Global Population Genetic Structure and Male-Mediated Gene Flow in the Green Sea Turtle (*Chelonia mydas*): Analysis of Microsatellite Loci

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ABSTRACT

We assessed the degree of population subdivision among global populations of green sea turtles, *Chelonia mydas*, using four microsatellite loci. Previously, a single-copy nuclear DNA study indicated significant male-mediated gene flow among populations alternately fixed for different mitochondrial DNA haplotypes and that genetic divergence between populations in the Atlantic and Pacific Oceans was more common than subdivisions among populations within ocean basins. Even so, overall levels of variation at single-copy loci were low and inferences were limited. Here, the markedly more variable microsatellite loci confirm the presence of male-mediated gene flow among populations within ocean basins. This analysis generally confirms the genetic divergence between the Atlantic and Pacific. As with the previous study, phylogenetic analyses of genetic distances based on the microsatellite loci indicate a close genetic relationship among eastern Atlantic and Indian Ocean populations. Unlike the single-copy study, however, the results here cannot be attributed to an artifact of general low variability and likely represent recent or ongoing migration between ocean basins. Sequence analyses of regions flanking the microsatellite repeat reveal considerable amounts of cryptic variation and homoplasy and significantly aid in our understanding of population connectivity. Assessment of the allele frequency distributions indicates that at least some of the loci may not be evolving by the stepwise mutation model.

THE ability to identify and define evolutionarily significant units (ESUs), or populations on independent evolutionary trajectories, is necessary in various aspects of biology ranging from ecology and conservation to population genetics (Waples 1995). Regardless of the ongoing debate concerning the relative importance of preserving neutral genetic or adaptive variation (Crandall *et al.* 2000; Moritz 2002), a clear understanding of population connectivity is critical to proper management strategies. A variety of molecular genetic markers allows for the detection of various levels of subdivision among populations and the tracking of the historical movements and interactions of individuals within and among populations. Six of the seven species of marine turtles are considered under the Endangered Species Act to be either threatened or endangered. The primary reason for this is believed to be human impact (Pritchard 1997). Conservation of marine turtle species exemplifies the importance of our ability to define ESUs and populations and to understand how they are related through gene flow.

We dedicate this article to Taísi Maria Sanches (August 5, 1965–March 25, 2000), an energetic, dedicated spirit and sea turtle researcher.

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Several studies of the green sea turtle (*Chelonia mydas*) have attempted to elucidate population subdivision in this globally distributed endangered species. Determining which populations are connected and particularly the strength of those connections has proven to be a challenging task. After leaving their natal beach, sea turtles are rarely seen again until they return to near shore foraging grounds as juveniles some years later, resulting in what Carr (1967) termed the "lost year." After reaching maturity, females are known to return to the same beach in successive nesting attempts with amazing accuracy and regularity (Carr and Ogren 1960; Limpus *et al.* 1984; Johnson and Ehrhart 1994). How this fidelity is established, however, was unknown. Carr (1967) suggested "natal homing" as the underlying mechanism whereby females were actually returning to their natal beach after potentially several decades at sea. Alternatively, the "social facilitation" model predicted that the close social contact between groups of turtles provides an opportunity for inter-rookery exchange of females. In this model, naive females accompany experienced females to nesting beaches regardless of the natal beach of origin (Hendrickson 1958; Owens *et al.* 1982). Studies based on tag return data indicate that green sea turtles also migrate extensively between nesting and feeding grounds (*e.g.*, Meylan 1982; Carr 1986).

Using mtDNA markers, Bowen *et al.* (1992) and Meylan *et al.* (1990) demonstrated female philopatry when they found significant population genetic subdivision

among green sea turtle nesting beaches. Bowen *et al.* (1992) found an extreme example of this relationship in turtle populations from Suriname and Ascension Island, whose rookeries share feeding grounds along the coast of Brazil. Here, the two nesting populations were fixed for different mitochondrial haplotypes, providing strong support for the natal homing hypothesis. Several other studies also have used mtDNA data to elucidate population subdivision in green turtles and other marine turtle species (Bass *et al.* 1996; Encalada *et al.* 1996; Bowen *et al.* 1997; Dutton *et al.* 1999). In general, these studies have revealed a significant level of population subdivision on both regional and global scales. Because of its maternally inherited nature, however, mtDNA is capable of providing information only on female genetic structure and behavior.

Male green sea turtles may provide a conduit for gene flow among populations. Some observational data indicate that males return to defined breeding sites or may accompany females to nesting sites (Dizon and Balazs 1982; Limpus 1993). Genetic information concerning male-mediated gene flow was lacking until work by Karl *et al.* (1992). Restriction fragment length polymorphisms (RFLPs) in single-copy nuclear DNA (scnDNA) revealed population subdivision between ocean basins, but only weakly subdivided populations within basins. In general, geographically proximate populations were indistinguishable from each other and many of the alleles were found worldwide, albeit at different frequencies. Pairwise population estimates of gene flow from the biparentally inherited nuclear loci revealed migration rates (Nm) consistently >1.0 and contrasting sharply with mtDNA estimates ($Nm = 0.0$ in 31 of 43 comparisons; Bowen *et al.* 1992; Karl *et al.* 1992). From this, Karl *et al.* (1992) concluded that on a regional scale there was a significant amount of male-mediated gene flow among maternally isolated populations. The same pattern was seen also in subsequent studies of green sea turtles in Australia (Fitzsimmons *et al.* 1997a,b), which demonstrated that the mixing was due to males and females from different natal beaches mating during migration events.

The difference in evolutionary rate for mtDNA *vs.* scnDNA loci, however, may be another possible explanation for the lack of concordance of the scnDNA and mtDNA results. Given a fourfold smaller genetic effective population size and a likely lack of mutation repair mechanism, it is generally considered that mtDNA evolves much faster than single-copy nuclear DNA (Brown *et al.* 1979). It is possible, therefore, that the different genetic patterns seen in mtDNA and scnDNA may be due to different evolutionary rates with scnDNA requiring a much longer period of time to accumulate a detectable amount of differentiation relative to mtDNA. The genetic similarities seen by Karl *et al.* (1992) among mtDNA-differentiated populations, therefore, could have been due simply to insufficient time since separation for the populations to accumulate differences at the scnDNA loci. A biparentally inherited gene that evolves at least as rapidly as mtDNA would be better able to differentiate recent population subdivision from ongoing male-mediated gene flow. Loci containing simple-sequence repeats (*i.e.*, microsatellites) have proven to be powerful tools in population genetics primarily because they evolve rapidly, are found throughout the nuclear genome, generally have several alleles per locus, and are typically inherited in a codominant, Mendelian fashion (Jarne and Lagoda 1996).

This study is a global evaluation of 337 individuals from 16 populations using four microsatellite markers originally developed by Fitzsimmons *et al.* (1995). A subset of the samples in this study was used by the previous global mtDNA study of Bowen *et al.* (1992) as well as the scnDNA study of Karl *et al.* (1992). Using these samples, we are able to compare estimates of population subdivision and gene flow across three different molecular marker types. More specifically, this study is concerned with the level of gene flow among nesting rookeries, the implications of male-mediated gene flow, and how gene flow affects the global population subdivision of green sea turtles.

MATERIALS AND METHODS

Green sea turtle samples: Nonsibling individual hatchlings were collected from 16 locations globally ($N = 337$), including nesting locations in the Atlantic, Pacific, and Indian Oceans as well as the Mediterranean Sea (Figure 1; Table 1). Samples were obtained from either a single hatchling or an egg from a given nest. These samples include individuals that were previously analyzed ($N = 256$) in Karl *et al.* (1992), which included 226 individuals previously analyzed in Bowen *et al.* (1992). In many cases, the number of samples collected was limited by United States import as well as country-specific export permit restrictions (see Bowen *et al.* 1992). Despite arguments for larger sample sizes, in 1994 officials at the National Marine Fisheries Sea Turtle Program determined that a sample size of 15 per location was sufficient for genetic analyses (including microsatellite surveys; B. W. Bowen, personal communication). Certain countries (*e.g.*, French Polynesia) levied additional size restrictions, making it impossible to obtain numerically large sample sizes for some populations.

Purified DNA used in previous studies was obtained by procedures outlined in Karl *et al.* (1992) and Bowen *et al.* (1992). Additional samples were obtained and DNA was extracted in a similar fashion from Lara Bay, Akamas Peninsula, Cyprus ($N = 16$), Atol das Rocas, Brazil (4), Aves Island, Venezuela (35), Tortuguero, Costa Rica (26), and Florida (2).

