Prospecting for extra-group matings

#### **1** Statistical methods for multivariate analyses

All statistical analyses were conducted using GenStat 5 (Lawes Agricultural Trust, 2 Harpenden, UK). Several analyses required the use of multivariate statistics, for which 3 4 Generalised Linear Mixed Models (GLMMs) were employed. These are similar to Generalised Linear Models (GLMs) except they allow both fixed terms and random terms to 5 be defined. Random terms allow the analysis to take account of repeated measures (Crawley 6 2002). We included both group and individual as random terms in both GLMMs, to control 7 for repeated measures at these levels. Model selection was conducted using reverse stepwise 8 9 elimination of fixed effects (as per Crawley 2002); all terms were initially entered into the model and then sequentially dropped until only terms whose elimination would have 10 significantly reduced the explanatory power of the model remained (thus yielding the 'final 11 model'). All two-way interactions were tested, but only those that were significant are 12 reported. The significance of a fixed effect was determined by dropping it from the final 13 model (if it was part of the final model), or adding it to the final model and then dropping it (if 14 it was not part of the final model). We present model tables for both our GLMMs below. 15

## **Table 1 – Factors affecting the proportion of time a male spent prospecting.**

A full description of the model and its terms is presented in the methods section of the paper. Briefly, the results are from a GLMM with binomial error structure, with days spent prospecting during the month set as the response term and total number of days in the month as the binomial total. The analysis used a sample of 2860 male-months, for 153 subordinate natal males, 51 subordinate immigrant males and 24 dominant immigrant males from our eight best-studied groups. Repeated measures of individuals and groups were controlled by fitting both as random factors.

Full Model	$\chi^2$	d.f.	Р
Age mid-month	331.67	1	< 0.001
Mean Monthly Female Fertility	227.94	1	< 0.001
Dominance and Dispersal Class	198.42	2	< 0.001
(Dom Immigrant, Sub Immigrant, Sub Natal)			
Mean Monthly Temperature	34.86	1	< 0.001
Mean Monthly Rainfall	7.59	1	0.006
Mean Monthly Body Weight <sup>1</sup>	3.21	1	0.073
Final model	Effect	S.E.	
Constant	-1.64	0.17	
Age mid-month	0.0030	0.00017	
Dominance and Dispersal Class	Figure 1a		
(Dom Immigrant, Sub Immigrant, Sub Natal)	in the paper		
Mean Monthly Female Fertility	3.09	0.20	
Mean Monthly Temperature	-0.036	0.0061	
Mean Monthly Rainfall	-0.0034	0.0012	

9

<sup>10</sup> <sup>1</sup>Residuals of body weight on age.

# 1 Table 2 – Do subordinate immigrant males and subordinate natal males differ in their

# 2 likelihood of siring intra- and extra-group young?

A full description of the model and its terms is presented in the methods section of the paper. 3 Briefly, the results are from a GLMM with binomial error structure, with whether or not (1 or 4 0) the male sired any offspring via the reproductive route in question (intra- or extra-group) 5 during his tenure in the dispersal class in question (natal or immigrant) set as the response 6 term, and the binomial total set to 1. The analysis used a sample of 162 tenures (123 natal, 39 7 immigrant) for 141 different males in our eight best-studied groups. Each tenure contributed 8 two data points (one for intra-group and one for extra-group reproduction). Repeated 9 measures of individuals and groups were controlled by fitting both as random factors. 10

Full Model	χ <sup>2</sup>	d.f.	Р
Time monitored in that dispersal class (days)	22.15	1	< 0.001
Dispersal class * Route to reproduction	6.47	1	0.011
Subordinate male dispersal class (natal, immigrant) <sup>1</sup>	8.66	1	0.003
Route to reproduction (intra-group, extra-group) <sup>2</sup>	8.35	1	0.004
Average age of male while monitored	0.63	1	0.63
Final model	Effect	S.E.	
Constant	-1.05	0.53	
Time monitored in that dispersal class (days)	0.0040	0.00084	
Dispersel along * Doute to reproduction	Figure 2b		
Dispersal class · Koule to reproduction	in the paper		

11

<sup>12</sup><sup>1,2</sup>The statistical significances of these two terms were calculated after removing the significant interaction between them from the model. <sup>1</sup> conveys that, on average, subordinate immigrant males were significantly more likely to sire offspring than subordinate natal males.
<sup>2</sup> conveys that, on average, subordinate males were more likely to sire extra-group offspring than within-group offspring. Effect sizes for these individual terms are not reported because the effects for the interaction between them are presented in Figure 2b in the paper.

Prospecting for extra-group matings

#### 1 Genetic sampling, extraction and analysis

Tissue samples were collected by removing a small piece of tail-tip skin from pups on emergence from the breeding burrow (at approximately three weeks of age) or from captured adults (see Young et al. 2005 for capture methods). Samples were stored in DMSO or 100mM EDTA 95% EtOH at -20°C. DNA was extracted using standard chelex or phenol/chloroform methods (Maniatis et al. 1982) and samples were stored in ddH2O at -20°C.

