Genetic Population Structure of Two Threatened South American River Turtle Species, *Podocnemis expansa* and *Podocnemis unifilis*

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ABSTRACT. – An electrophoretic analysis of *Podocnemis expansa* from Peru and Brazil revealed significant allele frequency differences among populations at four of five polymorphic loci. One locus also demonstrated significant allele frequency differences among individuals between Brazilian nesting beaches separated by only 80 km. Electrophoretic analysis of *P. unifilis* from Peru and Brazil, as well as from three nesting sites along the Caquetá River in Colombia exhibited higher levels of genetic variability than *P. expansa*, differing significantly in allele frequencies at eight of nine polymorphic loci. The three Colombian sub-populations differed as much genetically from each other as they did from Peruvian and Brazilian populations. The results argue that current conservation management practices, such as protecting only a small number of nesting beaches within a reserve, may be inadequate, and that transplanting juveniles from their natal beaches may be inappropriate.

KEY WORDS. – Reptilia; Testudines; Pelomedusidae; Podocneminae; *Podocnemis expansa*; *Podocnemis unifilis*; turtle; genetics; population structure; geographic variation; conservation; management; Brazil; Colombia; Peru

River turtles of the genus *Podocnemis* (family Pelomedusidae) have been a traditional food source for the inhabitants of much of lowland South America for centuries (von Humboldt, 1859; Bates, 1863; Coutinho, 1868). For most species, ample habitat remains, but the three largest species in genus (*P. expansa*, *P. unifilis*, and *P. lewyana*) are currently threatened by over-exploitation (Mittermeier, 1978; Smith, 1979; Johns, 1987; IUCN, 1989). *Podocnemis expansa* is particularly vulnerable to human hunting pressures due to its aggregated nesting behavior (Mosquera, 1945; Ramirez, 1956; Roze, 1964; Vanzolini, 1967; Ojasti, 1971; Alho and Padua, 1982a, b; von Hildebrand et al., 1988). The second largest species, *P. unifilis*, is sympatric with *P. expansa* throughout most of the Amazon and Orinoco drainages (Iversen, 1992), and becomes preferentially exploited when *P. expansa* is extirpated.

Information on the population structure of these two species is limited. Anecdotal evidence and some mark-recapture data suggest that female *P. expansa* in some populations may migrate hundreds of km each year between feeding ranges and nesting beaches (Roze, 1964; Ojasti, 1967; von Hildebrand et al., 1997). *Podocnemis unifilis* has less stringent nesting requirements than *P. expansa* (Foote, 1978; Fachin, 1992; Thorbjarnarson et al., 1993; Soini and Soini, 1995; Thorbjarnarson and Da Silveira, 1996) and radiotelemetry data from one population of *P. unifilis* (Bock et al., 1998) suggest that nesting females are less vagile.

In recent years, numerous management projects have been established for these two species in different areas (Brazil: Alho et al., 1979; Cantarelli and Herde, 1989; Cantarelli, 1997; Venezuela: Licata and Elguezabal, 1997; Thorbjarnarson et al., 1997; Peru: Soini and Coppula, 1995; Soini, 1996; Bolivia: Guayao, 1997; Colombia: Martinez and Muñoz, 1997; Páez and Bock, 1997). Most projects focus on guarding “key” nesting beaches and/or transferring nests to protect eggs from flooding and predation. In some projects, hatching turtles are released immediately at the site of collection of the eggs, while other projects hold hatchlings for weeks or months before release. Several projects routinely transfer hatchlings considerable distances from their natal beaches for release in putatively suitable juvenile habitat.

The advisability of these management procedures depends to a great extent on the demographic and genetic structure of the populations being managed. What may be appropriate for one species or population may not work for another. Sites et al. (1999) recently compared three *P. expansa* populations in Brazil using microsatellite and mtDNA markers. In this study, we used protein electrophoresis to characterize the genetic population structure of *P. expansa* and *P. unifilis* populations from Brazil, Colombia, and Peru, thereby providing additional information on the population genetics of these highly exploited species.

METHODS

Recently hatched turtles were collected from three regions of South America (Fig. 1). Sample sizes and locations were the result of compromises between biological and statistical design considerations on the one hand and logistic feasibility and permit limitations on the other.

