Conservation and Dynamics of Microsatellite Loci over 300 Million Years of Marine Turtle Evolution

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Microsatellite loci consisting of $(CA)_n$ repetitive arrays were obtained from three species of marine turtle, and primers were designed to test for polymorphism within species and the persistence of microsatellites across species. Homologous loci were found in each test of six marine species within two families (Cheloniidae and Dermochelyidae), as well as in a freshwater species (Emydidae, *Trachemys scripta*), which indicates a conservation of flanking sequences spanning approximately 300 million years of divergent evolution. The persistence of homologous microsatellites across marine turtles was confirmed by direct sequencing of loci across species and by the discovery of polymorphism in 24 of 30 cross species tests. The conservation of flanking sequences could be due to a slow rate of base substitution in turtle nuclear DNA, as previously reported for mtDNA. In contrast, the presence of up to 25 alleles per locus per species indicates that the replication slippage events responsible for changes in allele length operate as in mammals. Comparisons of alleles among species revealed that alleles of the same length may not be homologous due to mutations within the flanking sequences. Levels of heterozygosity were consistently higher in species from which the primers were designed, which suggests problems with cross-species comparisons of variability. Within species, microsatellite variation between divergent populations was consistent with results from previous mtDNA studies indicating the usefulness of microsatellites for comparing male- versus female-mediated gene flow.

Introduction

Microsatellite loci, sequences containing arrays of short (2–5 bp) tandem repeats with variable copy number (Tautz 1989), have proved to be powerful tools for gene mapping (Weber and May 1989; Weissenbach et al. 1992) and paternity studies (reviewed in Queller et al. 1993) because of their high heterozygosity. It has also been suggested that microsatellite loci have great potential for broader applications such as comparative gene mapping (Beckman and Soller 1990; Moore et al. 1991) and assessing genetic population structure within species (Bowcock et al. 1994; Roy et al. 1994). For this potential to be realized, we need a better understanding of how microsatellite loci evolve within and across species (Bruford and Wayne 1993; Valdes et al. 1993). On the one hand, it is possible that mutation rates are so high that common ancestry is obscured even within populations. On the other hand, there may be constraints on microsatellite loci resulting in excessive convergence of allelic states, leading to underestimation of allelic divergence among populations.

Information on the dynamics of microsatellite evolution has come mainly from studies of intraspecific polymorphism, predominantly in humans. Mutation rates estimated from pedigrees are in the range of 10^{-2}–10^{-5} (Edwards et al. 1992; Mahatani and Willard 1993), and changes in copy number of the repeats are thought to be due to slipped strand mispairing (Schlötterer and Tautz 1992). Mutations observed within pedigrees typically involve the gain or loss of one or a few repeat units (reviewed by Valdes et al. 1993, but see Kuhl and Caskey 1993), and comparisons of observed and simulated allele frequency distributions suggest broad agreement with stepwise mutation models (Shriver et al. 1993; Valdes et al. 1993) which allow for mutations that revert allele length to previous states. The potential for mechanical or selective constraints on microsatellite arrays is suggested by recent reports of mutational instability of long repeat arrays (reviewed in Kuhl and Caskey 1993).

In comparison to analyses of within-species polymorphism, little attention has been given to patterns of transpecific microsatellite evolution. The use of microsatellite loci across species depends on the conservation of priming sites within flanking sequences to enable amplification and on the maintenance of repeat arrays long

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enough to promote polymorphism (Weber 1990). Arrays may be reduced or extensively modified by slippage and base substitution, this process creating mosaics of interrupted or adjacent repeats (Levinson and Gutman 1987). Persistence of microsatellite arrays, albeit in modified form, has been reported among divergent mammalian families, that is, primates, artiodactyl, and rodents (Moore et al. 1991; Stallings et al. 1991). However, the proportion of loci that are polymorphic seems to drop rapidly with increasing evolutionary divergence in mammals. Of 48 microsatellite loci developed for cattle, 56% could be amplified, 42% were polymorphic in sheep (15 to 25-million-year divergence; see Irwin et al. 1991), and none could be amplified specifically in humans (80 to 100-million-year divergence; Marshall 1990) (Moore et al. 1991). Cetaceans showed more conservation; primers designed for an odontocetid whale were polymorphic in other toothed whales as well as in baleen whales, which indicates conservation of polymorphic microsatellites over 35–40 million years of evolution (Schlötterer et al. 1991). Systematic surveys of cross-species polymorphism for taxa other than mammals are generally lacking.

