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Nicole Valenzuela

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## GENETIC DIFFERENTIATION AMONG NESTING BEACHES IN THE HIGHLY MIGRATORY GIANT RIVER TURTLE (*PODOCNEMIS EXPANSA*) FROM COLOMBIA

NICOLE VALENZUELA<sup>1</sup>

Department of Ecology and Evolution, State University of New York at Stony Brook,  
Stony Brook, NY 11794, USA

and

Fundación Puerto Rastrojo, Carrera 10 No. 24-76 Of 1201, Santafé de Bogotá D.C., Colombia

**ABSTRACT:** I conducted a study of population subdivision and genetic diversity among populations of the giant river turtle *Podocnemis expansa* using microsatellite DNA markers. Turtles were sampled from four nesting beaches distributed within 100 km of one another along the Middle Caquetá River in Colombian Amazonia. I detected significant genetic differentiation within the Caquetá basin with Weir and Cockerham's  $F_{st}$  estimator,  $\theta$ , but not with rho-st estimator of Slatkin's  $R_{st}$ . I also detected differences in allele and genotype frequencies using probability and exact tests. Although significant, population subdivision appears subtle enough to pass undetected by  $R_{st}$  with the sample sizes available. This is presumably due to the relatively higher variance of  $R_{st}$ . The differentiation found among beaches within the Caquetá River is surprising given that individuals are able to migrate more than 400 km between nesting seasons, and females come to nest at the Caquetá River from common tributaries. Potential explanations of the subdivision found are the segregation of females into nesting family groups (including natal homing) or of males into mating family groups. The genetic subdivision has some geographic component such that the three beaches closest genetically are also the closest in distance along the river. I detected strong differentiation between Colombian and Brazilian populations using  $R_{st}$ , which I discuss in light of the available information about the biology of *P. expansa*. I also discuss the implications of these results to conservation issues of this endangered species.

**Key words:** Turtles; *Podocnemis expansa*; Population structure; Microsatellite; Amazonia; Endangered species

THE giant river turtle of the Amazon basin (*Podocnemis expansa*) constitutes an important economic and cultural resource for the indigenous peoples inhabiting the Middle Caquetá River in Colombian Amazonia. This highly migratory, colonial nesting species is used as food by the local inhabitants, both aborigines and non-indigenous settlers. The pressure imposed on the turtle populations due to harvesting has resulted in the placement of *P. expansa* on the United States Endangered Species List, and it is listed in the Appendix 2 of CITES. No study has been conducted to date describing the basic genetic parameters of Colombian populations, despite the implications of such information for conservation issues. It is particularly important to compare populations from Colombia to the larger ones existing in Brazil, to deter-

mine whether the reduction of this turtle in the study area has affected genetic variability. It is also important to determine the degree of substructuring that may exist within Colombia in order to identify distinct evolutionary or management units that might require individual conservation efforts. The present study represents the first such endeavor. Levels of genetic diversity within several nesting beaches along the Caquetá River and population subdivision across sampling sites were estimated from microsatellite data.

Microsatellites are repeated sequences of 2–4 oligonucleotides arranged in tandem, highly variable in the number of repeats, and flanked by more conserved sequences (Avisé, 1994). Discrete genotypes of these codominant markers are readily observed, allowing the estimation of number of alleles and levels of heterozygosity. Because some microsatellite loci can be highly variable, they have proved useful for studying population structure in spe-

<sup>1</sup> PRESENT ADDRESS: Department of Zoology and Genetics, Iowa State University, Ames, IA 50011, USA.

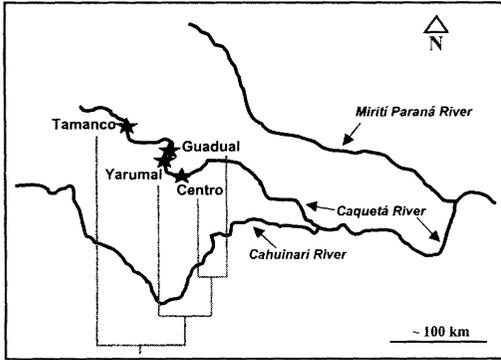


FIG. 1.—UPGMA dendrogram of Nei's (1978) genetic distances among nesting beaches.

cies whose genetic variability has been depleted to undetectable levels with other methods, or among populations which have shared a common ancestor or experienced gene flow in the recent past. For example, allozyme loci or the mitochondrial DNA genome (a single locus) may segregate few or no alternate alleles in small populations of endangered species, and yet microsatellite loci are often highly polymorphic in these same small populations (e.g., Allen et al., 1995; Amos et al., 1993; Paetkau and Strobeck, 1994; Pope et al., 1996).