In Peru, 21 hatching *P. expansa* were obtained randomly from a large pool of individuals from artificially incubated nests at the Cahuana Biological Station in the
Hatchlings were sacrificed to obtain muscle, liver, and blood samples. Most blood samples were separated into plasma and red blood cell fractions, and all samples were frozen in liquid nitrogen, transported on dry ice, and stored at -80°C at Ohio University. Samples were analyzed using standard horizontal starch gel electrophoresis (Murphy et al., 1996). The products of 23 presumptive structural loci were resolved, of which the following proved polymorphic in one or both species: Aconitate hydratase (ACOH, E.C. 4.2.1.3), Aspartate aminotransferase (AAT, E.C. 2.6.1.1), Esterase (EST), Fructose biphosphatase (FBP, E.C. 3.1.3.11), Fumarate hydratase (FUMH, E.C. 4.2.1.2), and Glucose-6-phosphate isomerase (GPI, E.C. 5.3.1.9). Peptidase-B (PEP-B, E.C. 3.4.11.13), Phosphoglucone dehydrogenase (PGDH, E.C. 1.1.1.44), and Purine-nucleoside phosphorylase (PNP, E.C. 2.4.2.1). Allozyme data were analyzed using BIOSYS I (Swofford and Selandor, 1981). Tests for deviations from Hardy-Weinberg genotype proportion expectations were conducted so as to achieve an experiment-wide significance level of p < 0.05 by adjusting the acceptance criterion level of tests at each locus using the sequential Bonferroni technique (Holm, 1979). Geographic and genetic distances between the sites were compared with a Mantel test (Mantel, 1967).

**RESULTS**

Five loci were polymorphic in *P. expansa* (0.95 common allele criterion). None of the 12 comparisons exhibited significant departures from Hardy-Weinberg expectations. Significant allele frequency heterogeneity was observed among the three populations at four loci (Table 1). Significant allele frequency differences were observed between the two Brazil sites at one locus (FBP, $\chi^2 = 9.15$, d.f. = 1, $p < 0.05$).

![Figure 1](image.png)

*Figure 1.* Location of the three geographic regions in South America from which juvenile *Podocnemis expansa* and *P. unifilis* individuals were collected. 1 = Río Caquetá, Colombia; 2 = Pacaya-Samiria reserve, Peru; 3 = Río Araguaiá, Brazil.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Peru</th>
<th>Brazil</th>
<th>$\chi^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pacaya River</td>
<td>Araguaia North</td>
<td>Araguaia South</td>
<td></td>
</tr>
<tr>
<td>ACOH</td>
<td>0.361</td>
<td>0.659</td>
<td>0.583</td>
<td>7.42</td>
</tr>
<tr>
<td></td>
<td>0.639</td>
<td>0.341</td>
<td>0.417</td>
<td></td>
</tr>
<tr>
<td>EST</td>
<td>0.789</td>
<td>1.000</td>
<td>1.000</td>
<td>22.35</td>
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<tr>
<td></td>
<td>0.211</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>FBP</td>
<td>1.000</td>
<td>0.464</td>
<td>0.741</td>
<td>32.89</td>
</tr>
<tr>
<td></td>
<td>0.000</td>
<td>0.536</td>
<td>0.259</td>
<td></td>
</tr>
<tr>
<td>PEP-B</td>
<td>0.952</td>
<td>0.881</td>
<td>0.817</td>
<td>4.18</td>
</tr>
<tr>
<td></td>
<td>0.048</td>
<td>0.119</td>
<td>0.183</td>
<td></td>
</tr>
<tr>
<td>PGDH</td>
<td>0.150</td>
<td>0.433</td>
<td>0.536</td>
<td>12.24</td>
</tr>
<tr>
<td></td>
<td>0.850</td>
<td>0.567</td>
<td>0.464</td>
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<tr>
<td></td>
<td>20.7</td>
<td>20.6</td>
<td>28.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.043</td>
<td>0.072</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>$n$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.7</td>
<td>20.6</td>
<td>28.6</td>
<td></td>
</tr>
<tr>
<td>$\chi^2$</td>
<td>79.08</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Unbiased Nei (1978) genetic distances between the three Podocnemis expansa populations sampled in Peru and Brazil (above the diagonal asterisks) and direct line geographic distances (km) between the sites (below the diagonal).

