Prospecting for extra-group matings 1

Statistical methods for multivariate analyses

All statistical analyses were conducted using GenStat 5 (Lawes Agricultural Trust, Harpenden, UK). Several analyses required the use of multivariate statistics, for which 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 be defined. Random terms allow the analysis to take account of repeated measures (Crawley 2002). We included both group and individual as random terms in both GLMMs, to control for repeated measures at these levels. Model selection was conducted using reverse stepwise 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 significantly reduced the explanatory power of the model remained (thus yielding the 'final model'). All two-way interactions were tested, but only those that were significant are reported. The significance of a fixed effect was determined by dropping it from the final model (if it was part of the final model), or adding it to the final model and then dropping it (if it was not part of the final model). We present model tables for both our GLMMs below.

1 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.

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

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^{1,2}The statistical significances of these two terms were calculated after removing the significant interaction between them from the model. 1 conveys that, on average, subordinate immigrant males were significantly more likely to sire offspring than subordinate natal males. ² 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.

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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 5 EDTA 95% EtOH at -20°C. DNA was extracted using standard chelex or phenol/chloroform 6 methods (Maniatis et al. 1982) and samples were stored in ddH2O at - 20° C.

8 Fifteen florolabelled microsatellite markers with 11 \pm 4 (mean \pm SD) alleles were used to assess paternity (AHT130, Fca045, Fca077, Fca232, Hg8.10, M54, Ssu7.1, Ssu8.5, Ssu10.4, Ssu11.12, Ssu12.1, Ssu13.8, Ssu13.9, Ssu14.14, Ssu14.18; see Table 3). To enable multiplexing we changed the annealing sites of markers Ssu7.1, Ssu8.5, Ssu11.12, Ssu12.1, and Ssu13.8 (Table 3). Touchdown 10µl PCR multiplexes contained three to four markers, labeled with fluorescent dyes HEX™, TET™ and FAM™ (PE Applied Biosystems®). All 14 PCR reactions contained 1.5µl Applied Biosystems® Gold BufferTM, 0.8µl 25mM MgCl₂, 1µl 10mM dNTP mix, 0.2µl Taq Gold™. The amount of marker varied between markers and multiplexes (from 0.25 to 0.8µl 10mM marker, equivalent to 2.5-8 pmol) and the annealing 17 temperature ranged from 48 to 58° C. Typical cycling conditions were 12min at 95° C, 20 x 18 (30s@95°C, 30s@58°C (-0.1°C/cycle), 1min@72°C), 15 x (30s@95°C, 30s@56°C, 19 1min@72°C), 10min@72°C. PCR products were run on a PE Applied Biosystems® ABI 377XL™ using TAMRA™ 500 as internal size standard and cellulose combs to prevent spillover. Results were extracted and analyzed with Genotyper™ software. Extracted lanes were compared to the raw gel image to eliminate false dye peaks.

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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.

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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 data) in an attempt to isolate genotyping errors. Since mother and siblings can reliably be assigned from observational data (Griffin et al. 2003), it was possible to rectify some of the identified genotyping errors by using simple logical rules based on Mendelian patterns of inheritance. In cases of single repeat errors (due to PCR stage misprint or variations in fragment mobility in gels), inferences from the genotypes of relatives allowed us to directly correct the genotype. Additionally, dropouts or poor amplification may cause false homozygotes. Wherever possible, we corrected such errors by looking at the raw gel to see if peaks just below the threshold had been omitted, or alternatively, from inferences made from the genotypes of known relatives. However, sometimes no correction could be made objectively, despite an obvious error, so these were left unaltered. Duplicate runs showed an overall error rate of about 3%, of which the majority could be corrected. When assigning paternity (see below) we therefore assumed an overall error rate in the final genotypes of 2%. 14 The overall amplification success was $78\% \pm 18\%$, yielding data for an average of 9.3 \pm 3.1 loci 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 5-15). Only individuals scored at 5-15 loci were considered in our genetic analyses.

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).

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.

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