In this paper we document the conservation of polymorphic microsatellite loci across the two extant families of marine turtles, Cheloniidae and Dermochelyidae. Microsatellite loci isolated from three genera of Chelonids were polymorphic across the family, spanning genera which diverged 12–75 million years ago (see Bowen et al. 1993) and also in the Dermochelyidae, thought to have diverged 100–150 million years ago (Zangerl 1980; Weems 1988). Additionally, we find persistence of some microsatellite loci in both marine and freshwater turtles. We take advantage of this unprecedented conservation to examine patterns of transpecies polymorphism across approximately 300 million years of divergent evolution.

Material and Methods

Isolation of Microsatellites

CA/GT microsatellites were obtained from libraries prepared from three species of marine turtles: green (*Chelonia mydas*), loggerhead (*Caretta caretta*), and hawksbill (*Eretmochelys imbricata*). Genomic DNA was isolated from muscle tissue by standard phenol/chloroform methods (Maniatis et al. 1982), digested with *Sau*3A1, run on a 1.5% low-melt agarose gel, and the 350- to 500-bp region was selected (Moore et al. 1994) and purified using a Prep-A-Gene kit (Bio-Rad). Turtle DNA was ligated to PUC19 plasmid (*Bam*HI cut and phosphatased, Pharmacia) and transformed into fresh competent cells prepared using a CaCl/RbCl method (Inoue et al. 1990). Transformed colonies were grown on Hybond N+ membranes (Amersham, Sydney) and duplicate transferred to Zeta-Probe blotting membranes (BioRad, North Ryde, New South Wales). A synthetic oligonucleotide probe, $(dA\text{-}dC)_n$-$(dG\text{-}dT)_n$ (Pharmacia, North Ryde, New South Wales), was prepared by random-priming (Feinberg and Vogelstein 1984) (Mega-Prime kit, Amersham, Sydney) with $\alpha^{32}P$ and overnight hybridization carried out at 65° (Moore et al. 1994). Filters were washed twice in 2 × SSC and 0.1% SDS for 20 minutes each, first at room temperature and then at 65°. A final wash was done in 0.2 × SSC and 0.1 × SDS at 65°. Positive colonies were identified by exposure to X-ray film and selected from the original filters to establish the initial microsatellite library. This library was then subjected to a second round of hybridization with the $(dA\text{-}dC)_n$-$(dG\text{-}dT)_n$ probe to confirm positive colonies. Ligated DNA was isolated from the positive clones and screened for microsatellites by cycle sequencing with $\gamma^{33}P$ (Murray 1989). Primer pairs were designed for dinucleotide microsatellites that contained >12 uninterrupted repeats using Oligo 4.0-s software (Rychlik 1992) to compare various 24-bp primer combinations selected at either end of the flanking region.

Extraction of DNA and Amplification of Microsatellites

Small amounts of blood (1–3 ml for adults, 50–200 µl for hatchlings) were collected from the dorsal cervical sinus. Total DNA was extracted from whole blood (5 µl) by proteinase K digestion (1 mg/ml) at 60° for 30 min in lysis buffer (300 µl; 100 mM Tris, 100 mM EDTA, 10 mM NaCl, 0.5% SDS), precipitation of the fractionate with NH_4 Acetate (150 µl; 7.5M), and ethanol precipitation (100%, 2×vol) of the DNA from the supernatant. Polymerase chain reactions (PCR) incorporated $\alpha^{32}P$ dCTP into the PCR products (Moore et al. 1991) and typically included an initial denaturation at 95° for 2.5 min, followed by 30 cycles consisting of 45 s at 95°, 1 min at 55°, 1 min at 72°, and a final extension for 5 min at 72°. Products were run through 6% denaturing sequence gels and their lengths determined from plasmid DNA which was cycle sequenced with $\gamma^{33}P$ (Murray 1989) and added to the gel as a size marker.

Analysis of cross-species polymorphism focused on four loci isolated from the green turtle and one locus from each of the other two species to reduce bias due to the source of the primers. We tested for polymorphism at homologous loci in six species within the two extant marine turtle families: the green turtle *Chelonia mydas,* the hawksbill *Eretmochelys imbricata,* the loggerhead *Caretta caretta,* the Pacific ridley *Lepidochelys olivacea,* and the flatback *Natator depressus* in the Cheloniidae; and the leatherback *Dermochelys coriacea* in the Dermochelyidae. We obtained samples from widely separated geographic populations within Australia (and globally for *Dermochelys*), including populations pre-