<table>
<thead>
<tr>
<th></th>
<th>Peru: Pacaya River</th>
<th>Brazil: Araguaia North</th>
<th>Brazil: Araguaia South</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peru: Pacaya River</td>
<td><strong>0.022</strong></td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>Brazil: Araguaia North</td>
<td>2600</td>
<td><strong>0.003</strong></td>
<td></td>
</tr>
<tr>
<td>Brazil: Araguaia South</td>
<td>2600</td>
<td>80</td>
<td><strong>0.003</strong></td>
</tr>
</tbody>
</table>

0.005). There was no significant association between the magnitudes of the geographic and genetic distances among sites (Mantel, r = 0.88, p > 0.10; Table 2).

For P. unifilis, only one of the 21 comparisons for the nine polymorphic loci exhibited a significant departure from Hardy-Weinberg expectations (a deficiency of heterozygote individuals at the PNP locus in Brazil). Significant interpopulation heterogeneity was detected among populations at eight loci (Table 3). A UPGMA cluster analysis of Nei (1978) genetic distances revealed a complex picture of differentiation among populations for P. unifilis (Fig. 2). Genetic variation was not statistically significantly related to geographic distances among the sites (Mantel, r = -0.003, p > 0.10; Table 4); as one Colombian population grouped with the Brazilian population and the remaining two Colombian populations grouped with the Peruvian population. However, the statistical power of both this and the P. expansa analyses were low. Sampling additional sites might well permit detection of significant geographic patterns in genetic structure for one or both species.

**DISCUSSION**

Despite evidence suggesting that P. expansa is a long-distance disperser (Roze, 1964; Ojasti, 1967; von Hildebrand et al., 1997), our results demonstrated significant regional genetic differences, as did the study by Sites et al. (1999). The suggestion of genetic differences among hatchlings obtained from nesting beaches some 80 km apart also implies either that adults in some populations may not migrate great distances, or that adults may wander extensively but return to breed at their natal beaches, as observed in sea turtles (Meylan et al., 1990; Bowen et al., 1992, 1993; Broderick et al., 1994; Allard et al., 1994; Bass et al., 1996; Encalada et al., 1996). Sites et al. (1999) also encountered significant genetic differences among three sites in the Araguaia River, Brazil, at one of the six microsatellite loci they examined.

In P. unifilis, inter-population differentiation was even more pronounced. Demes in Colombia separated by as little as 60 km exhibited genetic differences as great as those seen between populations separated by hundreds of km. In contrast to P. expansa, P. unifilis is less selective both in terms of its diet (Almeida et al., 1986; Fachin et al., 1995) and nesting microhabitat (Foote, 1978; Fachin, 1992; Thorbjarnarson et al., 1993; Soini and Soini, 1995; Thorbjarnarson and Da Silveira, 1996). This species may be able to satisfy both needs within a much more restricted area. A preliminary radiotelemetry study of adult P. unifilis demonstrated that females dispersed little after nesting, until they moved into the varzea forests with the annual floods. One female relocated the following year when the river returned to its banks was first encountered almost exactly where she had disappeared into the forest nine months before (Bock et al., 1998). This evidence seems to suggest that, despite its wide geographic range, most P. unifilis populations may be comprised of multiple reproductively isolated demes, even in the absence of obvious dispersal barriers.

The levels of allozyme variability maintained by these populations of Podocnemis (proportion of polymorphic loci [P] varying from 0.17 to 0.30 and mean proportion of loci heterozygous per individual [H] varying from 0.043 to 0.075) were lower than those documented for populations of other freshwater turtles. Although this result could have been an artifact of possibly including siblings in the population samples, the concordance of observed genotype frequencies to those expected under Hardy-Weinberg equilibrium in both species, and for P. unifilis the similarity of the
estimates of levels of genetic variability for the Brazilian, Peruvian, and Colombian populations (with the latter population sample comprised entirely on non-siblings), argues that these estimates are valid.

The low levels of genetic variability these populations express may be a reflection of their localized population structure, the exploitation they have faced in recent years, or both. The Peruvian population of *P. expansa* exhibited the lowest level of mean heterozygosity (H = 0.043) yet documented for a freshwater turtle population. This population has been estimated to contain only approximately 600 reproductive females (Soini, 1996) and has been maintained in recent years exclusively through artificial incubation of nests rescued from human depredation (Soini, 1995). However, more data on levels of genetic variation in other populations of this and other freshwater turtle species will be needed before the significance of these low levels of genetic variability may be confidently assessed.