#### MATERIALS AND METHODS

Samples came from 12–15 individuals from each of four nesting beaches (Fig. 1) along the Middle Caquetá River in Colombia ( $0^{\circ} 50.5' - 1^{\circ} 15' S$ ,  $71^{\circ} 30' - 71^{\circ} 48.7' W$ ). Each turtle was taken from a different clutch. I took samples of 0.5 cc of blood from the cervical sinus (Valenzuela et al., 1997) and preserved them in "Queen's" lysis buffer (Seutin et al., 1991), and I obtained DNA by standard phenol-chloroform extraction (Sambrook et al., 1989). I developed a DNA library and characterized three microsatellite loci (PE344, PE519, and PE1075) as described in Valenzuela (2000). I complemented these three loci with five additional microsatellite primers designed for *P. expansa* in Brazil [Pod1, Pod62, Pod79, Pod128, and Pod147 from Sites et al. (1999)], such that a total of eight variable loci were analyzed in the present

study. I screened individual genotypes using an ABI-Prism<sup>®</sup> 373 automatic sequencer (ABI) and ABI GeneScan<sup>®</sup> software (ABI).

I conducted several analyses for each nesting beach and across all sampling sites. These included assessment of allele frequencies and gene diversity, tests for conformity to Hardy-Weinberg equilibrium at each locus and across loci, and tests for linkage disequilibrium for each pair of loci. Tests for population subdivision include analyses of genic and genotypic differentiation for all pairs of beaches and across beaches, as well as for each locus separately and across loci. I carried out these analyses using GENEPOP 3.1c [Raymond and Rousset 1995a,b, ([www.cefe.cnrs-mop.fr](http://www.cefe.cnrs-mop.fr))], and FSTAT 2.7 (Goudet, 1995). I conducted probability tests as well as exact tests by the algorithms included in these programs. I calculated the frequency of potential null alleles for those loci that showed heterozygote deficiency according to the method of Brookfield (1996). I used formula 4 of Brookfield (1996) for this calculation, given that all individuals screened amplified one or two alleles per locus.

I calculated Weir and Cockerham's (1984)  $\theta$ , and rho-st (Michalakis and Excoffier, 1996; Rousset, 1996) as estimators of Wright's (1931)  $F_{st}$ , and Slatkin's (1995)  $R_{st}$ , respectively, using FSTAT 2.7 (Goudet, 1995) and RstCALC (Goodman, 1996). I used Nei's (1978) genetic distances as obtained using PopGene 1.21 [F. C. Yeh, R. C. Yang, and T. Boyle, ([www.ualberta.ca/~fyeh](http://www.ualberta.ca/~fyeh))] to build a dendrogram of genetic similarities among beaches by UPGMA.

#### RESULTS

The total number of alleles detected in Colombia is shown in Table 1, together with those detected in Brazil (Sites et al., 1999) for comparative purposes. Allele frequencies are depicted in Fig. 2. The Colombian population was not in Hardy-Weinberg equilibrium overall ( $\chi^2 = 154.8$ ,  $df = 64$ ,  $P < 0.0001$ ). Unbiased estimates of Hardy-Weinberg exact  $P$ -values indicated the presence of heterozygote deficiency in the entire population at loci Pod128, Pod147, and PE1075 ( $P < 0.006$ , after

TABLE 1.—Total number of alleles detected at each beach within the Caquetá River, across beaches at the Caquetá River, and at the Tapajós and Araguaia rivers in Brazil. Data for Brazil are from Sites et al. (1999). Localities are plotted in Figs. 1 and 3.

