GOVERNMENT OF WESTERN AUSTRALIA

Department of **Biodiversity, Conservation and Attractions**

Biodiversity and Conservation Science

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Genotyping of bilby scats collected from Roy Hill Mine Site, Pilbara, Western Australia.

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Background and field sampling

In 2018 Roy Hill detected bilby activity at their Pilbara mine site. Roy Hill collected scats on 2 August 2018 and 1 October 2018. More scats were collected 23-24 August 2018 during a site visit and inspection of the bilby activity area by Dr Martin Dziminski. In total 20 haphazardly collected scats were provided (Table 1), all of which were confirmed to be greater bilby (*Macrotis lagotis*). Samples were stored dry, at room temperature, in either 30ml tubes, approximately 1/3-filled with silica gel beads and topped with cotton wool (BIL0663 - BIL0666), or in envelopes (BIL0667 - BIL0673 and BIL0787 - BIL0796), 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

Genotyping using the eight loci identified one distinct individual. The site inspection confirmed the size of the activity area as corresponding to a typical activity area of one bilby.

Relatedness to samples collected nearby

The relatedness of this bilby was compared to two samples collected nearby (Table 3). Pairwise relatedness was compared by calculating the Lynch and Ritland (1999) LR estimator for each pairwise combination. This estimator has a range of -0.5 to 0.5. GenAlEx 6.5 (Peakall and Smouse 2006; Peakall and Smouse 2012) was used for calculations. Two monomorphic loci were removed. The bilby present at the Roy Hill mine site is more closely related to the bilby found at Roy Hill Station than the bilby found at FMG Christmas Creek mine (Table 4).

Recommendations

The bilby present at Roy Hill mine site may stay resident, move away, or more bilbies may move in to the area. This bilby moving into the area is a good example of the propensity of bilbies and bilby populations to move, as well as the generalist nature of bilbies being able to utilize many habitat types, even stony or gravely substrates such as where this bilby is located. During the assessment process no bilbies were found in the area, and the “Likelihood of occurrence and

status if present" was assessed as "Low-Resident: Little suitable habitat in the project area, although a few records exist from the local area" and it was concluded that "it is unlikely that this species will occur at Roy Hill" (Ecologia 2008; Ecologia 2009).

Roy Hill is monitoring bilby burrows with remote cameras and has a plan to conduct surveys prior to any clearance events (eg pipelines). A monitoring program implementing an array standardised 2 ha Sign Plots (Southgate *et al.* 2018) in combination with remote cameras is recommended to monitor the occupancy of bilbies. This would detect any bilbies moving onto the tenement and track the locations of activity of resident bilbies. A similar approach has been implemented by FMG and other bilby surveys nearby (eg Dziminski *et al.* 2018). DBCA can assist with providing advice for such a design. If occupancy increases, or more bilbies move in, then abundance monitoring can be undertaken if required (Dziminski and Carpenter 2017; Dziminski *et al.* 2018; Dziminski and Carpenter 2018).

Should management of the bilby habitat on Roy Hill mine site be undertaken, the following actions should be considered:

1. Fire management:
    a. Establishing and maintaining a suitable firebreak surrounding the managed area to prevent large wildfires destroying vegetation structure and food resources (Wright and Clarke 2007) and allowing easy predator access (McGregor *et al.* 2014; Doherty *et al.* 2015) within managed populations;
    b. Implementing patch mosaic burning to create fire age heterogeneity, increasing habitat and resource diversity for bilbies (Southgate and Carthew 2006; Southgate and Carthew 2007; Southgate *et al.* 2007).
2. Introduced predator management:
    a. Baiting the managed area and surrounding buffer zone with Eradicat® (Algar and Burrows 2004; Algar *et al.* 2013; Doherty and Algar 2015) coupled with supplementary trapping (Molsher 2002; Algar *et al.* 2013) and traditional hunting (Taylor 2015) to control feral cats and foxes. Ground baiting can be used within this area. The area is not included in the Fortescue Marsh Cat Baiting Program (DPaW 2015), however there may be scope to negotiate to have this area included in the annual program.
3. Livestock management (if applicable):
    a. Culling feral livestock (camels, horses, donkeys) within the managed populations;
    b. Negotiating to close or move artificial water points in the vicinity of managed populations in order to reduce localised cattle densities.

