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The panmixia paradigm of eastern Pacific olive ridley turtles revised: consequences for their conservation and evolutionary biology

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Abstract

Previous studies of the olive ridley *Lepidochelys olivacea* population structure in the tropical eastern Pacific have indicated the existence of a single panmictic population ranging from Costa Rica to Mexico. This information has been used to design specific management measures to conserve primary nesting beaches in Mexico. However, little is known about olive ridleys in the Baja California Peninsula, their northernmost reproductive limit, where recent observations have shown differences in nesting female behaviour and size of hatchlings relative to other continental rookeries. We used mtDNA control region sequences from 137 turtles from five continental and four peninsular nesting sites to determine whether such differences correspond to a genetic distinction of Baja California olive ridleys or to phenotypic plasticity associated with the extreme environmental nesting conditions of this region. We found that genetic diversity in peninsular turtles was significantly lower than in continental nesting colonies. Analysis of molecular variance revealed a significant population structure (Φ_{ST} = 0.048, P = 0.006) with the inclusion of peninsular samples. Our results: (i) suggest that the observed phenotypic variation may be associated with genetic differentiation and reproductive isolation; (ii) support the recent colonization of the eastern Pacific by *Lepidochelys*; (iii) reveal genetic signatures of historical expansion and colonization events; and (iv) significantly challenge the notion of a single genetic and conservation unit of olive ridleys in the eastern Pacific. We conclude that conservation measures for olive ridleys in Mexico should be revised to grant peninsular beaches special attention.

Keywords: conservation, control region, *Lepidochelys olivacea*, marine turtles, mitochondrial DNA, olive ridley

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Introduction

The olive ridley turtle *Lepidochelys olivacea* is a pantropical species common in the Pacific and Indian oceans, where major nesting beaches and foraging areas occur (Márquez 1990). The eastern Pacific represents the second most important nesting area worldwide, extending from the tip of the Baja California Peninsula to Costa Rica (Fritts *et al*. 1982; Márquez 1996; US National Marine Fisheries Service & US Fish and Wildlife Service 1998). However, most of the reproduction of the species in this region occurs in two main areas: 'La Escobilla' beach in Oaxaca, Mexico, and 'Nancite' beach in Costa Rica, where thousands of females come to nest en masse during a 3-day period, a phenomenon called 'arribada', from the Spanish word for 'arrival'. In southern Baja California (Baja California Sur, BCS henceforth), nesting is of solitary type, according to the low density of nests during beach surveys, indicating fewer nesting females in the area compared to the main nesting beaches (Plotkin *et al*. 1997; López-Castro *et al*. 2004).

During the 1960s and 1970s, olive ridleys were subject to severe exploitation in the eastern Pacific, and more than half

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of captures originated from BCS (Márquez & Villanueva 1982). Over-exploitation resulted in the collapse of eastern Pacific populations bringing them close to extinction (Márquez *et al*. 1976). Unofficial records indicate that more than 2 million olive ridleys were killed in this region (Briseño-Dueñas 1998). Consequently, the Mexican government implemented conservation measures in 1990: a total ban for all marine turtle species was established and a system of official and unofficial (NGO-related) turtle camps was developed (Márquez 1996). Since then, major conservation goals have been centred in surveying and protecting key rookeries where most of the arribadas take place. However, new biological information is needed to establish signs of recovery such as the identification of stocks, the genetic characterization of the populations in major nesting areas, and the pattern and degree of their connectivity (Briseño-Dueñas 1998).