Loci	Beaches within the Caquetá River				Rivers		
	Centro (n = 14)	Guadual (n = 14)	Tamanco (n = 12)	Yarumal (n = 15)	Caquetá (n = 55)	Gapajós (n = 25)	Araguaia (n = 69)
PE344	7	5	5	6	9	n/a	n/a
PE519	5	5	6	6	8	n/a	n/a
PE1075	6	2	3	5	8	n/a	n/a
Pod1	13	14	9	11	18	19	9
Pod62	8	6	5	8	10	8	5
Pod79	13	10	11	15	19	13	11
Pod128	9	10	10	11	16	20	6
Pod147	12	9	10	15	26	15	9

Bonferroni's correction for multiple comparisons). Independent multilocus exact tests performed for each nesting beach showed a deficiency of heterozygotes in all beaches except Centro, after Bonferroni's correction ( $P < 0.01$ ). Locus by locus analysis at each nesting beach showed heterozygote deficits for Pod128 at Guadual and Yarumal, and for Pod147 at Guadual, Tamanco, and Yarumal, but no significant deficiency for PE1075 at any separate beach. No excess of heterozygotes was found at any loci, at any nesting beach, nor across all beaches combined. These results are summarized in Table 2. There was no significant linkage disequilibrium between any pair of loci at any nesting beach or overall, thus each locus is considered independent of all others.

There were significant differences in allele and genotype frequencies among nesting beaches across loci using a probability test ( $\chi^2 = 48.30913$ ,  $df = 16$ ,  $P < 0.0001$ ; and  $\chi^2 \gg 50$ ,  $df = 16$ ;  $P < 0.0001$ , respectively). Tests of differentiation of the allele frequencies at each locus and across loci were also significant when using the unbiased estimate of the  $P$ -value of the probability test (Fisher's exact test) described by Raymond and Rousset (1995b) (Table 2).

Genotypic differentiation per locus was significant when using the unbiased estimate of the  $P$ -value of a log-likelihood ( $G$ ) based exact test described in Goudet et al. (1996). Likewise, genotypic differentiation across loci was significant when using the unbiased  $P$ -value obtained by Markov

chain method as described in version 3.1c of GENEPOP (Raymond and Rousset, 1995a) (Table 2). Randomization tests of alleles over all samples (thus assuming random mating within samples) and of genotypes among samples also showed significant differentiation among nesting beaches [Weir and Cockerham's (1984)  $\theta$ ,  $\theta = 0.007$ ,  $P < 0.001$  and  $P < 0.015$  respectively]. However, no significant differentiation was detected when using rho-st (Michalakis and Excoffier, 1996; Rousset, 1996). Rho-st values obtained when including data from Brazilian populations (Sites et al., 1999) are presented in Table 3 together with estimates of gene flow. I calculated Brookfield's (1996) estimates of the frequency of potential null alleles for those loci that showed heterozygote deficiency and obtained values  $>0$  for loci Pod128 and Pod147 ( $r = 0.07$  and  $r = 0.10$ , respectively). I then recalculated the  $F_{st}$  estimators of population subdivision excluding those two loci, and the randomization tests still showed significant subdivision among nesting beaches ( $P < 0.004$  when randomizing alleles, and  $P < 0.007$  when randomizing genotypes).

The dendrogram based on Nei's (1978) genetic distance using UPGMA has a subtle geographic component (Fig. 1). The three geographically proximate nesting beaches along the Caquetá River (Centro, Guadual, and Yarumal) were also most similar genetically, followed by Tamanco, which is the most geographically distant and genetically the most distinct. Alternatively, as recommended by de Queiroz and