Sincerely,

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Table 1. Bilby scat samples from the Roy Hill mine site.

Roy Hill ID	DBCA ID	Latitude	Longitude	Date Collected	Comments
Scat 1	BIL0663	-22.5150782	119.9405091	2/08/2018	
Scat 2	BIL0664	-22.5147249	119.9410748	2/08/2018	
Scat 3	BIL0665	-22.515248	119.9420865	2/08/2018	
Scat 4	BIL0666	-22.515505	119.941849	2/08/2018	
S001	BIL0667	-22.515348	119.941719	23/08/2018	
S002	BIL0668	-22.515356	119.941734	23/08/2018	
S003	BIL0669	-22.515388	119.941719	23/08/2018	
S004	BIL0670	-22.515087	119.940773	23/08/2018	
S005	BIL0671	-22.515247	119.94165	24/08/2018	
S006	BIL0672	-22.515478	119.941002	24/08/2018	
S007	BIL0673	-22.515553	119.940987	24/08/2018	
Sample # 1	BIL0787	-22.5119916	119.9429695	1/10/2018	New bilby burrow; Roy Hill Bilby Burrow 2
Sample # 2	BIL0788	-22.512001	119.9429502	1/10/2018	
Sample # 3	BIL0789	-22.5122713	119.9429754	1/10/2018	
Sample # 4	BIL0790	-22.5120831	119.9429034	1/10/2018	
Sample # 5	BIL0791	-22.5120688	119.9426991	1/10/2018	
Sample # 6	BIL0792	-22.5150297	119.9411881	1/10/2018	Old bilby burrow; Roy Hill Bilby Burrow 1
Sample # 7	BIL0793	-22.5149853	119.9411483	1/10/2018	
Sample # 8	BIL0794	-22.5149699	119.9410023	1/10/2018	
Sample # 9	BIL0795	-22.5151104	119.9407235	1/10/2018	
Sample # 8	BIL0796	-22.5149699	119.9410023	1/10/2018	Darker and smaller clump of pellets compared with others in Sample # 8; processed separately

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)

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Table 3. Bilby DNA samples collected nearby.

DBCA ID	Location	Latitude	Longitude	Date	Description
BIL0552	FMG Christmas Creek mine	-22.40918	119.81821	16/05/2017	Injured male bilby collected by FMG
RH02	Roy Hill Station	-22.80445	120.2526	10/08/2014	Scat collected

Table 4. Pairwise relatedness comparisons.

Sample 1	Sample 2	LRM*
Roy Hill Station bilby	Roy Hill mine bilby	0.122
Roy Hill Station bilby	FMG Christmas Creek mine bilby	-0.447
Roy Hill mine bilby	FMG Christmas Creek mine bilby	-0.421

*Lynch and Ritland (1999) LR estimator.

References

Algar, D., and Burrows, N. D. (2004). Feral cat control research: Western Shield review - February 2003. *Conservation Science Western Australia* **5**, 131–163.

Algar, D., Onus, M., and Hamilton, N. (2013). Feral cat control as part of Rangelands Restoration at Lorna Glen (Matuwa), Western Australia: the first seven years. *Conservation Science Western Australia* **8**, 367–81.

Carpenter, F., and Dziminski, M. A. (2017). Breaking down scats: degradation of DNA from greater bilby (*Macrotis lagotis*) faecal pellets. *Australian Mammalogy* **39**, 197–204.

Doherty, T. S., and Algar, D. (2015). Response of feral cats to a track-based baiting programme using *Eradicat* ® baits. *Ecological Management & Restoration* **16**, 124–130. doi:10.1111/emr.12158

Doherty, T. S., Dickman, C. R., Nimmo, D. G., and Ritchie, E. G. (2015). Multiple threats, or multiplying the threats? Interactions between invasive predators and other ecological disturbances. *Biological Conservation* **190**, 60–68. doi:10.1016/j.biocon.2015.05.013

DPaW (2015). Fortescue Marsh Feral Cat Baiting Program. Year 4 Annual Report. Department of Parks and Wildlife, Western Australia.

Dziminski, M. A., Bettink, K., Carpenter, F., Dickinson, R., MacKenzie, D. I., Shovellor, W., Taylor, B., Kitty, S., Hunter, R., Hunter, I., Smith, J., and Mamid, J. (2018). Greater Bilby Survey: La Grange Project Area. Report. Department of Biodiversity, Conservation and Attractions, Western Australia.