Molecular techniques: Four dinucleotide $(CA)_n$ microsatellite loci (CM3, CM58, CM72, and CM84) previously found to be variable in green turtles (Fitzsimmons *et al.* 1995) were amplified from genomic DNA via the polymerase chain reaction (PCR; Mullis and Wiss 1987; Saiki *et al.* 1988) in the presence of radioactively labeled dCTP (α-^{32}P or α-^{33}P). Fifteen-microliter PCR reactions included 0.033 μCi/μl of radioactively labeled dCTP, 0.003 mm dCTP, and 3.0 mm of each of the remaining three dNTPs; 0.625 unit of *Taq* polymerase (Promega, Madison, WI); 2.5 mm $MgCl_2$; 0.024 mm BSA (Boehringer Mannheim, Mannheim, Germany); and 0.41 μm of

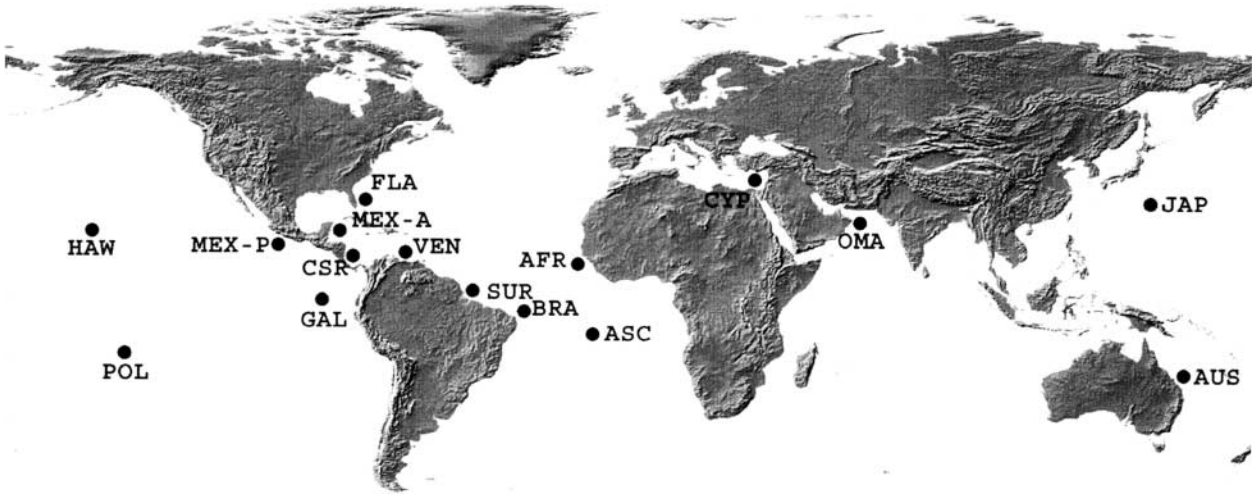

FIGURE 1.—Green sea turtle rookeries from which samples were collected. Sample sizes and location abbreviations are as in Table 1.

each primer. Thermal cycling consisted of 2 min denaturation at 95°, followed by 30 cycles of 30 sec at 95°, 30 sec at 55°, and 45 sec at 72°. A final extension step of 7 min at 72° also was performed. Following amplification, 2.5 μl of the sample was added to 2.5 μl of gel loading buffer (95% formamide, 20 mM EDTA, 0.05% xylene cyanol, and 0.05% bromophenol blue), heated for 5 min at 95°, and loaded on 6% denaturing acrylamide gels. Gels were run at 75 W for ~3 hr (time varied depending on the length of the target sequence). Allele sizes were established through comparison with a known DNA sequencing ladder run alongside the labeled PCR products. Gels were blotted onto filter paper, vacuum dried, and exposed to X-ray film. Exposure time varied depending upon the radioisotope used (α-^{32}P or α-^{33}P) and the strength of the emitting radioactive signal as estimated with a standard laboratory Geiger counter.

Locus CM72 was assessed for allele size homoplasy due to the high number of alleles observed. Twenty-five individuals representing all geographic sampling regions were amplified, cloned, and the microsatellite locus was sequenced in both directions. Total cell DNA was amplified using the previously described parameters with the CM72 primers. The amplification product was T-A subcloned using pBSK+ (Stratagene, La Jolla, CA) and DH5α competent cells (Life Technologies,

TABLE 1

Green turtle sample locations (n = 16)

Location	Sample size	Abbreviation
Atlantic-Mediterranean		
Ascension Island, UK	46	ASC
Atol das Rocas, Brazil	21	BRA
Aves Island, Venezuela	44	VEN
Hutchinson Island, Florida	21	FLA
Lara Bay, Akamas Peninsula, Cyprus	25	CYP
Matapica, Suriname	15	SUR
Pailoa, Guinea Bissau	19	AFR
Quintana Roo, Mexico	7	MEX-A
Tortuguero, Costa Rica	49	CSR
Total	247	
Pacific-Indian		
French Frigate Shoals, Hawaii	22	HAW
Heron Island, Queensland, Australia	16	AUS
Isabela Island, Galapagos, Ecuador	8	GAL
Michoacan, Mexico	7	MEX-P
Mopelia Atoll, French Polynesia	3	POL
Ogasawara Archipeligo, Japan	19	JAP
Ras Al Hadd, Oman	15	OMA
Total	90	
Grand total	337	

Sample size and location abbreviation are used throughout text.

Rockville, MD; Ausubel *et al.* 1994). Individual clones of alleles corresponding to the original gel-determined genotypes (32 clones) were amplified using the aforementioned conditions with M13 primers. Prior to the sequencing reaction, the PCR product was purified by centrifugal filtration with Millipore (Bedford, MA) Ultrafree-MC (30,000 NMWL) filter units. Clones were sequenced with a DYEnamic E-T Terminator cycle sequencing kit (Amersham Pharmacia Biotech, Piscataway, NJ) or a d-Rhodamine Terminator cycle sequencing reaction kit (PE Biosystems, Foster City, CA) following the manufacturer's protocol and run on a Perkin-Elmer (Norwalk, CT) automated ABI 310 sequencer. Sequences corresponding to unique alleles were deposited in GenBank under accession nos. AY197706–AY197723.

Statistical analysis: Deviations from Hardy-Weinberg genotype frequency equilibrium (HWE) were determined with an exact test using the program GENEPOP (Raymond and Rousset 1995) and a Markov chain analysis to estimate significance (5000 dememorization steps, 500 batches, and 5000 iterations per batch). Population genetic subdivision was assessed using both unbiased estimates of F_{ST} (Wright 1943; Weir and Cockerham 1984) and unbiased estimates of Slatkin's (1995) microsatellite-specific analog, R_{ST}, using the computer programs FSTAT (Goudet 1995) and RSTCALC (Goodman 1997), respectively. Chi-square values were used as significance estimators for F_{ST} as outlined in Workman and Niswander (1970). Significance of multiple test comparisons was corrected using the sequential Bonferroni method and $P = 0.01$ (Rice 1989), when appropriate.

R_{ST} values were calculated according to Slatkin (1995). Permutation tests were used to determine if the R_{ST} values were significantly different from zero. Estimates of gene flow (Nm) among populations were calculated on the basis of F_{ST} (and R_{ST}) values according to the equation

$$Nm = \frac{1}{4}\left(\frac{1}{F_{ST}} - 1\right)$$

(Wright 1951). The genetic distance measures $\delta\mu^2$ (Goldstein *et al.* 1995) and Nei's standard distance, D (Nei 1972), were calculated using the program MICROSAT (Minch 1995). Standard errors for each distance measure were calculated over 1000 bootstrap replication values.

To establish correlations between analogous measures of population subdivision and genetic diversity (F_{ST} and R_{ST}, $\delta\mu^2$, and D) a Spearman rank order correlation (Spearman 1904) was applied to each set of analogs. Gametic linkage equilibrium between all pairwise combinations of loci was estimated using the computer program Arlequin (Schneider *et al.* 2002). Similarity of allele frequency distributions between ocean basins was tested using the nonparametric Kolmogorov-Smirnov two-sample (KS) test (Sokal and Rohlf 1981; critical value is 0.2004 for $\alpha = 0.01$). The median allele frequencies at all loci were tested for statistical significance between ocean basins using a nonparametric Mann-Whitney rank sum test (Mann and Whitney 1947; $P < 0.005$).

To assess whether the microsatellite loci were evolving according to a stepwise (Kimura and Ohta 1978; Shriver *et al.* 1993; Valdes *et al.* 1993) or a two-phase (Di Rienzo *et al.* 1994) mutational model, we tested the per locus, within-ocean allele frequency distributions for unimodality (expected in the stepwise model). We first used a KS test to determine if the observed distributions deviated significantly from a standardized normal distribution with the same mean and variance. If normality is rejected, this suggests that the allelic distribution is either unimodal and not normally distributed or multimodal. To distinguish between the two, we generated 10,000 normal distributions using the sample size and variance of the observed distribution and a mean equal to the observed modal allele frequency. This gives the probability of observing by chance alone either empty cells (no alleles of that size observed) or an allele frequency equal to or greater than the observed. We considered the observed frequency significantly unlikely to be due to chance if the probability was $<0.5\%$. Since multimodality also can result from intraocean population subdivision, we analyzed the distributions both including and excluding populations with significant pairwise F_{ST} values.

Phylogenetic relationships: The phylogenetic relationships among green turtle populations were estimated using two distance measures (D or $\delta\mu^2$) and the neighbor-joining (Saitou and Nei 1987) tree-building algorithm implemented in the program PHYLIP (Felsenstein 1989). Bootstrapping (1000 replicates) was used to assess the significance of the nodes in the trees using the program MICROSAT (Minch 1995). The data were analyzed with and without populations with sample sizes <15 individuals. Sequences of the flanking regions of locus CM72 were aligned manually using Sequencher Version 3.1.1 (Gene Codes, Ann Arbor, MI) and gaps were inserted to maximize sequence similarity.