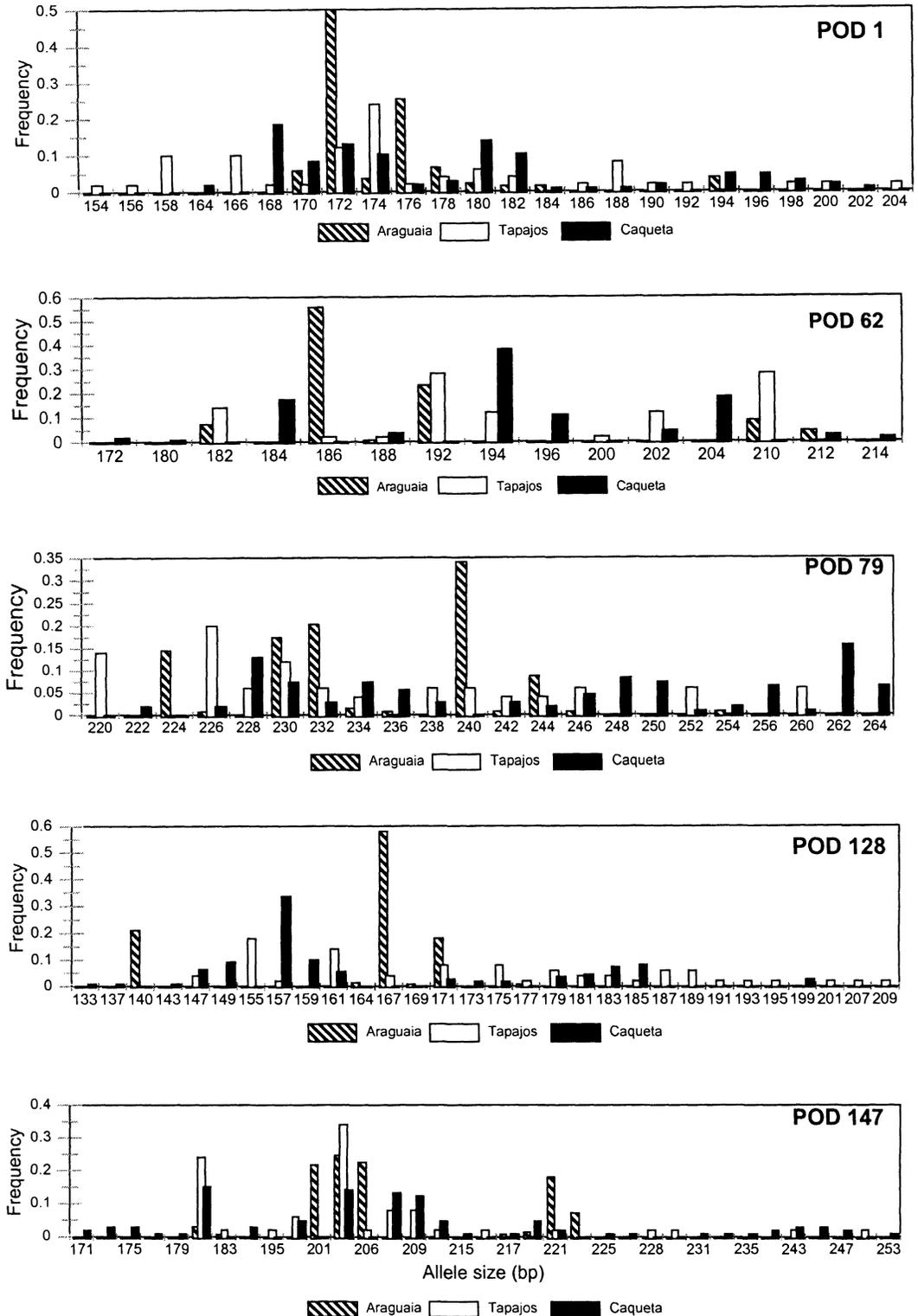


FIG. 2.—Histograms of allele frequencies at five microsatellite loci detected in the Caquetá River in Colombia (present study) and in the Araguaia and Tapajós rivers in Brazil (data from Sites et al., 1999).

TABLE 2.—*P*-values of the tests for Hardy-Weinberg (HW) equilibrium, genic/genotypic differentiation among nesting beaches, and observed heterozygosity per locus. For HW tests: ns = if in HW equilibrium; *P*-values shown when significant heterozygote deficiency was found.