Table 1
PCR Primers Designed to Amplify Marine Turtle Microsatellite Loci

Species/Locus	Primer (5′ to 3′)	Array	Range
Chelonia mydas/Cm3	AATACTACCATGAGATGGGATGTG ATTCTTTTCTCCATAAACAAGGCC	$(CA)_{13}$	6–38
C. mydas/Cm 58	GCCTGCAGTACACTCGGTATTTAT TCAATGAAAGTGACAGGATGTACC	$(CA)_{13}$	8–18
C. mydas/Cm72	CTATAAGGAGAAAGCGTTAAGACA CCAAATTAGGATTACACAGCCAAC	$(CA)_{33}$	25–49
C. mydas/Cm84	TGTTTTGACATTAGTCCAGGATTG ATTGTTATAGCCTATTGTTCAGGA	$(CA)_{15}$	6–21
Caretta caretta/Cc117	TCTTTAACGTATCTCCTGTAGCTC CAGTAGTGTCAGTTCATTGTTTCA	$(CA)_{17}$	8–23
Eretmochelys imbricata/Ei8	ATATGATTAGGCAAGGCTCTCAAC AATCTTGAGATTGGCTTAGAAATC	$(CA)_{19}$	8–22

NOTE.—The length of the original uninterrupted array is given along with the range in the number of repetitive units that were observed within that species.

viously shown to be distinct for mitochondrial DNA (e.g., eastern vs. western Australian populations of *Chelonia mydas,* cf. Norman et al. 1994; and of *Eretmochelys imbricata,* cf. Broderick et al. 1994). We also tested the PCR primers in a species of freshwater emydid turtle, *Trachemys scripta.* To confirm the identity of cross-species amplification products, PCR products from homozygous individuals at each locus were gel purified and cycle sequenced using both forward and reverse primers. Sequences were aligned using CLUSTAL V software (Higgins et al. 1992).

Statistical Analyses

Differences in observed heterozygosity between source and nonsource species were tested using arcsine-transformed values in Student's *t*-test and ANOVA. We examined the relationship between observed heterozygosity and elapsed evolutionary time, using transversion distances calculated from published cytochrome *b* mitochondrial DNA sequences (Bowen et al. 1993) as a surrogate for the latter. Cytochrome *b* transversion distances were estimated from a phylogeny obtained using CLUSTAL V (Higgins et al. 1992) alignments and PHYLIP (version 3.4; Felsenstein 1991). The significance of allele frequency differences among populations of green and hawksbill turtles was tested by comparing observed χ^2 values with those obtained from 1,000 randomized data sets (Roff and Bentzen 1989) as implemented in the MONTE routine of REAP (McElroy et al. 1992).

Results

Isolation of Microsatellites

A total of 142 positive colonies was identified from the three species after screening approximately 64,000 colonies. We sequenced 63 of these colonies and found 24 unique microsatellites: 11 from the green turtle, 7 from the hawksbill, and 6 from the loggerhead. Most (14) of the microsatellite loci were compound in structure, with both CA_n and related repeats joined: one mixed array extending for over 106 bp included series of CA_n and CT_n repeats. Of the remainder, seven microsatellites had perfect repetitive arrays, and three were imperfect (as defined by Weber 1990). Unbroken microsatellite arrays ranged from 7–34 repeat units with a mean of 16.

Seven sets of primers were designed from these sequences (table 1): four to amplify green turtle microsatellites, two for hawkbills, and one for loggerheads. One set of hawksbill primers amplified a continuous ladder of bands, possibly indicating a location of the locus within a highly repetitive region: this locus was dropped from further analysis. At the remaining six loci, the original number of uninterrupted CA repeats ranged from 13–33, three loci displayed perfect repeat sequences (Weber 1990), two (Cm3 and Ei8) were compound repeat sequences, and one (Cm84) contained additional mononucleotide repeat sequences (⩾10 repeats).

Microsatellite Amplification and Polymorphism

Within source species, each microsatellite locus was highly variable, with observed heterozygosity ranging from 0.757 (Ei8) to 0.932 (Cm3) and the total number of alleles from 9 to 25 (table 2). The numbers of repeats varied from 6 to 49 (table 1) under the assumption that the flanking sequences were of constant length. The Cm72 locus, which contained the longest repeat array, also was the most variable, and a positive relationship was found between the mean number of repeats within a source species and the number of alleles (linear regression, P=0.033, r^2=0.718, df=5).