RESULTS

Molecular markers: All loci were highly polymorphic, although to differing degrees. CM3 had 27 alleles, CM58 had 19 alleles, CM72 had 41 alleles, and CM84 had 42 alleles. In each ocean basin, all loci had from one (CM3) to three (CM72) common alleles and a larger number of alleles at lower frequencies (Figure 2). The average number of alleles per locus was generally the same in the Atlantic-Mediterranean Oceans (hereafter referred to as Atlantic) as in the Pacific-Indian Oceans (hereafter referred to as Pacific); however, not all alleles were found in both ocean basins. There were 9 (31.0%), 9 (34.6%), 6 (14.0%), and 13 (31.0%) ocean basin unique alleles for CM3, CM58, CM72, and CM84, respectively. Over all loci, however, neither ocean basin had a preponderance of unique alleles (18 in the Atlantic and 19 in the Pacific).

The median allele frequencies at all loci were statistically different between ocean basins. Comparing the Atlantic to the Pacific, the most similar allele frequency distributions occurred at CM3 where the most common allele (mode) in both ocean basins was 160 bp, although it occurred at very different frequencies (60.12 and 15.8% for the Atlantic and Pacific, respectively; Figure 2A). At all other loci, the modal alleles were different between the oceans (Figure 2, B–D). The results of the KS test of similarity of allele frequency distributions between ocean basins indicated that for all loci the Atlantic and Pacific allele distributions were highly significantly different (critical value = 0.2004 for $P \leq 0.01$; $D = 0.3842$, 0.2983, 0.4078, and 0.3478 for CM3, CM58, CM72, and CM84, respectively).

When considering both the ocean basins and loci separately, KS tests rejected normality of all allele frequency distributions ($P \leq 0.05$). Within ocean basins, only Florida and Cyprus indicated consistent significant differences in pairwise tests for subdivision (*i.e.*, F_{ST}).

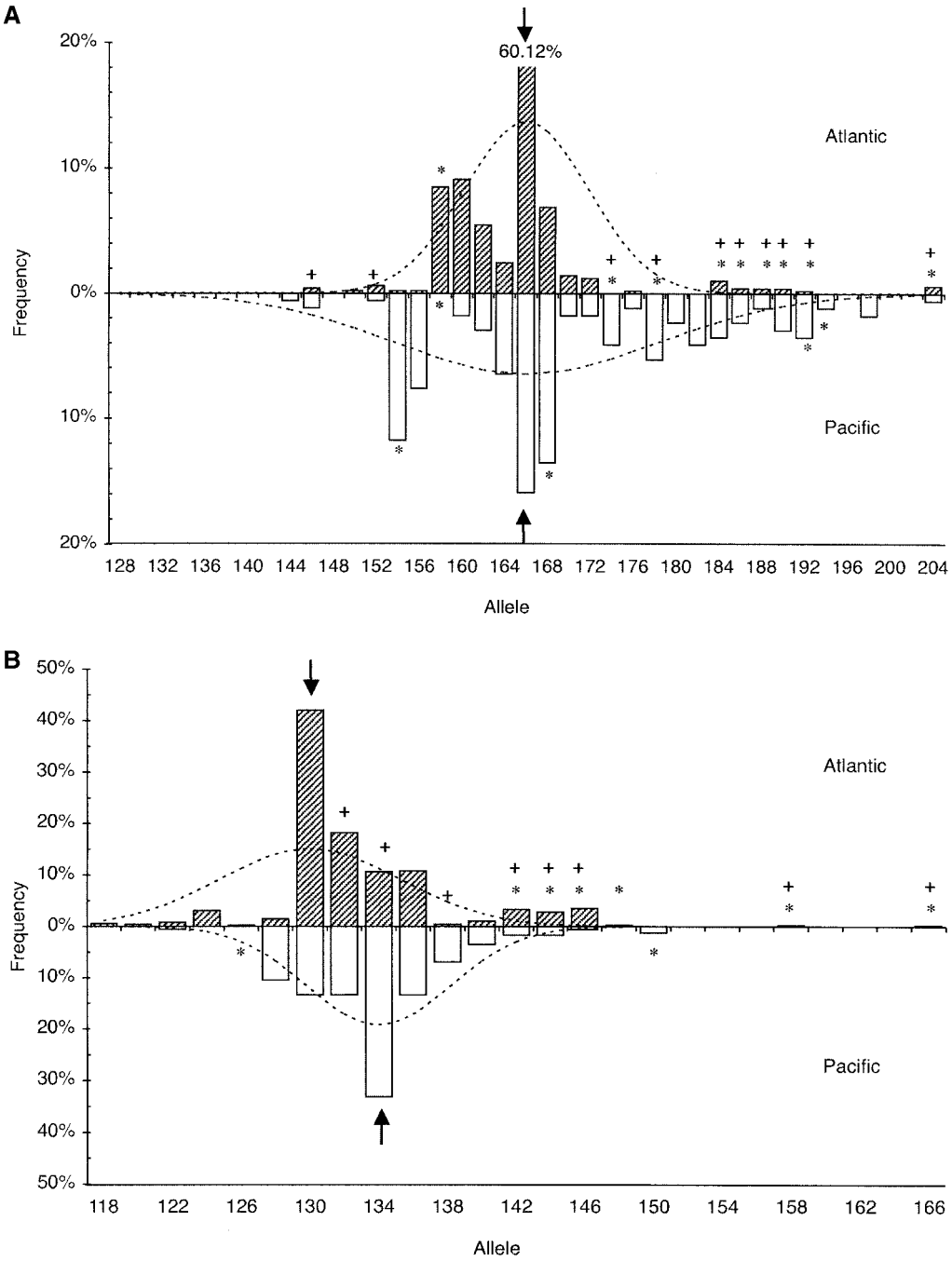

FIGURE 2.—Histograms of allele frequencies for Atlantic-Mediterranean and Indian-Pacific populations at each of the four loci sampled. (A) CM3; (B) CM58; (C) CM72; and (D) CM84. The frequency of allele 165 at locus CM3 is off the scale and the exact value is indicated. Dotted lines indicate the expected frequencies on the basis of the simulated standardized normal distribution with the same mean and variance as the observed data. Asterisks (all data) and crosses (Atlantic populations excluding Cyprus and Florida) indicate allele frequencies significantly different from those expected ($P \leq 0.005$) on the basis of simulation studies (see text). Modal allele frequencies used in the simulations are indicated with arrows.

After removing these two populations, KS tests again rejected normality. Since all distributions were significantly nonnormal, we then subjected them to the resampling test for deviations from unimodality. All loci in both oceans indicated at least some alleles at frequencies significantly different from those expected under unimodality (Figure 2). In the Pacific, most alleles at each locus corresponded to expectations except there was some indication for multimodality at CM3 and CM72. Both indicated a cluster of alleles that were seen more often than expected in a unimodal distribution (Figure 2, A and C). CM72 might have as many as three modes. In the Atlantic, all loci indicated several alleles at frequencies different from those expected and these deviations tended to be clustered as well. CM3, CM58, and CM84 all indicate an additional mode at larger allele sizes whether or not Cyprus and Florida were included (Figure 2, A, B, and D). CM72 strongly indicated two different modes at alleles 234 and 274 (Figure 2C). This was true even if Cyprus and Florida were excluded from the analysis and whether the mean of the standardized normal distribution was assumed to be 234 or 274.

Average heterozygosity across all populations and all loci was ~75%. The per locus heterozygosity varied ex-