Loci	Deviation from Hardy-Weinberg equilibrium					Genic differentiation	Genotypic differentiation	Observed heterozygosity
	Centro	Guadual	Tamanco	Yarumal	All beaches			
PE344	ns	ns	ns	ns	ns	ns	ns	0.645
PE519	ns	ns	ns	ns	ns	0.08812	ns	0.767
PE1075	ns	ns	ns	ns	0.0054	0.01355	0.0440	0.548
Pod1	ns	ns	ns	ns	ns	ns	ns	0.827
Pod62	ns	ns	ns	ns	ns	0.01392	0.0141	0.812
Pod79	ns	ns	ns	ns	ns	0.01662	0.0386	0.908
Pod128	ns	0.009	ns	0.005	0.00001	0.07850	ns	0.634
Pod147	ns	0.0009	0.0006	0.0045	0.00001	0.00674	ns	0.663
All Loci	ns	0.00001	0.0054	0.00001	0.00001	0.00001	0.00001	0.725

Good (1997), I used multidimensional scaling analysis on the genetic distance data (Kruskal, 1964a,b) and found the results to be comparable. The multilocus estimate of the overall effective number of migrants using private alleles (Slatkin, 1985) is  $Nm = 3.9$ , after correcting for sample size (Barton and Slatkin, 1986).

#### DISCUSSION

##### *Subdivision within the Caquetá River Basin*

Analysis of the four Colombian beaches suggests significant population subdivision within the Caquetá basin on the basis of Weir and Cokerham's (1984)  $F_{st}$  estimator,  $\theta$ , but not with the rho-st estimator of Slatkin's (1995)  $R_{st}$  (Michalakis and Excoffier,

1996; Rousset, 1996). In contrast, consistent results using both  $\theta$  and rho-st were obtained by Sites et al. (1999) in Brazil, where ample differentiation between Tapajós and Araguaia rivers was detected, but not among three nesting beaches examined within the Araguaia River.

Estimators of  $Nm$  based on Wright's  $F_{st}$ , assume an infinite island model of migration and (if gene flow is low) an infinite-allele or  $k$ -allele model of mutation. Therefore, estimators of  $F_{st}$ , like  $\theta$  are thought to be inappropriate for the analysis of microsatellite data. Mutations in microsatellite tandem repeat regions seem to follow step-wise or two-phase mutation processes and, thus, new variants are not independent of the progenitor state (Di

TABLE 3.—Pairwise rho-st values averaging over variance components, estimated gene flow ( $Nm$ ), and genetic distance (number of permutations = 1000); bold print indicates comparisons between nesting beaches in different river basins (Fig. 3).

Populations	rho-st (Var Comp)	$Nm$	<i>P</i>
Centro × Guadual	0.02217	11.0272	0.10500
Centro × Tamanco	-0.01590	not defined	0.63900
Centro × Yarumal	-0.03441	not defined	0.99700
<b>Centro × Araguaia</b>	0.09444	2.3971	<b>0.00000</b>
<b>Centro × Tapajós</b>	0.09826	2.2944	<b>0.00100</b>
Guadual × Tamanco	-0.01507	not defined	0.64200
Guadual × Yarumal	0.01644	14.9547	0.20200
<b>Guadual × Araguaia</b>	0.18359	1.1117	<b>0.00000</b>
<b>Guadual × Tapajós</b>	0.12417	1.7633	<b>0.00000</b>
Tamanco × Yarumal	-0.02039	not defined	0.76300
<b>Tamanco × Araguaia</b>	0.05072	4.6794	<b>0.03000</b>
<b>Tamanco × Tapajós</b>	0.05150	4.6041	<b>0.05000</b>
<b>Yarumal × Araguaia</b>	0.11077	2.0069	<b>0.00000</b>
<b>Yarumal × Tapajós</b>	0.10313	2.1742	<b>0.00000</b>
<b>Araguaia × Tapajós</b>	0.10371	2.1605	<b>0.00000</b>

Rienzo et al., 1994; Valdes et al., 1993; Weber and Wong, 1993).  $R_{st}$  is an analogue of  $F_{st}$  that incorporates microsatellite-specific modes of mutation (Slatkin, 1995). Goudet et al. (1996) studied the statistical power of several methods used to estimate the significance of  $F_{st}$  when sampling designs are balanced or unbalanced, but no study has been conducted to date to determine how those results apply to the  $R_{st}$  index. The discrepancy between the results that I obtained during this study with the two estimators is not entirely surprising given the lower statistical power of rho-st. This is due in part to the associated relative higher variance of the rho-st estimator (J. Goudet, personal communication), and it is likely that the discrepancy that I found between  $\theta$  and rho-st is caused in part by small sample sizes (sample sizes were 12, 14, 14, and 15 individuals, respectively).