Table 2
Numbers of Microsatellite Alleles and Sample Size, Size Range, and Observed Heterozygosity Values at Six Loci in Six Species of Marine Turtles

	Microsatellite Loci					
Species	Cm3	Cm58	Cm72	Cm84	Cc117	Ei8
Cheloniidae:						
Chelonia mydas:						
	21 (44)	9 (43)	25 (40)	19 (41)	17 (45)	1 (42)
r	138–202	130–148	237–295	325–365	228–266	170
h	0.932	0.744	0.900	0.707	0.844	0.000
Natator depressus:						
	6 (23)	3 (24)	4 (21)	3 (23)	3 (25)	3 (25)
r	171–185	127–131	232–240	319–345	232–250	186–216
h	0.478	0.125	0.286	0.391	0.400	0.240
Caretta caretta:						
	1 (43)	1 (43)	5 (44)	11 (42)	11 (43)	13 (44)
r	151	126	242–250	314–336	232–262	174–202
h	0.000	0.000	0.705	0.929	0.791	0.750
Lepidochelys olivacea:						
	1 (10)	1 (9)	2 (10)	6 (9)	3 (9)	6 (9)
r	163	126	235–239	328–338	216–234	186–226
h	0.000	0.000	0.900	0.444	0.111	0.444
Eretmochelys imbricata:						
	2 (38)	8 (36)	7 (39)	15 (34)	7 (36)	13 (37)
r	151–153	124–142	231–243	314–350	212–245	194–222
h	0.079	0.722	0.615	0.706	0.556	0.757
Dermochelyidae:						
Dermochelys coriacea:						
	6 (15)	3 (12)	1 (14)	4 (3)	11 (15)	6 (12)
r	169–187	119–125	225	348–354	224–252	192–254
h	0.533	0.417	0.000	0.667	0.530	0.250

Note.—Loci are designated by source species: Cm, *Chelonia mydas;* Cc, *Caretta caretta;* Ei, *Eretmochelys imbricata.* Sample sizes are shown in parentheses. *r,* bp range of allele length (bp); and *h,* observed heterozygosity.

All six primers amplified the microsatellite arrays of interest in each of the six species of marine turtle tested (e.g., fig. 1). Amplifications of nonsource species revealed polymorphism in 19 of 24 combinations within the Cheloniidae and five of six for the leatherback *Dermochelys coriacea* (table 2). Two of the green turtle loci (Cm3 and Cm58) were monomorphic in both *Caretta caretta* and *Lepidochelys olivacea* but revealed poly-

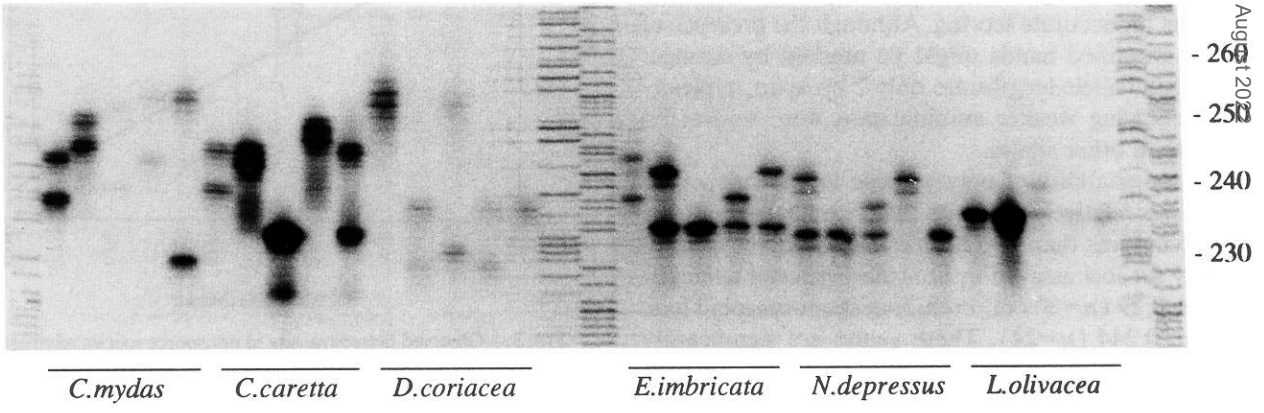

Fig. 1.—Autoradiograph of two marine turtle microsatellite loci, Cc117 and Cm72, each amplified in three species: *Chelonia mydas, Caretta caretta, Dermochelys coriacea,* and *Eretmochelys imbricata, Natator depressus,* and *Lepidochelys olivacea.* Allele sizes (bp) are indicated by reference to Bluescript plasmid sequence.