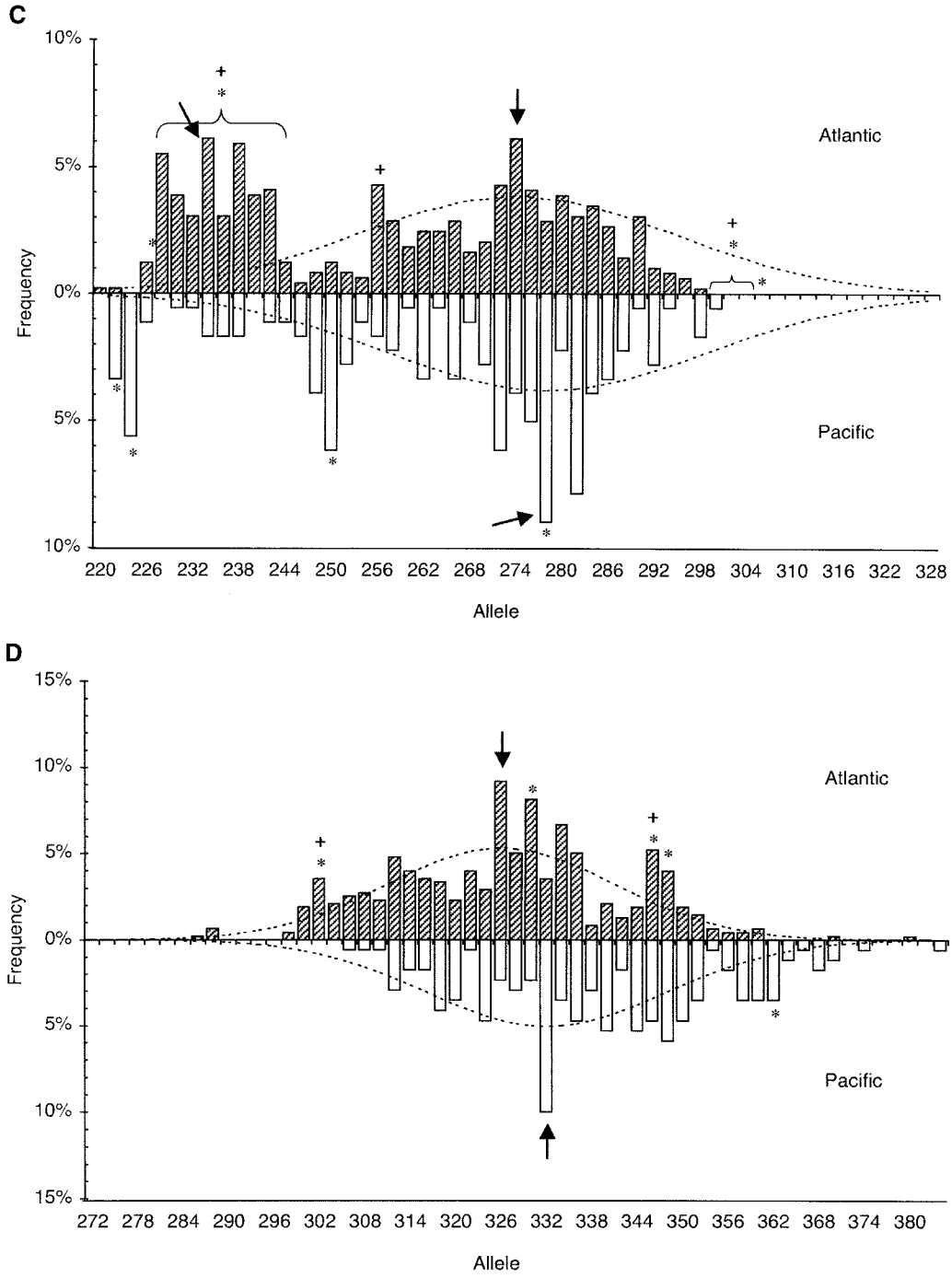

FIGURE 2.—*Continued.*

tensively among populations as well as across loci within populations (Table 2). Heterozygosity ranged from a low of 0.333 for the CM72 locus in Polynesia to a high of 1.0, which also was the high in at least one population for all loci except CM58 (Table 2). The highest observed heterozygosity recorded for CM58 was in the Galapagos Island rookery (87.5%). Mean heterozygosity per population ranged from 59.2% (Venezuela) to 85.7% (Quintana Roo). Although sample sizes varied considerably (N = 3–49), heterozygosity appeared to be unrelated (Table 2; R^2 = 0.0578). Deviations from HWE were present in only 6 of the 64 (9.4%) possible population-locus combinations. All of the F_{IS} values associated with these deviations were positive, indicating a heterozygote deficit when compared to expected values (data not shown). Of the 96 dilocus-by-population comparisons (6 locus comparisons for each of 16 populations), only one, CM3–CM84 in Australia, showed significant linkage disequilibrium after sequential Bonferroni correction at $P \leq 0.05$.

F_{ST} values for all pairwise comparisons indicated a general association among populations within ocean basins

TABLE 2

Genetic variation at microsatellite loci in green sea turtles

		CM3		CM58		CM72		CM84			
	N	*A*	*H*	*A*	*H*	*A*	*H*	*A*	*H*	Mean *H*	SE
Atlantic-Mediterranean											
Ascension	46	8	0.435	15	0.804	27	*0.652*	21	*0.783*	0.668	0.170
Brazil	21	9	0.524	10	0.714	19	0.762	21	0.810	0.702	0.125
Venezuela	44	9	0.367	9	0.592	26	0.857	26	*0.551*	0.592	0.202
Florida	21	9	0.714	8	0.476	16	0.857	22	0.857	0.726	0.180
Cyprus	25	5	0.400	7	0.760	18	*0.800*	14	0.680	0.660	0.180
Suriname	15	9	0.667	7	0.733	14	0.800	16	1.000	0.800	0.144
Africa	19	6	0.526	8	0.684	18	0.842	10	0.789	0.711	0.139
Quintana Roo	7	4	0.571	7	0.857	9	1.000	10	1.000	0.857	0.202
Costa Rica	49	11	0.694	11	0.673	31	0.939	26	*0.857*	0.791	0.128
Atlantic total	247	20	—	18	—	36	—	28	—	—	—
Atlantic mean	27.44	7.78	0.544	9.11	0.699	19.78	0.834	18.44	0.814	0.723	—
Atlantic SE	15.07	2.29	0.128	2.62	0.114	6.96	0.100	6.21	0.142	0.082	—
Pacific-Indian											
Hawaii	22	13	0.818	8	0.409	18	*0.636*	19	0.727	0.648	0.176
Australia	16	15	0.750	8	0.812	19	0.875	16	0.875	0.828	0.060
Galapagos	8	9	0.875	5	0.875	12	0.750	14	0.875	0.844	0.062
Michoacan	7	7	0.571	6	0.714	11	0.857	12	0.857	0.750	0.137
Polynesia	3	4	1.000	4	0.667	4	0.333	5	1.000	0.750	0.319
Japan	19	10	0.737	8	0.737	20	1.000	18	1.000	0.868	0.152
Oman	15	13	0.533	9	0.800	15	0.867	17	0.867	0.767	0.159
Pacific total	90	25	—	11	—	40	—	27	—	—	—
Pacific mean	13.3	11.0	0.754	6.8	0.721	15.0	0.780	15.3	0.834	0.768	—
Pacific SE	7.1	3.7	0.132	1.5	0.207	4.1	0.110	3.0	0.072	0.090	—
All populations											
Grand total	337	27	—	19	—	41	—	42	—	—	—
Grand mean	21.1	8.8	0.636	8.1	0.707	17.3	0.802	16.7	0.846	0.748	—
Grand SE	14.0	3.2	0.177	2.5	0.127	6.9	0.162	5.8	0.125	0.081	—

Sample size (*N*), number of alleles (*A*), and observed heterozygosity (*H*) are shown. Deviations from Hardy-Weinberg genotype frequency expectations are in italics ($P \leq 0.01$). All deviations were heterozygote deficits.

and divergence between them (Table 3). F_{ST} values among Atlantic populations ranged from 0.0087 to 0.0947, among Pacific populations from 0.0000 to 0.0676, and between ocean basins from 0.0181 to 0.1414. Of the 36 possible pairwise combinations among Atlantic populations, only 13 exhibited F_{ST} values that were significantly larger than zero (Table 3). Similarly, of the 21 pairwise estimates among all Pacific populations two revealed F_{ST} values significantly different from zero. When a sequential Bonferroni correction is applied only two of the Atlantic and none of the Pacific population pairwise F_{ST} values were significantly different from zero. In contrast, of the 63 interocean comparisons, 44 (70%) of the uncorrected and 9 (14.3%) of the corrected values were significant (Table 3). Pairwise estimates of population subdivision using the R_{ST} statistic produced similar results (Table 3). Intraoceanic pairwise estimates of gene flow (*Nm*) using either R_{ST} or F_{ST} were consistently larger than one and ranged from 2.55 to 9.03 (Table 3). Interocean *Nm* values also were greater than one, but generally less so (2.83–3.34). All microsatellite estimates of gene flow were larger than those observed in previous molecular studies (Bowen *et al.* 1992; Karl *et al.* 1992). In addition, genetic distances ($\delta\mu^2$) were generally greater for between-ocean comparisons than for within-ocean comparisons (data not shown).

Population phylogenetic relationships: The neighbor-joining trees using Goldstein *et al.*'s (1995; $\delta\mu^2$) and Nei's (1972) genetic distances (*D*; Figure 3) resulted in similar topologies and were primarily consistent with the mtDNA relationships set forth by Bowen *et al.* (1992) regardless of whether samples with only a small number of individuals were included or excluded. There was a phylogenetic split between the Atlantic and Pacific Ocean populations in both analyses, with the exception that Oman and Australia were placed with the Atlantic group in the $\delta\mu^2$ tree. The association between Australia and Oman was found also in the Karl *et al.* (1992)

TABLE 3

Population subdivision and gene flow in *C. mydas*

	Atlantic	Pacific	Atlantic *vs.* Pacific
Microsatellites (this study), $N = 337$			
F_{ST}	0.038 ± 0.023	0.024 ± 0.017	0.079 ± 0.030
Significant pairwise[a]	13(2) of 36	2(0) of 21	44(9) of 63
χ^2	69.55**	17.95*	155.22**
Nm	6.10	9.03	3.34
R_{ST}	0.026	0.065	0.065
Significant pairwise[a]	16(5) of 36	10(2) of 21	43(24) of 63
χ^2	51.72*	46.44**	175.98**
Nm	8.55	2.88	2.83
Nm (private alleles)	7.13	3.23	3.61
scnDNA RFLPs (Karl *et al.* 1992), $N = 256$			
F_{ST}	0.130	0.126	0.166
Significant pairwise	49 of 140	28 of 75	124 of 240
χ^2	238.76**	114.62**	502.93**
Nm	1.70	1.70	1.30
mtDNA/RFLP[b] (Bowen *et al.* 1992), $N = 226$			
G_{ST}	0.63	0.71	1.00
Significant pairwise	25 of 28	12 of 15	48 of 48
Nm	0.30	0.20	0.00

* $P \leq 0.05$; ** $P \leq 0.01$.

