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Patterns of Paternity in Clutches of Giant Amazon River Turtles, *Podocnemis expansa*, from Venezuela

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In recent years, molecular genetic data have shown that multiple paternity occurs in groups of offspring in many taxa. Though multiple paternity has been found in a wide variety of turtles (Pearse and Avise 2001), limited clutch size and/or small number of total clutches assayed has limited the evaluation of the frequency of multiple paternity within the population or the relative contributions of the respective sires. I have analyzed data from 32 clutches of the giant river turtle *Podocnemis expansa* from the Orinoco River in Venezuela and present here results on 29 of these clutches. Large clutch sizes of this turtle permit accurate assessment of multiple paternity, as well as skew in paternity within multiply-sired clutches(25 clutches, mean sample size=21.3, range, 10-25; 4 clutches mean sample size=72.25, range, 70-76). Results from this study add to the growing body of knowledge on turtle mating systems, and extend previous paternity research on this species (Valenzuela 2000).

Blood samples were obtained from 886 hatchlings from 32 clutches. All hatchlings were collected from nests from Isla Playlita del Refugio de la Tortuga Arrau, el medio Orinoco. DNA was extracted from the hatchling blood tissue, checked for quality, and used to amplify 7 microsatellite loci. (Sites et. al. 1999; Valenzuela 2000). PCR products were then analyzed using ABI 3100 and 3700 genotypers. These 7 loci are highly variable, and provide a combined exclusion probability of 0.9499. Clutches were individually evaluated to assess multiple paternity. Because DNA samples were not available from the mothers, parental inferences were made in the following manner:

First, all clutches were evaluated to ensure single maternity--indicated by the presence of one of two alleles in each individual at each locus. In many cases, the maternal genotype at some or all loci could be deduced from the segregation offspring genotypes and/or the presence of homozygous offspring.

Second, clutches were evaluated for evidence of multiple paternity. Each offspring was assumed to receive one maternal and one paternal allele, with the maternal allele randomly coming from one of her two alleles and the paternal allele from a variably sized pool of alleles (depending on the number of contributing males). A positive multiple paternity call was made when two or more loci showed evidence of five or more alleles. Homozygote scores indicate the mother and at least one candidate father both possess an allele; thus, these calls are considered as contributing two alleles. Five alleles at only one locus was not considered sufficient proof of multiple paternity, as the extra allele could be explained by the presence of a mutation.

Finally, in both maternity assessment, and when designating a clutch as singly sired, segregation of alleles was evaluated for Mendelian inheritance ratios. In singly sired clutches, both parental genotypes could be reconstructed, although the gender associated with each genotype could not be determined. In multiply-sired clutches, the maternal and paternal genotypes could both be determined, and hatchlings were assigned to one of the inferred fathers.

Results

- 3 of 29 (10.3%) of assayed and analyzed clutches exhibited multiple paternity. Two clutches show clear evidence of two sires; the third clutch was sired by a minimum of 2 males, and shows evidence of a third contributing father.
- Of the clutches exhibiting multiple paternity, paternity was highly skewed in one (4:67), and more evenly distributed in the other two (22:40 and 8:12).
- 9 alleles were assigned as mutations. In 6 of these instances, the mutated allele represented a fifth parental allele at a single locus. 9 mutations in 12,404 scores (886 individuals x 7 loci x 2 alleles) equals one mutation for every 1378 meiotic events, a relatively low rate for microsatellite loci.

Valenzuela (2000) found evidence of a high incidence of multiple paternity (100%) in two clutches of *Podocnemis expansa* from Colombia. This contrasts strongly with my results, which show that in the Orinoco population, multiple paternity frequency is much lower (10.3%). This difference is striking, and may provide evidence of different mating patterns in the two populations.

The ratio of offspring between sires in clutches 22 and 34 is consistent with expectations of multiple matings within the same season, whereas clutch 40 shows a pattern consistent with the sperm storage hypothesis (Pearse et al, 2002). However, the low frequency of multiple paternity in the present study does not provide enough data to allow for significant contribution to these topics.

A low frequency of multiple paternity, similar to that found in the Venezuelan population of *P. expansa*, has also been found in green turtles (Fitzsimmons 1998). However, the contrasting findings of Valenzuela (2000) suggest that the endangered status of the Venezuelan population may be affecting the mating system. This population has suffered large reductions in population size and has very low genetic diversity relative to other *P. expansa* populations (Pearse et al. in prep). Thus, in addition to ongoing efforts, specific genetic conservation measures may be needed to aid in its recovery.

Literature cited

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