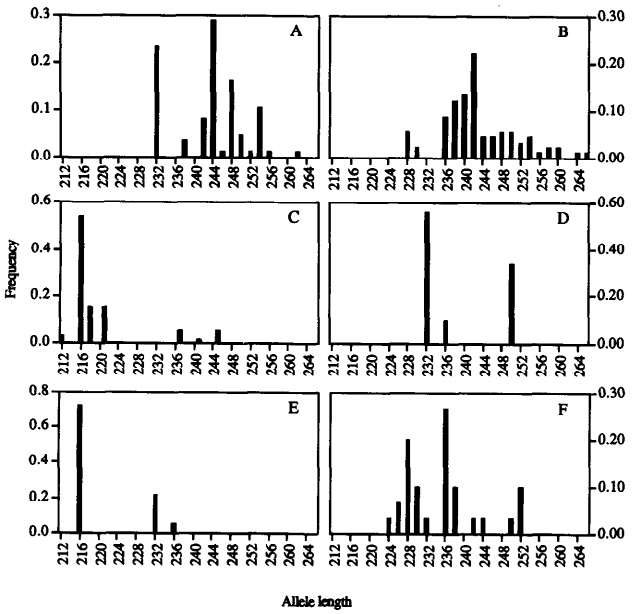

FIG. 2.—Allele frequency histograms for six species of marine turtle at the Cc117 microsatellite locus (derived from *Caretta caretta*). *A, C. caretta; B, Chelonia mydas; C, Eretmochelys imbricata; D, Natator depressus; E, Lepidochelys olivacea; F, Dermochelys coriacea.*

morphism in other species, including the leatherback. A third green turtle primer, Cm72, was uniform in the leatherback, and the hawksbill-derived locus, Ei8, was monomorphic in *Chelonia mydas*. The remaining three loci were variable across all marine turtles (e.g., fig. 2).

A greater proportion of samples from nonsource species failed to amplify at single loci relative to samples from source species, though this difference was not significant (one-tailed *t*-test; mean number of failures per locus was 0.048±.095 and 0.023±0.02 respectively, $P>0.5$). Amplifications from nonsource species were sometimes weaker than source species, although there was also a large variation in band intensity within species (fig. 1): both were problems which required reexposure of the gel for accurate scoring. Although the presence of weakly amplified bands might be masked by stronger bands if the allele lengths are only 2 bp apart, typically alleles showing weaker amplification were several bp longer than other alleles.

The variability of polymorphic loci in nonsource species was relatively low, with many combinations (10/24) having less than five alleles. The observed heterozygosity for loci assayed in nonsource species averaged 0.377 ± 0.29 ($n=30$) or, excluding monomorphic loci, 0.472 ± 0.244 ($n=24$). These values are significantly lower than the mean heterozygosity of the six loci analyzed from the source species (0.805±0.091; one-tailed *t*-tests on arcsine-transformed values, $P<0.005$). This observed decrease in heterozygosity in nonsource species remains significant even when the species with smaller samples sizes (<20 animals) are removed (df=22, $P=0.008$). For the four "Cm" loci, the overall decline in heterozygosity from source to nonsource species was not correlated (linear regression; $r=0.697$, df=5, $P=0.12$) with genetic distance, as measured by cytochrome b tranversion distances from *C. mydas* (fig. 3). Within nonsource species there was little evidence for a continued decline in heterozygosity with increased genetic distance from the source species: even with small sample sizes, heterozygosity was relatively high in *D. coriacea*. In contrast to the general pattern, heterozygosity was greatest in nonsource species at the Cc117 locus for both green and leatherback turtles.

The frequency distributions of allele lengths within species were complex, with the same loci showing unimodal distributions in some species and multimodal distributions in others (e.g., fig. 2), irrespective of the number of alleles. Allele distribution, however, was constrained as most (74%) alleles were separated from their nearest neighbors by only 2 bp, and the largest distance between alleles at any locus was 24 bp. Loci with <6 alleles had a greater frequency of nonadjacent (distances >2 bp) alleles than loci with ≥6 alleles (0.31 and 0.14, respectively). Although the maximum range in allelic lengths across species at a particular locus was not large (≤84 bp; table 2), there was significant divergence in the mean allele length among species at all but one locus (Cm3) (ANOVA, df=5, $P<0.005$).