[a] The number in parentheses is after Bonferroni correction (not available for previously published results).

[b] No mtDNA haplotypes were shared between ocean basins.

scnDNA study. As with the previous study, within-ocean basin associations were less pronounced than between-, and, in general, geographically proximate locations clustered together albeit with only low to moderate bootstrap support for most nodes.

Allelic homoplasy: To identify incidents of potential homoplasy in the microsatellite allele sizes, 32 alleles at locus CM72 were sequenced in both directions, resulting in ~202 nucleotides of flanking region and a variable number of internal repeat units (depending on allele size). The microsatellite alleles were named to indicate the amplified fragment size and population of origin (*e.g.*, FLA 250 is a 250-bp allele from a Florida individual) and flanking sequence haplotypes were designated with letters. Overall, in the flanking sequences there were 21 variable sites resolving 18 haplotypes. Most of the variation (17 sites) observed was single-nucleotide substitution, although a few small indels also were present (Table 4). The flanking sequences of 10 different-size microsatellite alleles were identical and designated haplotype A (two alleles, AFR-280 and CSR-280, were identical in both sequence and size). This sequence was found in association with both Atlantic and Pacific microsatellite alleles and one of the haplotype A microsatellite alleles (280) was found in both Africa and Venezuela. Six of the 21 Atlantic microsatellite alleles shared a two-nucleotide deletion at position 124–125 not found in the Pacific (Table 4). Two of these (CSR 234 and BRA 240) had additional, unique mutations and one (FLA 250) shared an A-to-G transition with an allele (BRA 230) without the deletion. A four-nucleotide deletion and a C-to-T transition (position 223) were shared between two of the three Oman alleles. The third Oman allele shared an A-to-G transition with a different-sized Galapagos allele at position 217. A single transversion (position 233) united different microsatellite alleles from Africa, Suriname, and Ascension. Overall, a considerable amount of cryptic variation and homoplasy was found. In several cases, putatively identical microsatellite alleles (*e.g.*, FLA 250 and JAP 250; Table 4) actually contained different numbers of simple sequence repeats but the alleles were identical in size due to compensatory flanking region deletions (nucleotides 124–125 were deleted in FLA 250). They also contained substitutional differences within the flanking sequences. Other similarly sized microsatellite alleles also were clearly different when the flanking regions were considered (Table 4; JAP 230, BRA 230, and SUR 230).

DISCUSSION

Microsatellite mutation: The generation of new microsatellite alleles is thought to be largely due to polymerase slippage during replication (Tautz and Rentz 1984; Levinson and Gutman 1987; Stephan 1989; Weber 1990; Caskey *et al.* 1992; Schlötterer and Tautz 1992; Strand *et al.* 1993). Evolution at microsatellite loci should then follow a stepwise mutation model (SMM) in which new alleles are formed by the gain or loss of a single repeat unit (Kimura and Ohta 1978; Shriver *et al.* 1993; Valdes *et al.* 1993). Allele frequency distributions are therefore expected to be unimodal and

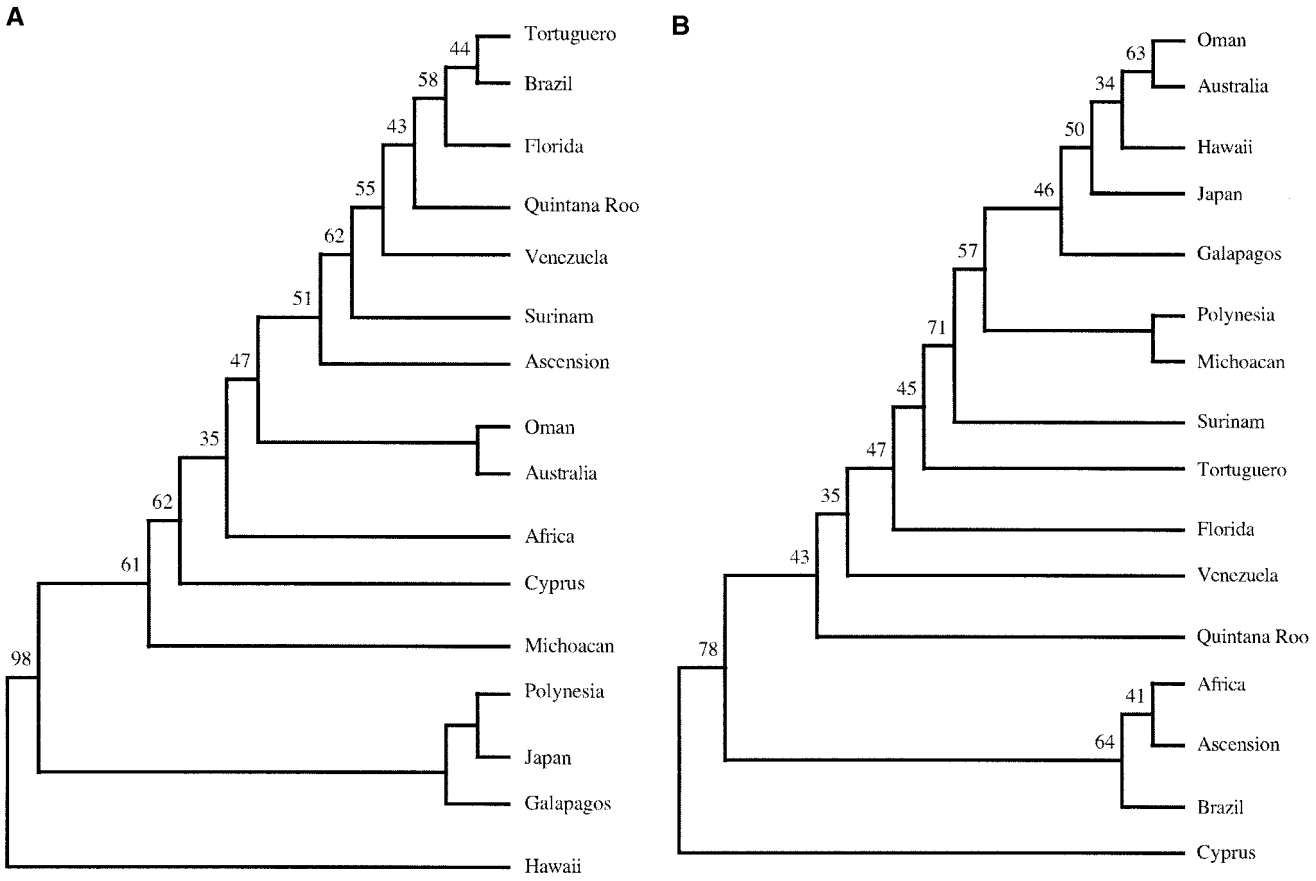

FIGURE 3.—Bootstrap consensus neighbor-joining dendrograms of all sampled populations. (A) $\delta\mu^2$ (GOLDSTEIN *et al.* 1995) measure of genetic distance. (B) NEI's (1972) standard genetic distance (D). Bootstrap values were assigned to nodes on the basis of the percentage of times that node was represented in 1000 bootstrap resampling replicate trees. Atlantic Ocean and Pacific Ocean rookeries cluster separately with the notable exception of Australia and Oman clustering with the Atlantic Ocean populations (see text for discussion).

symmetrical with alleles identical in state (IIS) but not necessarily identical by descent (IBD).

Alternatively, new alleles at microsatellite loci may be generated via unequal crossing over during recombination (JEFFREYS *et al.* 1988; JARNE and LAGODA 1996). These loci would then evolve according to the infinite alleles model (IAM; KIMURA and OHTA 1978). A simulation study by DI RIENZO *et al.* (1994), however, found that a two-phase mutation model (TPM) best fit the microsatellite evolutionary process for several loci in humans. In the TPM, typically a new allele is generated by the gain or loss of a single repeat unit; however, infrequent, large changes in allele size also occur. The resulting allele frequency distributions, therefore, tend to be asymmetrical and multimodal in nature. Identifying the correct mutational model is analytically important since the probability of scoring alleles IIS and not IBD will affect the choice of approach and robustness of the results. Furthermore, size homoplasy due to indels in flanking sequences mimicking changes in the microsatellite array itself increases the frequency of alleles IIS but not IBD, thus further complicating analysis (ESTOUP *et al.* 1995; GARZA and FREIMER 1996; GRIMALDI and CROUAU-ROY 1997; ORTI *et al.* 1997).

