JZAR JOURNAL OF ZOO AND AQUARIUM RESEARCH
www.jzar.org

Research article

Using behavioural observations and genetic markers to characterise the flexible reproductive system in an ex-situ population of carmine bee-eaters (*Merops nubicus nubicus*)

Gina M. Ferrie[1,2]*, Tyler Hether[1], Ocean R. Cohen[1], Eran Brusilow[1,2], Christy Sky[2], Shawnlei Breeding[2], Katherine A. Leighty[2], Paul Schutz[2], Chelle Plasse[2], Tammie L. Bettinger[2], and Eric A. Hoffman[1]

[1]Department of Biology, University of Central Florida, Orlando, Florida, USA
[2]Animals, Science and Environment, Disney's Animal Kingdom®, Walt Disney World Resort, Lake Buena Vista, Florida, USA
**Correspondence: Gina Ferrie; Gina.M.Ferrie@disney.com; +1 407-938-2023*

Keywords: behaviour, colony, parentage, population, relatedness, reproduction

Supporting Information
Expanded Methods and Results for Molecular Data Collection and Parentage Analysis

Article history:
Received: 5 Mar 2017
Accepted: 28 Sept 2017
Published online: 31 Oct 2017

Genetic data collection

To isolate species-specific microsatellite loci for genetic analyses, random DNA fragments (~200–2500 bp) were generated using degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR), using the K6-MW primer and protocol (Macas et al. 1996; Degner et al. 2009). Microsatellite enrichment of the PCR-amplified genomic fragments employed a 3'-biotinylated (GATA)8 repeat motif bound to streptavidin-coated particles (Promega Corporation, Madison, Wisconsin, USA) enriched via magnetic separation. Enriched DOP-PCR products were made double-stranded by a subsequent DOP-PCR and cloned using TOPO TA cloning kit (Invitrogen, Carlsbad, California, USA). Clones positive for (CA)n or (GATA)n microsatellites were identified using the screening procedure of Cabe and Marshall (2001). In brief, two PCRs were conducted per colony: one PCR included T3 and T7 primers while the second included the (GATA)8 primer in addition to the T3 and T7 primers. The product of the PCR reactions was visualised on a 2.0% agarose gel and positive clones (those containing microsatellites) were identified by a distinctive smear in the (GATA)8 reaction. Positive clones were then sequenced (Applied Biosystems 3730 DNA Analyzer, Applied Biosystems, Life Technologies Corporation, Carlsbad, California, USA) and PCR primers were developed from flanking regions of DNA surrounding the microsatellite repeats. In total, microsatellite primer sets were developed for eight loci.

PCRs for all loci were performed in 20 µL reactions containing 1 µL of template DNA diluted to 20 ng µL-1, 2 µL of 10X PCR buffer, 1.25 µL of 25 mM of MgCl2, 200 µM of each dNTP, 0.5 µL of 10 µM M13 labeled (Schuelke 2000) forward primer and 1 µL of 10 µM reverse primer, 1 µL of 10 µM fluorescently-labeled M13 primer, 0.2 µL dimethyl sulfoxide, and 1 unit of Taq polymerase. The fluorescently-labeled dyes were ABI DS-30 dye set (6-FAM, HEX, NED; Applied Biosystems, Life Technologies Corporation, Carlsbad, California, USA). PCRs were performed using a BioRad MyCycler thermalcycler (Bio-Rad Laboratories, Hercules, California, USA). The initial denaturing step was 94°C for 4 minutes, followed by 35 cycles of 30 seconds at 94°C, 30–35 seconds at the annealing temperature, and 45 seconds at 72°C, then a final extension cycle at 72°C for 7 min, and a hold at 4°C. Annealing temperatures for all loci are listed in Table 1. PCR products were visualised on a 2% agarose gel before genotyping.

PCR products were sized using an ABI PRISM® 3730 DNA Analyzer (Applied Biosystems, Life Technologies Corporation, Carlsbad, California, USA) in 5 µL multi-plexed reactions at the University of Arizona Genetics Core (Tucson, Arizona, USA). Alleles were sized with respect to size standard ROX (DS-30, Applied Biosystems, Life Technologies Corporation, Carlsbad, California, USA) using the Peak-Scanner Software (v1.0, Applied Biosystems, Life Technologies Corporation, Carlsbad, California, USA). Allele sizes were checked for accuracy by double-genotyping some individuals at each locus.