7

Fifteen florolabelled microsatellite markers with 11±4 (mean±SD) alleles were used to assess 8 paternity (AHT130, Fca045, Fca077, Fca232, Hg8.10, M54, Ssu7.1, Ssu8.5, Ssu10.4, 9 Ssu11.12, Ssu12.1, Ssu13.8, Ssu13.9, Ssu14.14, Ssu14.18; see Table 3). To enable 10 multiplexing we changed the annealing sites of markers Ssu7.1, Ssu8.5, Ssu11.12, Ssu12.1, 11 12 and Ssu13.8 (Table 3). Touchdown 10µl PCR multiplexes contained three to four markers, labeled with fluorescent dyes HEX<sup>™</sup>, TET<sup>™</sup> and FAM<sup>™</sup> (PE Applied Biosystems®). All 13 PCR reactions contained 1.5µl Applied Biosystems® Gold Buffer™, 0.8µl 25mM MgCl<sub>2</sub>, 1µl 14 10mM dNTP mix, 0.2µl Tag Gold<sup>™</sup>. The amount of marker varied between markers and 15 multiplexes (from 0.25 to 0.8µl 10mM marker, equivalent to 2.5-8 pmol) and the annealing 16 temperature ranged from 48 to 58°C. Typical cycling conditions were 12min at 95°C, 20 x 17  $(30s@95^{\circ}C, 30s@58^{\circ}C, (-0.1^{\circ}C/cycle), 1min@72^{\circ}C), 15 \times (30s@95^{\circ}C, 30s@56^{\circ}C)$ 18 1min@72°C), 10min@72°C. PCR products were run on a PE Applied Biosystems® ABI 19 377XL<sup>™</sup> using TAMRA<sup>™</sup> 500 as internal size standard and cellulose combs to prevent 20 spillover. Results were extracted and analyzed with Genotyper<sup>™</sup> software. Extracted lanes 21 were compared to the raw gel image to eliminate false dye peaks. 22

Prospecting for extra-group matings

## 1 **Table 3 - Microsatellite statistics**

2 The annealing sites of markers Ssy7.1, Ssu8.5, Ssu11.12, Ssu12.1 and Ssu13.8 were redesigned to allow multiplexing (changes within brackets).

3 After Bonferroni correction, only one marker deviated significantly from Hardy-Weinberg equilibrium, possibly because its low variation (only

4 three alleles) made it susceptible to random effects.

5

Marker	Ref	Sequences 5'-3' Forward/Reverse	# ind	# alleles	Size range	H <sub>OBS</sub>	H <sub>EXP</sub>	р
AHT130	1	CCTCTCCTGGTAAGTGCTGC TGGAACACTGGTCCCCAG	639	17	122-156	0.77	0.73	0.44
Fca 045	2	TGAAGAAAAGAATCAGGCTGTG GTATGAGCATCTCTGTGTTCGTG	270	11	158-178	0.80	0.64	0.08
Fca 077	2	GGCACCTATAACTACCAGTGTGA ATCTCTGGGGAAATAAATTTTGG	677	10	96-114	0.61	0.59	0.47
Fca 232	2	ATGACCATCTCAAACTTCATGG AGCTGAGTTTGCGTTTATCATG	714	12	90-118	0.62	0.64	0.36
HG 8.10	3	AATTCTGAAGCAGCCCAAG GAATTCTTTTCTAGCATAGGTTG	263	5	190-225	0.37	0.39	0.43
M54		CAACATGGAGCCTCCTTGGG TCTAAACCAGACTAGAAAGTG	560	10	120-128	0.74	0.63	0.22
*Ssu 7.1 (+18 bp)	4	ATCCCTTAATGCATAGGCACAC CCTGCTAGTCTTCTCCGTGCG	676	11	158-178	0.77	0.63	0.14
*Ssu 8.5 (+44 bp)	4	GTCAGGTGCTTAACTGACTGG TGGAGTCACTCATTTGGTTTTG	492	17	252-284	0.86	0.74	0.04
Ssu 10.4	4	CTCCAGTTCTTTTCCCTGGAG CATTGGGTGCACACTGTCTCC	674	17	108-140	0.79	0.73	0.35
*Ssu11.12 (-11 bp)	4	CAGGAAATTTTCATCCTGGTAG AGCTTTATTTTTCTCTGTGGCA	689	14	102-128	0.75	0.69	0.40
*Ssu 12.1 (+26 bp)	4	TTGTTTAAGCCACCCAGTCTG TCCTGGTGACACAAACAATGC	674	13	158-182	0.78	0.67	0.17
*Ss 13.8 (+69 bp)	4	GATCAGTGAGAACAGAAGTGC ACCTCCTCCTCCAGATGCATC	407	11	204-228	0.68	0.64	0.48
Ss 13.9	4	TGGAAGTAGGTAGAAGACATTT AGGGATGAGAAGACCACCCTC	200	3	130-134	0.66	0.25	0.00
Ss 14.14	4	GCATTAAACTATAATTTGCTGAG CCCGAGGACAGAGACAAAATG	219	9	111-127	0.80	0.62	0.02
Ssu 14.18	4	GATCACCTAAAATTGCACTACT TCAACCTGCAGGTTTCAGACC	192	8	129-149	0.64	0.57	0.41
Average±SD			490±208	11±4	N/A	0.71±0.12	0.61±0.13	N/A