While the general population genetic analytical methods available to this study required an assumption of either the SMM or the IAM models, some of our results suggest that the alternative two-phase model may be more appropriate. Our per locus per ocean analyses indicate that, at least for CM72, allele frequency distributions deviate significantly from unimodality with two and sometimes three different modes. At all loci, multiple alleles appear to be over- or underrepresented although these deviations are not always clustered as seen for CM72 (Figure 2). In general, there is a much weaker indication of multimodality in the Pacific than in the Atlantic. We believe, however, that this is not due to different evolutionary processes but more likely a result of the smaller sample size from the Pacific ($N = 90$) relative to the Atlantic (247), resulting in a reduced ability to detect significant differences in allele frequencies. Alternatively, it is possible that the Pacific data are robust and that these loci are evolving under the SMM, but there is undetected population subdivision in the

TABLE 4

Allelic homoplasy at locus CM72

Haplotype	Population	Allele (bp)	Variable sites	GenBank accession no.
A	AFR	278		
	AFR	280		
	ASC	226		AY197706
	ASC	242		
	ASC	294		
	CSR	238		
	CSR	280		
	GAL	284		
	HAW	258		
	JAP	230		
	MEX-A	262		
B	AFR	256	A-C/233	AY197707
	ASC	280	A-C/233	
C	BRA	230	A-G/126, Δ275	AY197708
D	BRA	222	T-G/31, INS A/208, A-G/217, C-T/226, A-G/254	AY197709
E	BRA	240	Δ/124-125, T-C/248	AY197710
F	AFR	242	Δ/124-125	AY197711
	CSR	256	Δ/124-125	
	FLA	270	Δ/124-125	
G	FLA	250	Δ/124-125, A-G/126, C-T/265	AY197712
H	ASC	234	Δ/208	AY197713
I	SUR	230	MS C-T/149	AY197714
J	SUR	278	A-C/233, G-C/122	AY197715
K	CSR	234	Δ/124-125, A-G/95	AY197716
L	GAL	238	A-G/217	AY197717
	OMA	252	A-G/217	
M	GAL	268	T-C/85	AY197718
N	JAP	250	T-C/208, A-G/99, MS A-G/162 and C-T/173	AY197719
O	JAP	242	T-C/208	AY197720
P	JAP	274	Δ/35	AY197721
Q	OMA	272	Δ/208-211, C-T/223	AY197722
R	OMA	270	Δ/208-211, C-T/223, MS A-G/182	AY197723

The alleles are designated by size (in base pairs). The type of mutation is separated from the position with a slash (*e.g.*, A-C/233 is adenine-to-cytosine transversion at position 233). Deletions are indicated by Δ, insertions by INS, and mutations in the microsatellite region by MS.

Atlantic and not in the Pacific, resulting in Atlantic multimodality. We do not, however, believe that this is likely. Both R_{ST} and F_{ST} estimates of microsatellite loci indicate only very weak population subdivision within either ocean basin and do not appear to be different in the Atlantic than in the Pacific (Table 3). Only small or no differences in gene flow within ocean basins were noted in two previous molecular studies (Bowen *et al.* 1992; Karl *et al.* 1992). Although the mtDNA study by Bowen *et al.* (1992) did detect within-ocean maternal lineage subdivision not seen in the scnDNA study, the degree of gene flow within the Atlantic ($Nm = 0.3$) was comparable to that seen within the Pacific ($Nm = 0.2$). In addition, in the current analysis when populations that were demonstrated to be subdivided were excluded, our results were unchanged. Furthermore, population subdivision strong enough to result in significant multimodality would also likely create significantly different allele frequencies and thus significant pairwise F_{ST} values. In addition, it seems unlikely that the considerably geographically smaller and younger Atlantic Ocean would be more subdivided than the Pacific. We cannot, however, eliminate population subdivision as a contributing cause of multimodality observed in our data. Even so, our data indicate that these loci are not purely evolving under a simple SSM of mutation and it seems possible that at least some loci (*e.g.*, CM72) may be following a two-phase mutational model. While establishing this unequivocally calls for additional investigation, our results do provide some empirical indication for the possibility of a general two-phase model of evolution at microsatellite loci and a general approach for detecting multimodality in allele frequency distributions.

Population genetic substructure: Five of 36 possible Atlantic population-locus combinations and one of the 28 Pacific combinations deviate from HWE (Table 2).

All deviations are heterozygote deficits and may indicate local inbreeding. Alternatively, null alleles could result in heterozygote deficits. Data from the flanking regions indicate that mutations to the flanking regions and homoplasy are indeed occurring. However, the magnitude and effect of these are difficult to estimate. Although we have no direct, independent indication of null alleles, they may nonetheless be affecting our analyses by reducing our ability to detect population subdivision. Regardless, we believe that the sporadic population-locus HWE deviations observed here are artifactual and do not indicate persuasive problems with this study.

In our analyses, we have used algorithms assuming an IAM (F_{ST} and D) and a SMM (R_{ST} and $\delta\mu^2$). Differences between the outcomes of these two approaches are minimal. Estimates of F_{ST} and R_{ST} appear to be correlated with each other ($r^2 = 0.177$; $P = 0.05$), although these statistics tend to track each other less than measures of genetic distance (see below). Both F_{ST} and R_{ST} statistics indicate a higher level of inter- *vs.* intraoceanic population subdivision (Table 3) and are principally in agreement with Karl *et al.* (1992) and Bowen *et al.* (1992). Between-ocean basin subdivision is indicated also by a larger number of significant pairwise differences in genetic distance observed between as opposed to within oceans (data not shown). Genetic distance estimates (Nei's D and Goldstein's $\delta\mu^2$) seem to be correlated ($r^2 = 0.32$; $P = 0.00$) better than F_{ST} and R_{ST} estimates. Accordingly, there are generally only marginal differences in the tree topologies (Figure 3, A and B). A neighbor-joining consensus bootstrapped tree of Nei's D indicates a separation between the Atlantic and Pacific populations and within-ocean relationships tend to reflect geography (Figure 3A) regardless of inclusion or exclusion of samples with small numbers of individuals. The $\delta\mu^2$ distance topology is consistent with Nei's D with the notable exception of an Australia and Oman clade clustering just within the Atlantic. This apparent mixing between the Atlantic and Indian Oceans seems counterintuitive and has been considered, by some, "impossible to interpret" and indicating that "DNA analysis was fundamentally useless in determining the real affinities. . ." (Pritchard 1999, p. 1001). Because of prevailing cold water conditions, the southern tip of Africa is commonly assumed to be an absolute barrier to mixing of Atlantic and Pacific tropical and subtropical species and even more so for the southern tip of South America. Thus, Africa and South America provide geographic barriers to gene flow between the oceans. Nonetheless, both here and in Karl *et al.* (1992) there is a curious clustering of Indian-Pacific and eastern Atlantic populations. This, however, may not be unreasonable given the connection between the southwestern-flowing Indian Ocean Agulhas and the northern-flowing South Atlantic Benguela currents near the tip of Africa (Gordon 1985; Lutjeharms and Gordon 1987). A growing number of studies have documented an Indian and East Atlantic phylogeographic connection in other species as well (Chow *et al.* 2000; Bowen *et al.* 2001; Lessios *et al.* 2001). If even an occasional wayward turtle was able to escape the Indian Ocean around the southern tip of Africa, it could account for this curious clustering in these analyses. While green sea turtles do not nest in South Africa, all age classes of turtles are frequently found stranded along the Indian Ocean coast as far south as the city of Cape Town (G. Hughes, personal communication). Furthermore, these strandings do not appear to be correlated with the nesting activity of turtles on nearby Indian Ocean Europa and Comores Islands beaches, indicating that this is not a seasonal phenomenon associated with nesting. The very presence of the stranded turtles in the Atlantic opens the possibility that gene flow around the southern tip of Africa could account for the repeated placement of the Australia and Oman clade within the Atlantic clade. That mtDNA of green turtles does not reflect this may simply be due to its maternally inherited nature. For example, a Pacific male turtle could round the tip of Africa and mate with an Atlantic female. This female would then return to her natal Atlantic beach to lay her eggs and carry with her nuclear DNA from the Indian-Pacific Ocean. As such, Indian-Pacific nuclear DNA and not mtDNA would leak into the Atlantic Ocean basin. Female green turtles traversing the tip of Africa, while capable of mating, should attempt to return to their natal beaches to nest, thus maintaining the ocean basin-specific mtDNA pattern. Failed attempts to renavigate the tip of Africa likely would result in a failure to find suitable nesting habitat. Bowen *et al.* (1989, 1992) and Meylan *et al.* (1990) clearly have shown that social facilitation in choosing a nesting beach does not normally occur in green turtles and a naive Indo-Pacific female trapped in the Atlantic (or vice versa) is less likely to locate suitable nesting sites. It is a real possibility, therefore, that Pacific-Atlantic association of marine turtles is far from impossible to interpret, but reflects a real biological phenomenon.

In a scnDNA study, Karl *et al.* (1992) documented male-mediated gene flow among maternally distinct populations sampled globally. In particular, they highlighted the degree of mixing between nesting populations on Suriname and Ascension Island, which are distinct in mtDNA but share a feeding ground along the coast of Brazil. The estimated degree of nuclear gene flow between these two populations was relatively high ($Nm = 5.0$) compared to mtDNA ($Nm = 0.0$). We estimate levels of gene flow between these populations, using microsatellite data, to be 3.9 (with R_{ST}) to 9.3 (with F_{ST}). A limitation of the previous scnDNA study was that the level of variation found in the nuclear DNA was fairly low and may have resulted in overestimates of gene flow due to lack of variation or retention of ancestral polymorphisms. With 7–27 alleles at each locus in these populations (Table 2) and high mutation rates

generally assigned to microsatellite loci, lack of variation or *de novo* evolution clearly is not a factor in this study. In addition, identical deletions in the flanking regions of the CM72 locus were found in both populations (Table 4). Considering all the data, male-mediated gene flow is clearly occurring between these populations and likely others as well. This does not suggest, however, that males do not demonstrate natal site fidelity, but merely that green turtle nesting beaches are genetically isolated maternally but not paternally. As indicated in Fitzsimmons *et al.* (1997a), males may exhibit natal homing but still mate with females from other nesting beaches as females and males migrate through nonnatal areas.