Slatkin (1995) showed that  $F_{st}$  tends to underestimate the amount of true genetic differentiation when it is applied to microsatellite data. The values of estimators like  $\theta$  depend on the level of within-population homozygosity, which decreases with increasing mutation rates, as in the case of microsatellite markers (Hedrick, 1999). Given the significant population subdivision in the Caquetá basin detected by this conservative estimator of  $F_{st}$  ( $\theta$ ), I conclude that there is real genetic differentiation among the nesting beaches studied, but that such differentiation is subtle enough to pass undetected by rho-st, particularly with the small sample sizes available. Significant differences in allele and genotype frequencies were also detected by the probability and exact tests, in agreement with the result obtained using the  $F_{st}$  estimator.

The subdivision that I found within the Caquetá basin is not due to an artificial heterozygote deficiency caused by the presence of null alleles. Null alleles are non-amplifying alleles resulting from mutations in the flanking region that prevent the primers from annealing adequately during the PCR reaction (Pemberton et al., 1995). Null alleles can generate an artificial heterozygote deficiency that can be

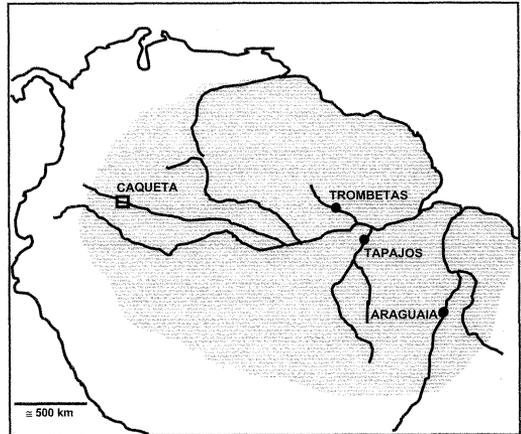


FIG. 3.—Distribution of *Podocnemis expansa* (shaded area) showing the study site (rectangle) and some populations from Brazilian rivers used for comparisons in the text (dots).

misinterpreted as arising from population subdivision in the form of the Wahlund effect (Brookfield, 1996; Pemberton et al., 1995). In order to account for this possibility, I excluded from the analysis those loci that showed heterozygote deficiency and a frequency of potential null alleles larger than zero, and I still detected significant differentiation by the  $F_{st}$  estimator.

The value of  $F_{st}$  ( $\theta$ ) estimated within the Caquetá River ( $\theta = 0.007$ ) is smaller than that found by Sites et al. (1999) between the widely separated (Fig. 3) Araguaia and Tapajós rivers ( $F_{st}$  ( $\theta$ ) = 0.13). Karl et al. (1992) also report larger  $F_{st}$  values for comparisons of populations of the marine turtle *Chelonia mydas* across its global range using RFLP of anonymous nuclear loci ( $F_{st} = 0.13$  and  $F_{st} = 0.17$ ). Higher  $F_{st}$  values can be expected in these two studies given their large spatial scale. Likewise, Scribner et al. (1986) found significant  $F_{st}$  values for *Pseudemys scripta* from naturally subdivided pond habitats using allozymes ( $F_{st} = 0.02$  to  $F_{st} = 0.05$ ) which are closer but still larger than  $F_{st}$  values for the Caquetá River.

The genetic differentiation that I found within the Caquetá River is surprising. The nesting beaches studied are all confined to a 100-km section of the Caquetá River. Data of mark-and-recapture studies indicate that adult females nesting at these