References 1) (Holmes et al. 1995); 2) (Menotti-Raymond et al. 1999); 3) (Goodman 1997); 4) (Griffin et al. 2001). \*modified annealing sites.

Individual genotypes were compared to those of their relatives (where know from life-history 1 data) in an attempt to isolate genotyping errors. Since mother and siblings can reliably be 2 assigned from observational data (Griffin et al. 2003), it was possible to rectify some of the 3 identified genotyping errors by using simple logical rules based on Mendelian patterns of 4 inheritance. In cases of single repeat errors (due to PCR stage misprint or variations in 5 fragment mobility in gels), inferences from the genotypes of relatives allowed us to directly 6 correct the genotype. Additionally, dropouts or poor amplification may cause false 7 homozygotes. Wherever possible, we corrected such errors by looking at the raw gel to see if 8 peaks just below the threshold had been omitted, or alternatively, from inferences made from 9 the genotypes of known relatives. However, sometimes no correction could be made 10 objectively, despite an obvious error, so these were left unaltered. Duplicate runs showed an 11 overall error rate of about 3%, of which the majority could be corrected. When assigning 12 13 paternity (see below) we therefore assumed an overall error rate in the final genotypes of 2%. The overall amplification success was  $78\% \pm 18\%$ , yielding data for an average of  $9.3 \pm 3.1$  loci 14 15 per individual. Of the 673 pups that emerged in our 15 study groups between 1997 and 2002 inclusive, 499 (74.1%) were successfully sampled and genotyped at >4 loci (median 9; range 16 5-15). Only individuals scored at 5-15 loci were considered in our genetic analyses. 17

18

#### 19 Cervus simulation parameters for EGP prevalence calculations

The Cervus simulation parameters for each litter were set as follows. Number of candidate fathers: the number of known candidates fathers for the litter plus four (to be conservative, by allowing for possible interactions with males from an additional and completely unknown group containing the modal number of candidates). Number of related candidate fathers: five males related at 0.25 (estimated from known candidates). Proportion of candidates sampled: 89.5% of known candidate fathers had been genotyped (at 5-15 loci), but, to be conservative, we calculated a lower value (averaging 75.5%) to reflect the possibility of interactions with
four additional unknown (and hence unsampled) males from outside our study population (see
above). Proportion of loci typed: calculated as an average of 70%. Genotyping error rate: set
at 2% (see above for calculation).

5

#### 6 Cervus simulation parameters for subordinate male reproductive rate calculations

The Cervus simulation parameters were set as described for the previous Cervus run (see above) except that the removal of extra-group dominant males from the candidate files in this case reduced the number of related candidate males from five to four and the modal number of candidates from an unknown group (used in the number of candidate males and proportion unsampled calculations) from four to three.

12

#### 13 **References**

- 14 Crawley, M. J. 2002 Statistical Computing. Chichester, UK: John Wiley & Sons, Ltd.
- Goodman, S. J. 1997 Dinucleotide repeat polymorphisms at seven anonymous microsatellite
   loci cloned from the European Harbour Seal (*Phoca vitulina vitulina*). In *Animal Genetics*, vol. 28, pp. 310-311.
- Griffin, A., Nurmberger, B. & Pemberton, J. M. 2001 A panel of microsatellite markers
   developed for meerkats (*Suricata suricatta*) by cross-species amplification and
   species-specific cloning. In *Molecular Ecology Notes.*, vol. 1, pp. 83-85.
- Griffin, A. S., Pemberton, J. M., Brotherton, P. N. M., McIlrath, G., Gaynor, D., Kansky, R.,
   O'Riain, J. & Clutton-Brock, T. H. 2003 A genetic analysis of breeding success in the
   cooperative meerkat (*Suricata suricatta*). *Behav. Ecol.* 14, 472-480.
- Holmes, N. G., Dickens, H. F., Parker, H. L., Binns, M. M., Mellersh, C. S. & Sampson, J.
  1995 18 Canine Microsatellites. In *Animal Genetics*, vol. 26, pp. 132-133.
- Maniatis, T., Fritsch, E. F. & Sambrook, J. 1982 *Molecular Cloning. A Laboratory Manual.* New York: Cold Spring Harbor Press.