Surprisingly few studies on allozyme variation in freshwater turtle populations have been conducted during the past three decades, in marked contrast to the situation for other vertebrate groups. This is especially true considering the attention turtle biologists have given to intraspecific variation in life history characteristics. However, evidence for marked population structuring similar to our findings has been demonstrated with allozyme data for *Chrysemys picta* (Scribner et al., 1993) and *Trachemys scripta* (Scribner et al., 1984, 1986; Smith and Scribner, 1990). More recently, analyses of mtDNA restriction site and sequence data for freshwater turtles in the southeastern USA (Walker et al., 1995, 1997; Walker and Avise, 1998) also revealed extensive intraspecific geographic variation with strong local population structure. Thus, pronounced genetic differentiation among populations may be more typical of freshwater turtles than is generally assumed.

Until more information is available on the genetic population structure of freshwater turtle species, it would seem prudent for *Podocnemis* turtle management projects to refrain from releasing hatchlings long distances from their natal beaches. In addition, projects attempting to manage *P. expansa* should be aware that holding hatchling turtles in captivity in attempts to prevent early juvenile mortality, they may also be disrupting key behavioral processes necessary for the development of typical migratory behavior at the time of first reproduction. Finally, *Podocnemis* management projects that focus on only one or a few nesting beaches in a region may not be providing sufficient protection to the overall local genetic diversity present in the regional population.

**Table 4.** Unbiased Nei (1978) genetic distances between the five *Podocnemis unifilis* populations sampled in Peru, Brazil, and Colombia (above the diagonal asterisks) and direct line geographic distances (km) between the sites (below the diagonal).

<table>
<thead>
<tr>
<th>Peru: Yanayacu River</th>
<th>Brazil: Araguaia North</th>
<th>Colombia: Bernardo beach</th>
<th>Colombia: Cahuinari beach</th>
<th>Peru: Tres Islas beach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yanayacu</td>
<td>0.046</td>
<td>0.028</td>
<td>0.023</td>
<td>0.040</td>
</tr>
<tr>
<td>Araguaia</td>
<td>0.046</td>
<td>0.037</td>
<td>0.022</td>
<td>0.019</td>
</tr>
<tr>
<td>Bernardo beach</td>
<td>0.037</td>
<td>0.040</td>
<td>0.019</td>
<td>0.036</td>
</tr>
<tr>
<td>Cahuinari beach</td>
<td>0.028</td>
<td>0.022</td>
<td>0.019</td>
<td>0.022</td>
</tr>
<tr>
<td>Tres Islas beach</td>
<td>0.023</td>
<td>0.040</td>
<td>0.036</td>
<td>0.022</td>
</tr>
<tr>
<td>Colombia: Tres Islas beach</td>
<td>0.036</td>
<td>0.019</td>
<td>0.036</td>
<td>0.022</td>
</tr>
</tbody>
</table>

**RESUMEN**

Análisis electroforéticos de *P. expansa* de Brasil y Perú revelaron diferencias significativas en las frecuencias alélicas entre las poblaciones, para cuatro de cinco loci polimórficos. También demostramos diferencias significativas en las frecuencias alélicas de un locus entre individuos obtenidos...
en diferentes playas de anidación en Brasil separadas por tan solo 80 km. Análisis electroforéticos de *P. unifilis* de Brasil, Perú, y de tres sitios de anidación a lo largo del río Caquetá en Colombia presentaron mayores niveles de variabilidad genética que *P. expansa*, difiriendo significativamente en las frecuencias alélicas de ocho de los nueve alelos polimórficos. Las tres subpoblaciones colombianas se diferencian en la misma magnitud unas de otras genéticamente, como lo hacen con respecto a las poblaciones de Perú y Brasil. Estos resultados indican que las prácticas de manejo conservacionista actuales, en las que se protegen únicamente algunas pocas playas de anidación dentro de las reservas, pueden estar siendo inadecuadas, y el transplante de juveniles desde sus playas natales a otros sitios podría ser inapropiado.

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