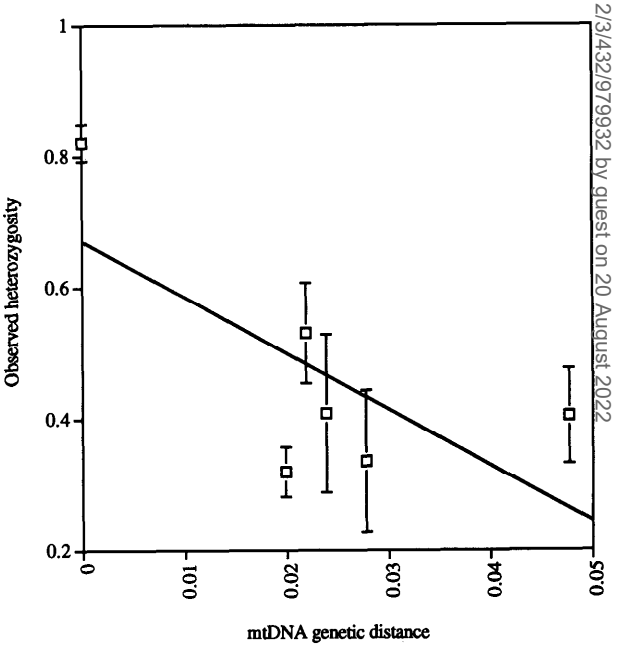

FIG. 3.—Observed heterozygosity in nonsource species relative to the source species at marine turtle microsatellites with increased genetic distance from source species. Genetic distance is calculated as cytochrome *b* transversions, and heterozygosity is calculated as the average observed values for the Cm3, Cm58, Cm72, and Cm84 loci; showing ±1 SE. The linear regression is described as follows: $y = -8.53x + 0.67$, $r^2 = 0.49$, $P = 0.12$.