Comparison among molecular markers: Forces such as migration, mutation, and genetic drift may affect different types of genetic markers in distinctly different ways. The ability to detect a consistent signal across different markers depends on marker-specific factors such as the degree of homoplasy and mutational mechanisms and rates (Mariette *et al.* 2001). Here, we can compare the inferred degree of global population genetic subdivision in the same set of green sea turtle samples for three different molecular markers. Overall, these studies are remarkably consistent with each other and the known biology and biogeography of the green sea turtle. All show a split between oceanic groups and (where appropriate) comparable estimates of male-mediated gene flow (Table 3). Because of a faster rate of evolution, it was expected that microsatellites might detect population subdivision not indicated in the scnDNA study (Karl *et al.* 1992) due to retention of ancestral polymorphisms. This was clearly not the case with scnDNA actually estimating a higher level of population subdivision between ocean basins ($F_{ST} = 0.166$) than microsatellites ($F_{ST} = 0.058$, $R_{ST} = 0.065$). Three possibilities (not mutually exclusive) might account for this result.

The most likely possibility is that the rate of mutation at microsatellite loci is too high relative to the length of time separating the populations and results in homoplasy. Unless sequenced, microsatellite alleles represent a hidden amalgam of various alleles. Orti *et al.* (1997) report data that suggest that microsatellite allele size-based analysis of population structure proves inconsistent and inaccurate due to flanking sequence indels resulting in size homoplasy. Here, we addressed this concern by sequencing 5% of the alleles at the most variable locus in this study, CM72. Deletions were definitely present; however, they were not common and are unlikely to result in the large differences in inferred subdivision (Table 4). However, more important is a second type of homoplasy that is harder to detect. If repeats are being inserted and deleted at such a rate that alleles IIS are likely not IBD, then subdivided populations would follow a progression of being genetically identical immediately after separation, increasing in divergence by accumulation of new alleles, and then decreasing in divergence as new alleles mutate to existing alleles in the population. This type of homoplasy is similar to saturation in gene sequences and could similarly be accounted for except that we lack an appropriate mutational model upon which to base the necessary corrections. Bowen *et al.* (1992) estimated that the Atlantic and Pacific populations have been separated for at least 1.5–3.0 million years, and it seems reasonable that size homoplasy may account for some of the reduction in the magnitude of population subdivision seen in microsatellite loci as compared to scnDNA.

A second explanation for the relatively low microsatellite F_{ST} values is that it is simply an inappropriate statistic with which to estimate population subdivision using microsatellites due to the large number of alleles generally found at these loci (Hedrick 1999). In essence, a high level of within-population heterozygosity overshadows differences between populations, resulting in an underestimation of subdivision (Wright 1978). It is also possible that the lower population subdivision detected by microsatellites is a result of a relatively recent burst of male-mediated gene flow between the ocean basins. This, however, is the most difficult possibility to fully support. To our knowledge, no tracking studies able to detect movement between ocean basins have been done. Nonetheless, the results presented here may provide some motivation to attempt such studies. Although simply documenting the movement of individuals between oceans does not necessarily equate with gene flow, it would at least bolster the possibility. As microsatellite loci can detect early stages of population divergence, they also can detect initial stages or low levels of admixture (Lougheed *et al.* 2000). The clustering of Indian populations with the Atlantic clade and the routine stranding of green turtles around the southern tip of Africa may in fact reflect low-level interocean mixing.

Finally, it is possible that a bias such as disruptive selection may occur in the scnDNA estimates in Karl *et al.* (1992), causing an overestimation of the population subdivision. We believe that this is unlikely. The reasons for this are thoroughly discussed in Karl *et al.* (1992) and essentially rely on the likely neutrality of these markers. In any case, this comparison underscores the need for assessment of multiple genetic markers when estimating the degree of population subdivision of natural populations. By using nuclear markers, sex-specific mixing was detected. By using both quickly and slowly evolving nuclear markers, population connectivity over a broader time span was assessed.

Overall, this study supports and advances previous research on male-mediated gene flow in green sea turtles. Intraoceanic populations are clearly connected by gene flow principally through males. The putatively surprising connection between the Indian and Atlantic Oceans is once again found and likely represents a real, biological phenomenon. However, further studies (*e.g.*, satellite tagging near South Africa) are needed to better

document the pattern, frequency, and fate of wayward interocean migrants. Assessment of the microsatellite flanking sequences indicates that allele homoplasy due to indels in these regions may result in difficulty for traditional analytical methods. Allele frequency distributions indicate that, at least in some cases, microsatellite loci may be evolving by the two-phase mutational model.

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LITERATURE CITED

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman *et al.*, 1994 *Current Protocols in Molecular Biology*, Vol. 1. John Wiley & Sons, Hoboken, NJ.

Bass, A. L., D. A. Good, K. A. Bjorndal, J. I. Richardson, Z. M. Hillis *et al.*, 1996 Testing models of female reproductive migratory behavior and population structure in the Caribbean hawksbill turtle, *Eretmochelys imbricata*, with mtDNA sequences. Mol. Ecol. **5:** 321–328.

Bowen, B. W., A. B. Meylan and J. C. Avise, 1989 An odyssey of the green sea turtle: Ascension Island revisited. Proc. Natl. Acad. Sci. USA **86:** 573–576.

Bowen, B. W., A. B. Meylan, J. P. Ross, C. J. Limpus, G. H. Balazs *et al.*, 1992 Global population structure and natural history of the green sea turtle (*Chelonia mydas*) in terms of matriarchal phylogeny. Evolution **46:** 865–880.

Bowen, B. W., A. M. Clark, F. A. Abreu-Grobois, A. Chaves, H. A. Reichart *et al.*, 1997 Global phylogeography of the ridley sea turtles (*Lepidochelys* spp.) as inferred from mitochondrial DNA sequences. Genetica **101:** 179–189.

Bowen, B. W., A. L. Bass, L. A. Rocha, W. S. Grant and D. R. Robertson, 2001 Phylogeography of the trumpetfishes (*Aulostomus*): ring species complex on a global scale. Evolution **55:** 1029–1039.

Brown, W. M., M. J. George and A. C. Wilson, 1979 Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA **76:** 1967–1971.

Carr, A., 1967 *So Excellent a Fishe: A Natural History of Sea Turtles.* Scribner, New York.

Carr, A., 1986 Rips, FADS and little loggerheads. Bioscience **36:** 92–100.

Carr, A., and L. Ogren, 1960 The ecology and migrations of sea turtles, IV: the green turtle in the Caribbean Sea. Bull. Am. Mus. Nat. Hist. **121:** 1–48.

Caskey, C. T., A. Pizzuti, Y.-H. Fu, J. R.G. Fenwick and D. L. Nelson, 1992 Triplet repeat mutations in human disease. Science **256:** 784–788.

Chow, S., H. Okamoto, N. Miyabe, K. Hiramatsu and N. Barut, 2000 Genetic divergence between Atlantic and Indo-Pacific stocks of bigeye tuna (*Thunnus obesus*) and admixture around South Africa. Mol. Mol. Ecol. **9:** 221–227.

Crandall, K. A., O. R. P. Bininda-Emonds, G. M. Mace and R. K. Wayne, 2000 Considering evolutionary processes in conservation biology. TREE **15:** 290–295.

Di Rienzo, A. D., A. C. Peterson, J. C. Garza, A. M. Valdes, M. Slatkin *et al.*, 1994 Mutational processes of simple-sequence repeat loci in human populations. Proc. Natl. Acad. Sci. USA **91:** 3166–3170.

Dizon, A. E., and G. H. Balazs, 1982 Radio telemetry of Hawaiian green turtles at their breeding colony. Mar. Fish. Rev. **44:** 13–20.

Dutton, P. H., B. W. Bowen, D. W. Owens, A. Barragan and S. K. Davis, 1999 Global phylogeography of the leatherback turtle (*Dermochelys coriacea*). J. Zool. **248:** 397–409.

Encalada, S. E., P. N. Lahanas, K. A. Bjorndal, A. B. Bolten, M. M. Miyamoto *et al.*, 1996 Phylogeography and population structure of the Atlantic and Mediterranean green turtle *Chelonia mydas*: a mitochondrial DNA control region sequence assessment. Mol. Ecol. **5:** 473–483.

Estoup, A., L. Garney, M. Solignac and J.-M. Cornuet, 1995 Microsatellite variation in the honey bee (*Apis mellifera* L.) populations: hierarchical genetic structure and test of the infinite allele and stepwise mutation models. Genetics **140:** 679–695.

