Appendix 3

Quality control in genetic analyses

From the 776 leopard groupers sampled, we selected 500 individuals for DNA extraction and PCR amplification that met our criteria related to the candidate parents/progeny age groups, as explained in the Methods section. From these, we performed strict quality controls, since errors in genotyping and the presence of missing data can strongly bias the results of parentage and sibship analyses (Jones et al., 2010). First, we eliminated those individuals that showed low quality or quantity of genomic DNA, likely because their tissues were not properly fixed in the field and that failed to consistently amplify via PCR, as assessed by agarose gel electrophoresis. Second, we discarded individuals that failed to amplify for at least 11 microsatellite loci, those that showed more than two alleles per locus, or the presence of more than one individual DNA, indicative of DNA contamination, and those individuals for which electropherograms showed noisy or ambiguous results. Our final dataset included 282 leopard grouper individuals genotyped at 13 loci (138 from BK and 144 from PL; see Appendix 4. Figure A4.1 for sampling details and Appendix 5. Table A5.1 for the raw genotype data of each individual), with an average of 1.47% missing data. The 13 genotyped loci were highly variable, averaging 11 alleles per locus, while observed and expected heterozygosity were 0.851 and 0.825, respectively (Appendix 6. Table A6.1). Based on the level of polymorphism, the combined genotypes for the 13 loci provided a probability of individual identity 2.0^{-22}, and a probability of identifying full sibs of 4.3^{-7}. From 130 tests of HWE among the 13 loci within each population, we found only two cases of significant deviations ($P < 0.0003$). We did not find any significant instances of significant LD among 78 tests conducted between each pair of loci (all P values > 0.0006).

References:

Jones, A. G., C. M. Small, K. A. Paczolt, and N. L. Ratterman 2010. A practical guide to methods of parentage analysis. *Molecular Ecology Resources*, 10: 6-30.