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Genotyping of bilby scats collected from the Tanami Desert, Northern Territory.

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Background and field sampling

Low Ecological Services provided 17 scat samples to the Department of Biodiversity, Conservation and Attractions for genotyping, all of which were confirmed to be greater bilby (*Macrotis lagotis*) scats (Table 1). Samples were opportunistically collected between 24 - 30 March 2019. Samples were stored dry, at room temperature, in 30ml tubes, approximately 1/3-filled with silica gel beads and cotton wool, until DNA extraction was undertaken.

Laboratory analyses

DNA extractions were undertaken following the protocol in Carpenter and Dziminski (2017). Genomic DNA was extracted from scats using the Qiagen QIAamp Fast DNA Stool Mini Kit with some modifications from Piggott and Taylor (2003) to the recommended procedures included in the kit. DNA was screened using eight highly polymorphic microsatellite markers Table 2. These were multiplexed into two polymerase chain reactions (PCR) using the Qiagen Multiplex PCR Plus Kit. PCR amplification was performed using cycling conditions modified from the Qiagen Multiplex PCR Plus Kit. The PCR product was then analyzed on an ABI3730XL Sequencer, sized using Genescan-500 LIZ internal size standard, and genotyped using Genemapper Software 5.

Genotyping

Allele matching was completed using the R package '*AlleleMatch*' (Galpern *et al.* 2012). Unclassified samples and samples that matched multiple unique genotypes were examined manually and excluded if they could not be matched or classified as new unique genotypes. Any remaining mismatched alleles were flagged and examined manually to determine if they were genotyping errors.

Of the 17 supplied samples, 16 samples yielded DNA and 15 amplified at enough loci to include in identity analysis (Table 3). Genotyping using the eight loci identified two distinct individuals (Table 3).

Recommendations

Since the samples were collected opportunistically across a limited area, abundance analyses were unable to be completed. Maximum likelihood spatially explicit capture-recapture analyses (SECR: Efford 2004) are used to calculate densities and numbers of animals within activity areas or across larger tracts of occupied habitat selected for survey. These analyses require systematically collected samples from within delineated activity areas or a selected defined portion of land that is spatially continuously occupied. Two previous studies (Dziminski *et al.* 2018; Dziminski *et al.* 2019) are attached as examples should abundance need to be sampled in the future.

Sincerely,

Dr Martin Dziminski
Research Scientist

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Table 1. Bilby scat samples provided by Low Ecological Services.

Low Ecol ID	DBCA ID	Latitude	Longitude	Date Collected	Comments
SN01	D0255	-20.533783	130.264067	24/03/2019	
SN02	D0256	-20.53295	130.264017	24/03/2019	
SN04	D0257	-20.5331	130.2635	26/03/2019	
SN05	D0258	-20.532617	130.2646	26/03/2019	
SN06	D0259	-20.5326	130.264183	26/03/2019	
SN07	D0260	-20.533017	130.263033	26/03/2019	
SN08	D0261	-20.5335	130.263417	26/03/2019	
SN09	D0262	-20.53255	130.263733	27/03/2019	
SN10	D0263	-20.53275	130.2634	27/03/2019	
SN11	D0264	-20.530017	130.243283	27/03/2019	
SN12	D0265	-20.532483	130.263367	27/03/2019	
SN13	D0266	-20.537898	130.258857	28/03/2019	
SN20	D0267	-20.538267	130.257613	28/03/2019	
SN21	D0268	-20.535903	130.258671	28/03/2019	
SN22	D0269	-20.536792	130.256401	29/03/2019	
SN23	D0270	-20.536587	130.256251	29/03/2019	
SN24	D0271	-20.533092	130.263377	30/03/2019	
SN01	D0255	-20.533783	130.264067	24/03/2019	
SN02	D0256	-20.53295	130.264017	24/03/2019	
SN04	D0257	-20.5331	130.2635	26/03/2019	
SN05	D0258	-20.532617	130.2646	26/03/2019	
SN06	D0259	-20.5326	130.264183	26/03/2019	

Table 2. Microsatellite markers used in PCR.

Locus	Primer set	Fluorescent label	Reference
Multiplex 1			
B02	BIL02	6-FAM	Moritz et al. (1997)
B17	BIL17intF	VIC	Moritz et al. (1997) and Smith et al. (2009)
B56	BIL56intF	PET	Moritz et al. (1997) and Smith et al. (2009)
B66	BIL66	NED	Moritz et al. (1997)
Multiplex 2			
B55	BIL55	6-FAM	Moritz et al. (1997)
B22	BIL22	VIC	Moritz et al. (1997)
B41	BIL41intF	PET	Moritz et al. (1997) and Smith et al. (2009)
B63	BIL63	NED	Moritz et al. (1997)

Table 3. Individuals identified from scat samples.

Individual	Low Ecol ID	Latitude	Longitude
1	SN02	-20.53295	130.264017
1	SN04	-20.5331	130.2635
1	SN05	-20.532617	130.2646
1	SN06	-20.5326	130.264183
1	SN07	-20.533017	130.263033
1	SN08	-20.5335	130.263417
1	SN09	-20.53255	130.263733
1	SN10	-20.53275	130.2634
1	SN12	-20.532483	130.263367
1	SN13	-20.537898	130.258857
1	SN20	-20.538267	130.257613
1	SN21	-20.535903	130.258671
1	SN22	-20.536792	130.256401
2	SN11	-20.530017	130.243283

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