Parentage and relatedness analysis

Using CERVUS, which uses a pair-wise likelihood comparison based approach to assign parentage, an allele frequency analysis was performed on the eight polymorphic loci discussed above, which were then used to calculate multilocus parental exclusion probabilities (Selvin 1980). Then, a parentage analysis

simulation was run of the parent pair with known sexes, including the a priori probabilities of sampling of candidate parents shown in the main text. This simulation calculates the critical delta of each assignment, which is a derivative of the likelihood score used in parentage analysis and provides a threshold for assigning varying levels of confidence in the parentage analysis (Kalinowski et al. 2007). Finally, a parentage analysis was conducted of the parent pair with known sexes, including a list of candidate sires and dams for each offspring. To be included in analysis, the minimum number of loci typed per individual was four. This analysis assigns the most likely candidate parent pair using the likelihood score, and the predetermined confidence levels of 95% and 80%.

COLONY (v2.0; Jones and Wang 2009) was also used, which implements a full-pedigree maximum likelihood method to assign parentage and sib-ship among individuals with multi-locus genotypes, to run a parentage analysis and examine family relationships. For the COLONY analysis, a polygamous mating system was chosen which assumes no inbreeding and allows for maternal-only and paternal-only sib-ships (half-sibs). A full likelihood analysis was carried out with a long run length, which considers more configurations in the simulated annealing process (Jones and Wang 2009) and no sib-ship prior. The eight microsatellite marker types were input, including an error rate of 0.02 (Wang 2004). The genotypes of the 50 offspring, 11 candidate sires and 10 candidate dams were input as separate files. In both programmes, parentage is assigned with confidence levels of 95% and 80% confidence.

The program Coancestry (Wang 2011) implements seven different methods to estimate pair-wise relatedness between individuals and allows for comparison between estimators using simulated data with predefined relationships. First, an analysis was conducted using simulated genotypes with the observed allele frequencies at each locus, the proportion of genotypes missing at each locus, and the genotyping error rate of 0.02 (Wang 2004) to calculate correlation coefficients between estimated and true simulated relatedness values. One hundred dyads each of six known relationships (parent–offspring, full-sibs, half-sibs, first cousins, second cousins, and unrelated) were simulated with genotyping errors accounted for in the likelihood calculations. Ninety-five percent confidence intervals (CIs) were calculated with 1000 bootstraps. To determine which method of calculating relatedness was most representative of the data, correlations between the relatedness values of simulated and known relationships in JMP Pro 9.0.0 (2010 SAS Institute, Cary, North Carolina, USA) were tested. To obtain relatedness estimates between individuals in the colony, an analysis of the empirical data was conducted with observed allele frequencies. One hundred reference individuals used in the triadic likelihood methods and 1000 bootstrapping samples were included to calculate 95% CIs. Finally, potential error in genotyping was accounted for with the error rate of 0.02 at all loci (Wang 2004).

Based on the comparisons in Coancestry between simulated genotypes and relatedness values between known relationships, the TrioML method of calculating relatedness (Wang 2007) yielded the highest correlation between genetic (simulated) and true estimates ($r = 0.76$, $p < 0.001$) and also had the lowest variance (0.03) of the seven methods. When the dataset contains relatively unrelated individuals, the triadic likelihood method of calculating relatedness best accounts for genotype error in the data (Wang 2007) and gives the most prudent method of inferring relationships between pairs of individuals (Doutrelant et al. 2011).

Results

See table

References

Cabe P.R., Marshall K.E. (2001) Microsatellite loci from the house wren (Troglodytes aedon). Molecular Ecology Notes 1: 155–156.

Degner J.F., Hether T.D., Hoffman E.A. (2009) Eight novel tetranucleotide and five cross-species dinucleotide microsatellite loci for the ornate chorus frog (Pseudacris ornata). Molecular Ecology Resources 9: 622–624.

Doutrelant C., Dalecky A., Covas, R. (2011) Age and relatedness have an interactive effect on the feeding behavior of helpers in cooperatively breeding sociable weavers. Behaviour 148: 1399–1417.

Macas J., Gualberti G., Nouzova M., Samec P., Lucretti S., Dolezel J. (1996) Construction of chromosome-specific DNA libraries covering the whole genome of field bean (Vicia faba L). Chromosome Research 4: 531–539.

Schuelke M. (2000) An economic method for the fluorescent labeling of PCR fragments: A poor man's approach to genotyping for research and high-throughput diagnostics. National Biotechnology 18: 233–234.

Selvin S. (1980) Probability of nonpaternity determined by multiple allele codominant systems. American Journal of Human Genetics 32: 276–278.

Wang J. (2004) Sibship reconstruction from genetic data with typing errors. Genetics 166: 1963–1979.

Wang J. (2007) Triadic IBD coefficients and applications to estimating pairwise relatedness. Genetic Research 89: 135–153.

Wang J. (2011) Coancestry: a program for simulating, estimating and analyzing relatedness and inbreeding coefficients. Molecular Ecology Resources 11: 141–145.