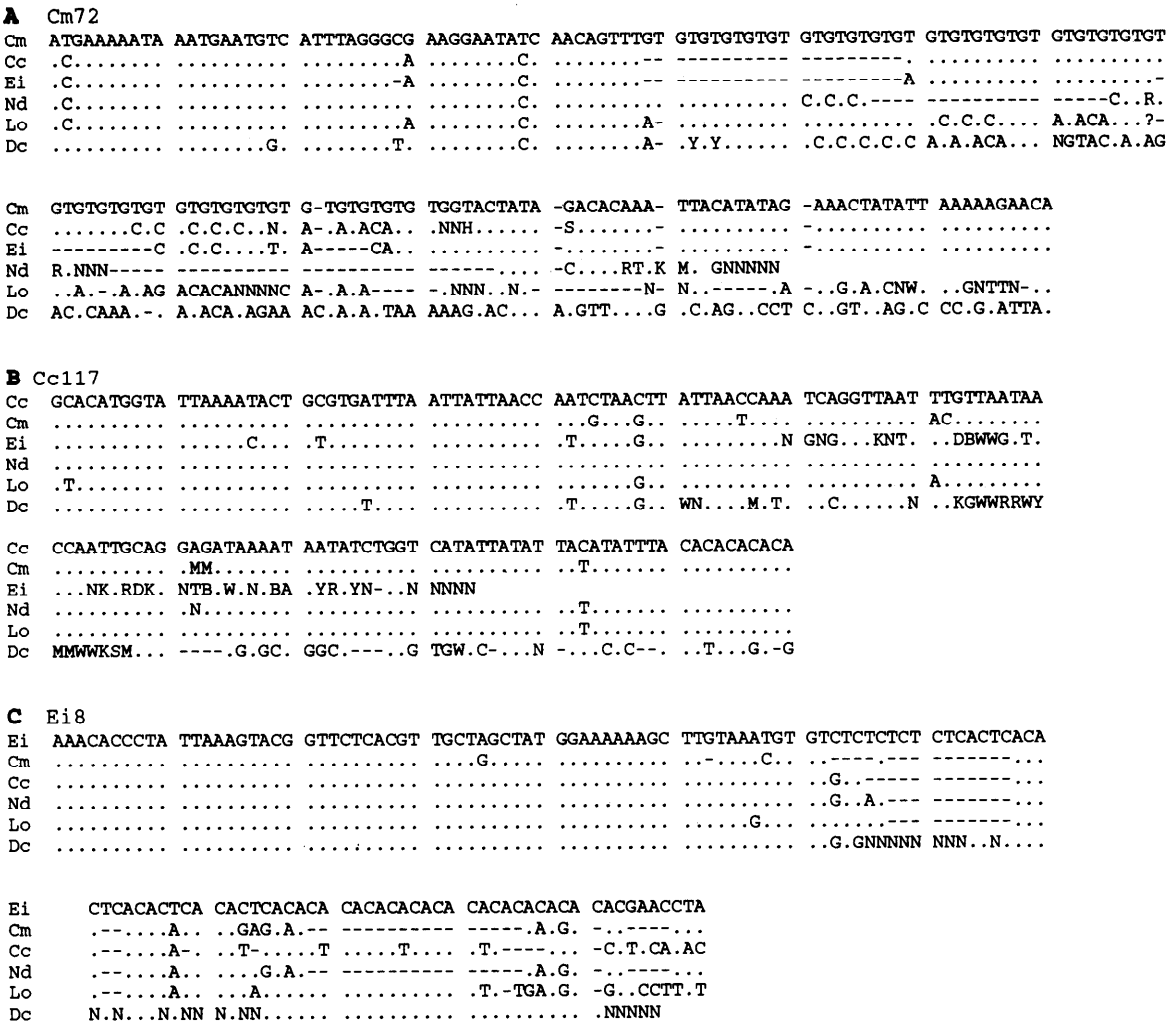

FIG. 4.—Partial sequences of PCR products at homologous microsatellite loci in six species of marine turtles showing homology of products and the presence of insertions and deletions in flanking regions. *A,* Cm72 locus derived from *Chelonia mydas; B,* Cc117 locus derived from *Caretta caretta; C,* Ei8 locus derived from *Eretmochelys imbricata.* Other abbreviations are as follows: Lo, *Lepidochelys olivacea;* Nd, *Natator depressus;* and Dc, *Dermochelys coriacea.* Colons indicate identical sequences; dashes indicate indels. All sequences shown begin 7 bp downstream of the appropriate primer. Coding for ambiguous bases is as follows: R, A/G; Y, C/T; M, A/C; W, A/T; S, C/G; K, G/T; B, C/G/T; D, A/G/T; H, A/C/T; N, A/C/G/T.

Within species, comparisons of allele frequencies between eastern and western Australian nesting populations of green and hawksbill turtles revealed significant heterogeneity. Nesting female green turtles at Heron Island ($n=20$) in the southern Great Barrier Reef were genetically divergent at two of five loci (Cm3 and Cm58; $P<0.05$ in each Monte Carlo χ^2 simulation) from those at North West Cape ($n=14$) in western Australia. This heterogeneity was confirmed for four of four loci in analyses with larger sample sizes (N. FitzSimmons, L. C. Pope, C. J. Limpus, and C. Moritz, unpublished manuscript). Similarly, nesting hawksbills from Milman Island ($n=20$) in the northern Great Barrier Reef and Rosemary Island ($n=19$) in western Australia were genetically divergent at four of six loci (Cm3, Cm58, Cm84, and Ei8; $P<0.001$ in each Monte Carlo χ^2 simulation).

Flanking Sequences

Amplification products from homozygous individuals of nonsource species were sequenced directly to confirm their identity and to examine whether the flanking sequences were conserved in length. Sufficient flanking sequences were obtained to confirm that each of the amplification products for all loci was homologous across species, and data from three loci—Cm72, Ei8, and Cc117—provided clear evidence for insertions and deletions in the flanking sequence of the nonsource species relative to the source species (fig. 4). Thus, microsatellite alleles of the same length in different species do

not necessarily have the same number of repeats. These sequences also revealed examples of the evolution of interrupted and concatenated arrays (e.g., CA_nCG_n in Cm72; fig. 4). The absence of polymorphism for the Cm72 locus in *Dermochelys coriacea* and for the Ei8 locus in *Chelonia mydas* are associated with substantial shortening of the repeat arrays, as well as base substitutions in *D. coriacea.* However, some species with similarly shortened arrays were polymorphic (e.g., *Eretmochelys imbricata* at Cm72 and *Natator depressus* at Ei8; fig. 3 and table 2).

All nonsource species showed new allelic states (i.e., products of novel length) in comparison to the allele lengths observed in the respective source species (e.g., fig. 2). Across species, allele lengths often differed by an odd number of bp, which indicates an indel event in addition to changes in copy number of the CA repeats. A shift in allele length by 1 bp within a species was only seen at one locus (e.g., alleles 212–218 and 221–245 for Cc117 in *E. imbricata;* fig. 2) in which the indel event has distinguished the short versus long alleles.

Discussion

Conservation of Microsatellite Polymorphism

The ability to amplify microsatellite loci and the persistence of polymorphism across extant marine turtles represents the retention of microsatellite flanking regions and polymorphism over an evolutionary period an order of magnitude greater than previously reported (Schlötterer et al. 1991) and contrasts markedly with the evolutionary transience reported for minisatellite loci (Gray and Jeffreys 1991). We have yet to determine the outer limits of application for these primers: all loci could be amplified in a freshwater turtle (*Trachemys scripta*), and three of the six were variable. Therefore, it appears some of these microsatellites originated in an ancestral lineage that predated the split of marine from freshwater turtles. Considering that generation times of marine turtles may range from 15 to 40 years (Limpus and Walter 1980; Frazer 1986), this represents a conservation of polymorphism at homologous loci for over at least 3 million generations within each lineage.