beaches migrate from common tributaries to the Caquetá basin, such as the Cahuiñarí River >200 km from the study site (von Hildebrand et al., 1997). Low dispersal capability can be ruled out as the cause of the genetic pattern found and leaves behavior as one possible explanation. The genetic differentiation that I found could result from groups of related females segregating along the Caquetá River into the different nesting beaches, or from related males mating with groups of females that nest in a particular beach (even if the females are not related to each other). The first case would occur if there is a high degree of female philopatry to natal beaches (natal homing), such that daughters will tend to nest at the same site as their mothers and grandmothers. Although some authors have postulated that *P. expansa* is philopatric (Alho et al., 1979; Ojasti, 1971), mark-recapture data from the study area (von Hildebrand et al., 1997) and the ephemeral nature of some nesting beaches in the Caquetá basin (Duivenvoorden and Lips, 1993) argue against it. Because microsatellites are nuclear markers inherited from both parents, it is impossible to tease apart the potential contribution of the behavior of each sex to this result. Analysis of mitochondrial DNA, which is a maternally inherited marker and thus gives information about matrilineal population structure, should be contrasted with the microsatellite data in order to distinguish male versus female contributions. This study is ongoing.

#### *Differentiation of Colombian and Brazilian Populations*

Although rho-st was unable to detect the differentiation among the Caquetá beaches with available sample sizes, this estimator detected strong population subdivision between Colombian and Brazilian river basins (Table 3). This extensive genetic differentiation was expected given the large geographic distance that separates Colombian populations from the Tapajós and Araguaia rivers (>1350 and >2400 km, respectively), and in this case I detected differences using rho-st despite the small samples sizes from Colombia.

Evidence of differentiation among the Caquetá, Tapajós, and Araguaia rivers is also visible in the histograms of sample allele frequencies of the five common loci analyzed in this study and by Sites et al. (1999) (Fig. 2). Population subdivision is expected in a species as widespread as *Podocnemis expansa* (Fig. 3) due simply to isolation by distance. Additionally, it is known that some geographic features such as strong rapids and large waterfalls in rivers constitute effective barriers for the distribution of the species (K. Oshbar, unpublished). Some of these obstacles have been overcome presumably even by unusual land migration in the past (Pritchard and Trebbau, 1984), such that some turtle populations are found on both sides of some of these barriers. Nonetheless these geographic hurdles pose some impediment to unrestrained gene flow and help isolate populations that otherwise could be reached by water by individuals. Mark-recapture data from the Caquetá study region indicate that females are able to migrate at least 422 km between nesting episodes (von Hildebrand et al., 1997).

Colombian and Brazilian populations appear to be distinct evolutionary significant units that differ in several genetic, morphological, and other biological aspects. The incubation period reported for the Caquetá River is longer than that reported for Trombetas River in Brazil, a river not far from the Tapajós (Alho et al., 1985; Valenzuela et al., 1997; N. Valenzuela, unpublished; Fig. 3). Estimates of body size of adult females are larger for Colombian populations (von Hildebrand et al., 1997). Incubation time dissimilarities may be the result of varied climatic conditions at the two sites. Female size variation could be a phenotypically plastic response to distinct environmental conditions: e.g., food availability at each site. Longer incubation times in Colombia could also be explained in part by the larger size attained by females compared to Brazil, given that larger females of *P. expansa* tend to dig deeper nests which in turn experience colder and less fluctuating temperatures that increase time to hatching (N. Valenzuela, unpublished). It is also

possible that biological differences between these populations are at least, in part, the result of genetic adaptations to local conditions, reflected as well in the divergence of microsatellite allele frequencies observed between Caquetá, Tapajós, and Araguaia basins. Theoretically, migration rate estimates ( $Nm$ ) of 2 or smaller gives room for genetic divergence resulting from genetic drift (Hart and Clark, 1989), whereas  $Nm$  of 4 or larger have been postulated to reflect panmixia among populations under a stepping stone or island models of population subdivision (Kimura and Maruyama, 1971). In general,  $Nm$  estimates between Colombian and Brazilian beaches are  $<4$  indicating little genetic exchange among populations, whereas  $Nm$  estimates among Colombian beaches are all  $>4$  or not defined (Table 3). Reported values of  $Nm \sim 4.6$  for the Tamanco  $\times$  Araguaia and Tamanco  $\times$  Tapajós comparisons (Table 3) may be the result of size homoplasmy rather than gene flow between these distant river basins. Two of the five loci used for this analysis (Pod79 and Pod147) have compound repeats of two different dinucleotide motifs (Sites et al., 1999) such that alleles of identical size but different sequence could result from certain combinations of repeat sizes for each dinucleotide motif [e.g.  $(CT)_{10}(CA)_{10}$  versus  $(CT)_{12}(CA)_8$ ].