Felsenstein, J., 1989 PHYLIP—phylogeny inference package (version 3.2). Cladistics **5:** 164–166.

Fitzsimmons, N. N., C. Moritz and S. S. Moore, 1995 Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. Mol. Biol. Evol. **13:** 432–440.

Fitzsimmons, N. N., C. J. Limpus, J. A. Norman, A. R. Goldizen, J. D. Miller *et al.*, 1997a Philopatry of male marine turtles inferred from mitochondrial DNA markers. Proc. Natl. Acad. Sci. USA **94:** 8912–8917.

Fitzsimmons, N. N., C. Moritz, C. J. Limpus, L. Pope and R. Prince, 1997b Geographic structure of mitochondrial and nuclear gene polymorphisms in Australian green turtle populations and male-biased gene flow. Genetics **147:** 1843–1854.

Garza, J. C., and N. B. Freimer, 1996 Homoplasy for size at microsatellite loci in humans and chimpanzees. Genome Res. **6:** 211–217.

Goldstein, D. B., A. R. Linares, L. L. Cavalli-Sforza and M. W. Feldman, 1995 Genetic absolute dating based on microsatellites and the origin of modern humans. Proc. Natl. Acad. Sci. USA **92:** 6723–6727.

Goodman, S. J., 1997 Rst Calc: a collection of computer programs for calculating estimates of genetic differentiation from microsatellite data and determining their significance. Mol. Ecol. **6:** 881–885.

Gordon, A. L., 1985 Indian-Atlantic transfer of thermocline water at Agulhas retroflection. Science **227:** 1030–1033.

Goudet, J., 1995 Fstat version 1.2. A computer program to calculate F-statistics. J. Hered. **86:** 485–486.

Grimaldi, M.-C., and B. Crouau-Roy, 1997 Microsatellite allelic homoplasy due to variable flanking sequences. J. Mol. Evol. **44:** 336–340.

Hedrick, P. W., 1999 Perspective: highly variable loci and their interpretation in evolution and conservation. Evolution **53:** 313–318.

Hendrickson, J. R., 1958 The green sea turtle, *Chelonia mydas* (Linn.) in Malaya and Sarawak. Proc. Zool. Soc. Lond. **130:** 455–535.

Jarne, P., and P. J. L. Lagoda, 1996 Microsatellites, from molecules to populations and back. TREE **11:** 424–429.

Jeffreys, A. J., N. J. Royle, V. Wilson and Z. Wong, 1988 Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. Nature **322:** 204–209.

Johnson, S. A., and L. M. Ehrhart, 1994 Nest-site fidelity of the Florida green turtle, p. 83 in *Proceedings of the Thirteenth Annual Symposium on Sea Turtle Biology and Conservation*, edited by B. A. Schroeder and B. E. Witherington. National Oceanic and Atmospheric Administration Technical Memo, Miami.

Karl, S. A., B. W. Bowen and J. C. Avise, 1992 Global population genetic structure and male-mediated gene flow in the green turtle (*Chelonia mydas*): RFLP analysis of anonymous nuclear loci. Genetics **131:** 163–173.

Kimura, M., and T. Ohta, 1978 Stepwise mutation model and distribution of allelic frequencies in a finite population. Proc. Natl. Acad. Sci. USA **75:** 2868–2872.

Lessios, H. A., B. D. Kessing and J. S. Pearse, 2001 Population structure and speciation in tropical seas: global phylogeography of the sea urchin *Diadema*. Evolution **55:** 955–975.

Levinson, G., and G. A. Gutman, 1987 High frequency of short frame shifts in poly-CA/GT tandem repeats borne by bacteriophage M13 in *Escherichia coli* K-12. Nucleic Acids Res. **15:** 5323–5338.

Limpus, C. J., 1993 The green turtle, *Chelonia mydas*, in Queens-

land—breeding males in the southern Great Barrier Reef. Wild. Res. **20:** 513–523.

Limpus, C. J., A. Fleay and M. Guinea, 1984 Sea turtles of the Capricornia Section, Great Barrier Reef, pp. 61–78 in *The Capricornia Section of the Great Barrier Reef: Past, Present and Future*, edited by W. T. Ward and P. Saenger. Royal Society of Queensland and Australian Coral Reef Society, Brisbane, Australia.

Lougheed, S. C., H. L. Gibbs, K. A. Prior and P. J. Weatherhead, 2000 A comparison of RAPD versus microsatellite DNA markers in population studies of the Massasauga rattlesnake. Genetics **91:** 458–463.

Lutjeharms, J. R. E., and A. L. Gordon, 1987 Shedding of an Agulhas ring observed at sea. Nature **325:** 138–140.

Mann, H. B., and D. R. Whitney, 1947 On a test of whether one of two random variables is stochastically larger than the other. Annu. Rev. Math. Stat. **30:** 103–104.

Mariette, S., D. Chagné, C. Lézier, P. Pastuszka, A. Raffin *et al.*, 2001 Genetic diversity within and among *Pinus pinaster* populations: comparison between AFLP and microsatellite markers. Heredity **86:** 469–479.

Meylan, A. B., 1982 Sea turtle migration—evidence from tag returns, pp. 91–100 in *Biology and Conservation of Sea Turtles*, edited by K. A. Bjorndal. Smithsonian Institution Press, Washington, DC.

Meylan, A. B., B. W. Bowen and J. C. Avise, 1990 A genetic test of the natal homing versus social facilitation models for green turtle migration. Science **248:** 724–726.

Minch, E., 1995 *Microsat Version 1.4*. Stanford University, Stanford, CA.

Moritz, C., 2002 Strategies to protect biological diversity and the evolutionary processes that sustain it. Syst. Biol. **51:** 238–254.

Mullis, K. B., and S. B. Wiss, 1987 Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol. **155:** 335–350.

Nei, M., 1972 Genetic distance between populations. Am. Nat. **106:** 283–292.

Orti, G., D. E. Pearse and J. C. Avise, 1997 Phylogenetic assessment of length variation at a microsatellite locus. Proc. Natl. Acad. Sci. USA **94:** 10745–10749.

Owens, D. W., M. A. Grassman and J. R. Hendrickson, 1982 The imprinting hypotheses and sea turtle reproduction. Herpetologica **36:** 17–20.

Pritchard, P. C. H., 1997 Evolution, phylogeny, and current status, pp. 2–28 in *The Biology of Sea Turtles*, edited by P. L. Lutz and J. A. Musick. CRC Press, New York.

Pritchard, P. C. H., 1999 Status of the black turtle. Conserv. Biol. **13:** 1000–1003.

Raymond, M., and F. Rousset, 1995 Genepop (version 1.2): population genetic software for exact tests and ecumenicism. J. Hered. **86:** 248–249.

Rice, W. R., 1989 Analyzing tables of statistical tests. Evolution **43:** 223–225.

Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi *et al.*, 1988 Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239:** 487–491.

Saitou, N., and M. Nei, 1987 The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:** 406–425.

Schlötterer, C., and D. Tautz, 1992 Slippage synthesis of simple sequence DNA. Nucleic Acids Res. **20:** 211–215.

Schneider, S., D. Roessli and L. Excoffier, 2002 *Arlequin v. 2.000: A Software for Population Genetics Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Geneva.

Shriver, M. D., L. Jin, R. Chakraborty and E. Boerwinkle, 1993 VNTR allele frequency distributions under the stepwise mutation model: a computer simulation approach. Genetics **134:** 983–993.

Slatkin, M., 1995 A measure of population subdivision based on microsatellite allele frequencies. Genetics **139:** 457–462.

Sokal, R. P., and F. J. Rohlf, 1981 *Biometry*. W. H. Freeman, New York.

Spearman, C., 1904 The proof and measurement of the association between two things. Am. J. Psych. **15:** 72–101.

Stephan, W., 1989 Tandem-repetitive noncoding DNA: forms and forces. Mol. Biol. Evol. **6:** 198–212.

Strand, M., T. A. Prolla, R. M. Liskay and T. D. Petes, 1993 Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. Nature **365:** 274–276.

Tautz, D., and M. Rentz, 1984 Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res. **17:** 4127–4138.

Valdes, A. M., M. Slatkin and N. B. Freimer, 1993 Allele frequencies at microsatellite loci: the stepwise mutation model revisited. Genetics **133:** 737–749.

Waples, R. S., 1995 Evolutionary significant units and the conservation of biological diversity under the Endangered Species Act. Am. Fish. Soc. Symp. **17:** 8–27.

Weber, J. L., 1990 Informativeness of human (dC-dA)n.(dG-dT)n polymorphisms. Genomics **7:** 517–524.

Weir, B. S., and C. C. Cockerham, 1984 Estimating F-statistics for the analysis of population structure. Evolution **38:** 1358–1370.

Workman, P. L., and J. D. Niswander, 1970 Population studies on southwestern Indian tribes. II. Local genetic differentiation in Papago. Am. J. Hum. Genet. **22:** 24–49.

Wright, S., 1943 Isolation by distance. Genetics **28:** 139–156.

Wright, S., 1951 The genetical structure of populations. Ann. Eugen. **15:** 323–354.

Wright, S., 1978 *Evolution and the Genetics of Populations, Vol. 4: Variability Within and Among Natural Populations*. University of Chicago Press, Chicago.

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