The conservation of the priming sites flanking the turtle microsatellite loci could be explained if the slow rate of nucleotide substitution reported for mtDNA (Avise et al. 1992; Bowen et al. 1993) also applies to nuclear sequences. A low rate of base substitution within the repeat arrays could also account for these loci being polymorphic over such long evolutionary periods due to a reduced probability of repeat arrays being interrupted by base substitutions which tend to reduce the level of polymorphism (Weber 1990). In contrast, the replication slippage process that likely underlies microsatellite variation (Schlötterer and Tautz 1992) appears to be operating as in mammals to produce the high levels of polymorphism at these loci. Particularly within source species, the heterozygosity levels were as high or higher than values reported for mammals (Paetkau and Strobeck 1994; Roy et al. 1994; Taylor et al. 1994).

Evolutionary Dynamics and Implications for Population Studies

The opportunity to examine the magnitude and pattern of polymorphism across species spanning a wide range of evolutionary divergence has provided insights into the dynamics of microsatellite variation. Across species, the distributions of allele lengths were complex and often multimodal (fig. 2). Divergence in mean allele length across species at five loci suggest evolutionary shifts within the different lineages as observed in several homologous human and nonhuman primate microsatellite loci (Bowcock et al. 1994). The variation within species was consistent with the results from previous mtDNA studies (Broderick et al. 1994; Norman et al. 1994) in that both green and hawksbill turtles showed significant differences in allele frequency between eastern and western Australian rookeries. This empirical result, together with the large number of alleles per locus, indicate that microsatellites will be a useful tool for analyses of gene flow in marine turtle populations, including studies of paternity. With regard to the latter, we note that all individuals ($n = 178$) sampled with the exception of two identical pairs of flatbacks had unique genotypes, and in source species most (81.6%) alleles observed were rare (frequency< 0.01).

A strong pattern in the data was for loci to be most variable in their respective source species. To some extent, this may reflect an underlying higher heterozygosity of green turtles from which four of the six loci were derived as the bias was not as strong in the loci derived from the loggerhead and hawksbill turtles. However, the selection of loci with >12 uninterrupted arrays of repeats in the source species is expected to bias estimates of heterozygosity upward in source relative to nonsource species (Bruford and Wayne 1993; Taylor et al. 1994). Some nonsource lineages are likely to have shortened or interrupted arrays (e.g., fig. 4); at the extreme, the microsatellite array was completely missing in *Trachemys scripta* at the Cc117 locus, which probably indicates a separate evolution of the microsatellite within marine turtles. Because of this, comparisons of genetic variability between genera are not appropriate unless several loci derived from each genera are studied. Additionally, the presence of null alleles may confound the interpretation our data (Callen et al. 1993), particularly if mutations within the priming sites of nonsource species result in a greater proportion of null alleles within those species: the likelihood of this occurring will be better understood as these loci are applied in studies of mating systems.

Insertions and deletions in the flanking sequences as well as in the microsatellite array are contributing to increases in the number of alleles across species. Further analysis is needed to correct for this effect in order to make inferences about the dynamics of the repeat arrays among species. This same observation argues against the use of microsatellites for phylogenetic analysis among species because alleles of the same length may not be homologous (see also Bruford and Wayne 1993). For example, measures of genetic distance (using NTSYS, version 1.80: Hillis unbiased, Wright's, and Roger's modified; Rohlf 1993, and $1-P_s$; P_s=proportion of shared alleles) from microsatellite data were not well correlated to cytochrome *b* mtDNA transversion distances (r=0.12–0.50, df=14, P=0.06–0.50), and construction of neighbor-joining trees failed to identify leatherbacks as being substantially more distant from the other species. Additionally, the length of the PCR product could bias the number of allelic states observed as longer flanking sequences may increase the probability of discovering genetic variation in cross-species comparisons. Within species, the frequency of length changes in the flanking sequences has not been determined; however, single base shifts in allele length were rare, which suggests that it is less of a problem.

The primers described here permit the amplification of homologous and highly variable microsatellite loci across all species of marine turtle. These loci will be very useful for analyses of diversity and mating systems within populations and of the distribution of genetic variation among populations. Nuclear gene polymorphism in marine turtles has previously been assayed by allozyme electrophoresis (see, e.g., Bonhomme et al. 1987) and RFLP analysis of PCR-amplified anonymous single-copy loci (Karl et al. 1992). Analysis of microsatellites has revealed far higher levels of polymorphism than either alternative and is more efficiently applied across species than the RFLP analysis. Microsatellite analysis will therefore provide a valuable tool to complement assays of sequence variation in mtDNA and nDNA in studies of the population and conservation biology of marine turtles.

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