#### *Implications for Conservation*

The strong and significant differentiation detected in all pairwise comparisons between Colombian and Brazilian populations provides grounds to consider each of those river basins as evolutionary units worthy of independent protection efforts. Within the Caquetá River, the biological relevance of differentiation may be that it results from behavioral segregation in the absence of any direct physical barriers. This behavioral component makes the inclusion of all nesting beaches in a management plan necessary to avoid the conceivable population genetic biases that could result if only some genetic lineages are protected within Colombia.

The total number of alleles detected in Colombia across all nesting beaches is sim-

ilar to and even higher than that found in Brazilian populations of *P. expansa* (Table 1). Sample sizes used by Sites et al. (1999) were larger than in this study ( $n = 69$  from Araguaia and  $n = 25$  from Tapajós). This is an encouraging result because one of the concerns of environmental organizations was that the decline in census numbers in Colombian populations might have resulted in a substantial loss of alleles. Current population size estimates for the Middle Caquetá River range between 1500 and 3000 nesting females (von Hildebrand et al., 1997). Accounts of the number of females exploited between 1950's and late 1970's suggest that several thousand females were extracted annually, causing a noticeable reduction of the nesting population (von Hildebrand et al., 1997). Ojasti (1971) mentioned that in the Orinoco basin, numbers of *P. expansa* decreased from 330,000 nesting females estimated by Humboldt (1820) to 13,800 estimated by Ojasti (1967). In the Trombetas River in Brazil, Valle et al. (1973) report that 7200 females nested in 1966. Alho (1985) calculated the average for the Trombetas River as 5184 females between 1978 and 1985, and 353 females for the Tapajós River. Although not yet studied, it is possible that these populations also suffer from similar levels of loss of genetic diversity and heterozygote deficiency.

Heterozygote deficiency was observed in the Colombian population at three of the eight loci analyzed here, in contrast with Brazilian populations which were in Hardy-Weinberg equilibrium (Sites et al., 1999). This deficiency could be the product of inbreeding due to a reduction of population numbers, because inbreeding can have the effect of modifying genotypic frequencies by rearranging alleles into excesses of homozygotes even when allele frequencies themselves remain virtually unchanged (Hartl and Clark, 1989). Because inbreeding would affect all loci simultaneously, I tested whether the number of loci that showed heterozygote deficiency, even if not significant, was larger than expected by chance (Table 4) using a chi-square test. The chi-square value was significant ( $\chi^2_{[1]} = 8$ ;  $P < 0.005$ ) which in-

TABLE 4.—Sign of the difference between observed and expected heterozygosity per locus per population. The chi-square test for the 32 internal cells (4 populations  $\times$  8 loci) is significant.

Loci	Observed-expected heterozygosity					Observed-expected No. negative cells	Observed-expected No. positive cells
	Centro	Guadual	Tamanco	Yarumal	All beaches		
PE344	—	—	—	—	—		
PE519	—	—	+	+	+		
PE1075	+	—	—	—	—		
Pod1	—	—	—	—	—	24-16	8-16
Pod62	+	+	+	—	+		
Pod79	+	—	—	—	—		
Pod128	—	—	—	—	—		
Pod147	+	—	—	—	—		
All Loci	—	—	—	—	—	$\chi^2_{[1]} = 8; P < 0.005$	

dicates that more loci than expected by chance exhibited heterozygote deficiency, a result that is consistent with the inbreeding hypothesis. Whether Brazilian populations have suffered considerable loss of genetic variability because of reduced sizes can only be answered by comparing populations of different sizes and different levels of disturbance. It can be stated, however, that the population decline of *P. expansa* in Colombia appears not to have had effects so drastic as to make conservation programs futile. There seems to be a considerable amount of genetic variation still available (at least compared with the Tapajós and Araguaia rivers in Brazil) and heterozygote deficiency, if caused by inbreeding, could be alleviated by the efforts to boost census numbers with surveillance, head-start, and artificial incubation programs.

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