PIC values ranged from 0.51 to 0.80 (Table 1), indicating considerable variability within our sample (Mukesh et al., 2011).

Although it is commonly assumed that microsatellite loci differ among individuals only in the number of units of a single repeat (Guyer and Collins, 1993), many studies have shown that their sequence variation may be more complex (Bull et al., 1999). Five of the cross-amplified *C. pumilus* s. l. loci contained the same repeat motif as *T. brasiliensis*. The repeat motif of locus TabrA10, however, was a tetranucleotide (TAGA) in *C. pumilus* s. l. compared with a diucleotide (GA) in *T. brasiliensis*. We also recovered a short stretch of a second tetranucleotide repeat (TGGA) adjacent to the TAGA repeat at locus TabrA10. Thus, it appears that this locus in *C. pumilus* s. l. may be a compound microsatellite (Weber, 1990) which arose by mutation and replication slippage (Tautz and Schlötterer, 1994) in the period since *C. pumilus* s. l. and *T. brasiliensis* last shared a common ancestor. Analyses of complex microsatellites can underestimate variability, as sequencing has revealed differences between such alleles which are identical in length (Bull et al., 1999).

In conclusion, the six polymorphic microsatellite loci reported here are sufficiently variable to prove useful in analyses of mating and paternity studies, as well as in studies of population genetic structure of *C. pumilus* s. l. from south eastern Africa, and possibly other members of the *C. pumilus* species complex from Africa and the western Indian Ocean region.

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