Dziminski, M. A., and Carpenter, F. (2018). The conservation and management of the bilby (*Macrotis lagotis*) in the Pilbara: Annual Report 2018. Annual Report. Department of Biodiversity, Conservation and Attractions, Western Australia.

Dziminski, M. A., and Carpenter, F. (2017). The conservation and management of the bilby (*Macrotis lagotis*) in the Pilbara: Progress Report 2017. Annual Report. Department of Parks and Wildlife, Western Australia.

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Ecologia (2008). Roy Hill Iron Ore Project Proposed Infrastructure Supplementary Level 1 Terrestrial Vertebrate Fauna Survey. Unpublished report. Ecologia Environment, Western Australia.

Ecologia (2009). Roy Hill Iron Ore Project Vertebrate Fauna Assessment. Unpublished report for Hancock Prospecting Limited. Ecologia Environment, Western Australia.

Lynch, M., and Ritland, K. (1999). Estimation of Pairwise Relatedness With Molecular Markers. *Genetics* **152**, 1753.

McGregor, H. W., Legge, S., Jones, M. E., and Johnson, C. N. (2014). Landscape Management of Fire and Grazing Regimes Alters the Fine-Scale Habitat Utilisation by Feral Cats Ed P. Adam. *PLoS ONE* **9**, e109097. doi:10.1371/journal.pone.0109097

Molsher, R. L. (2002). Trapping and demographics of feral cats (Felis catus) in central New South Wales. *Wildlife Research* **28**, 631–36.

Moritz, C., Heideman, A., Geffen, E., and McRae, P. (1997). Genetic population structure of the Greater Bilby *Macrotis lagotis*, a marsupial in decline. *Molecular Ecology* **6**, 925–936. doi:10.1046/j.1365-294X.1997.00268.x

Peakall, R., and Smouse, P. E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**, 288–295. doi:10.1111/j.1471-8286.2005.01155.x

Peakall, R., and Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research--an update. *Bioinformatics* **28**, 2537–2539. doi:10.1093/bioinformatics/bts460

Piggott, M. P., and Taylor, A. C. (2003). Extensive evaluation of faecal preservation and DNA extraction methods in Australian native and introduced species. *Australian Journal of Zoology* **51**, 341-355.

Smith, S., McRae, P., and Hughes, J. (2009). Faecal DNA analysis enables genetic monitoring of the species recovery program for an arid-dwelling marsupial. *Australian Journal of Zoology* **57**, 139–148. doi:10.1071/ZO09035

Southgate, R., and Carthew, S. (2007). Post-fire ephemerals and spinifex-fuelled fires: a decision model for bilby habitat management in the Tanami Desert, Australia. *International Journal of Wildland Fire* **16**, 741–754. doi:10.1071/WF06046

Southgate, R., and Carthew, S. M. (2006). Diet of the bilby (*Macrotis lagotis*) in relation to substrate, fire and rainfall characteristics in the Tanami Desert. *Wildlife Research* **33**, 507–519. doi:10.1071/WR05079

Southgate, R., Dziminski, M. A., Paltridge, R., Schubert, A., and Gaikhorst, G. (2018). Verifying bilby presence and the systematic sampling of wild populations using sign-based protocols – with notes on aerial and ground survey techniques and asserting absence. *Australian Mammalogy*. doi:https://doi.org/10.1071/AM17028

Southgate, R., Paltridge, R., Masters, P., and Carthew, S. (2007). Bilby distribution and fire: a test of alternative models of habitat suitability in the Tanami Desert, Australia. *Ecography* **30**, 759–776. doi:10.1111/j.2007.0906-7590.04956.x

Taylor, P. (2015). Pintubi cat hunters to take skills across Australia. *The Australian*. Available at: http://www.theaustralian.com.au/news/pintubi-cat-hunters-to-take-skills-across-australia/news-story/e80f111d790ac0f47c2134bb6a63b40d [accessed 13 October 2016]

Wright, B. R., and Clarke, P. J. (2007). Resprouting responses of *Acacia* shrubs in the Western Desert of Australia – fire severity, interval and season influence survival. *International Journal of Wildland Fire* **16**, 317. doi:10.1071/WF06094

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