Previous mtDNA surveys have found a phylogeographic pattern featuring four main lineages. One is found in the eastern coast of India and recent genetic evidence suggests it is probably ancestral to all populations of olive ridleys (Shanker *et al*. 2004). A second one is found in the Indian Ocean and western Pacific, and probably gave origin to the third lineage residing in the Atlantic. The fourth lineage resides in the eastern Pacific, and probably originated by colonization from the western Pacific (Bowen *et al*. 1998; Shanker *et al*. 2004). As for the eastern Pacific, previous mitochondrial and nuclear DNA surveys indicate that nesting colonies in this region comprise a solitary panmictic population (Briseño-Dueñas 1998; López-Chávez 2000). However, there is evidence indicating that turtles from Baja California are unique. Peninsular turtles exhibit ethological (i.e. nesting behaviour) and morphological (i.e. larger hatchlings) differences from continental colonies (López-Castro *et al*. 2004). It is uncertain, however, whether these reflect the existence of reproductive isolation and local adaptation or are just a phenotypic plastic response to the extreme peninsular environmental conditions (i.e. low humidity and high temperatures), which differ greatly from those found in the continental beaches during the nesting season.

Here we use mitochondrial control region sequences to address the question of genetic differentiation of the BCS nesting colonies and of whether the observed phenotypic differences between peninsular and continental turtles reflect genetic differentiation and reproductive isolation or are the product of phenotypic plasticity.

Materials and methods

Sample collection

Four nesting locations of *Lepidochelys olivacea* were sampled in BCS during 2002 and 2003: Punta Tinaja and San Cristobal in the Pacific coast, and Punta Arena and Punta Colorada in the Gulf of California (Fig. 1). Samples consisted of eggs and skin from dead hatchlings or nesting females. To avoid resampling, females were tagged on the right front flipper and their clutch was not sampled. Where no female was observed, only one dead hatchling or egg was collected per nest within the 15-day internesting period. Samples were preserved in 96% ethanol and aseptic procedures were used to avoid cross-contamination and protect turtles from infection. Genetic data from the continental nesting beaches is from Briseño-Dueñas (1998) with subsequent revisions (A. Abreu-Grobois, personal communication).

DNA purification, amplification and sequencing

Total genomic DNA was extracted using standard proteinase K digestion and purified with a lithium chloride salting-out protocol, followed by organic extraction using chloroform–isoamyl alcohol, and subsequent ethanol precipitation. An 871-base pair (bp) fragment including part of the tRNA-Thr, the intervening tRNA-Pro, and the first 806 bp of the 5′ end of the mitochondrial control region was amplified with primers LCM-15382 (GCTTAACCCTAAAGCATTGG, A. Abreu-Grobois, personal communication) and H879lo (GGGTTTAGTTAAAAAATACGG, designed for this study) using HotStartTaq Mastermix according to manufacturer protocols (QIAGEN). Polymerase chain reaction (PCR) thermal cycling consisted of an initial *Taq* activation of 15 min at 95 °C, followed by 35 cycles of: 95 °C for 15 s, 56 °C for 45 s, and 72 °C for 90 s. Electrophoresis in a 2% agarose gel was used to confirm amplification. PCR products were prepared for sequencing with exonuclease and shrimp alkaline phosphatase (ExoSAP IT, United States Biochemical Co.). Both strands of purified PCR products were cycle-sequenced using the BigDye Terminator version 3.1 following manufacturer protocols, and products were analysed in an ABI 3100 Gene Analyser (Applied Biosystems). Base calling of complementary strands was carried out using SEQUENCHER 2.0 (Gene Codes Co.) and CODONCODE ALIGNER 1.2 (CodonCode Co.). Subsequent analyses were performed on 487 bp overlapping with the other eastern Pacific haplotypes.

Data analyses

mitochondrial DNA control region sequences of olive ridleys were downloaded from GenBank (http://www.ncbi.nlm.nih.gov) and obtained from the literature (Bowen *et al*. 1998; Briseño-Dueñas 1998). This resulted in 31 haplotypes of variable size; however, because each source used its own nomenclature they had to be aligned (CLUSTAL_X, Thompson *et al*. 1997) and compared to determine the number of distinct haplotypes. Subsequently, sequences from BCS were included in the alignment and the orthologous regions

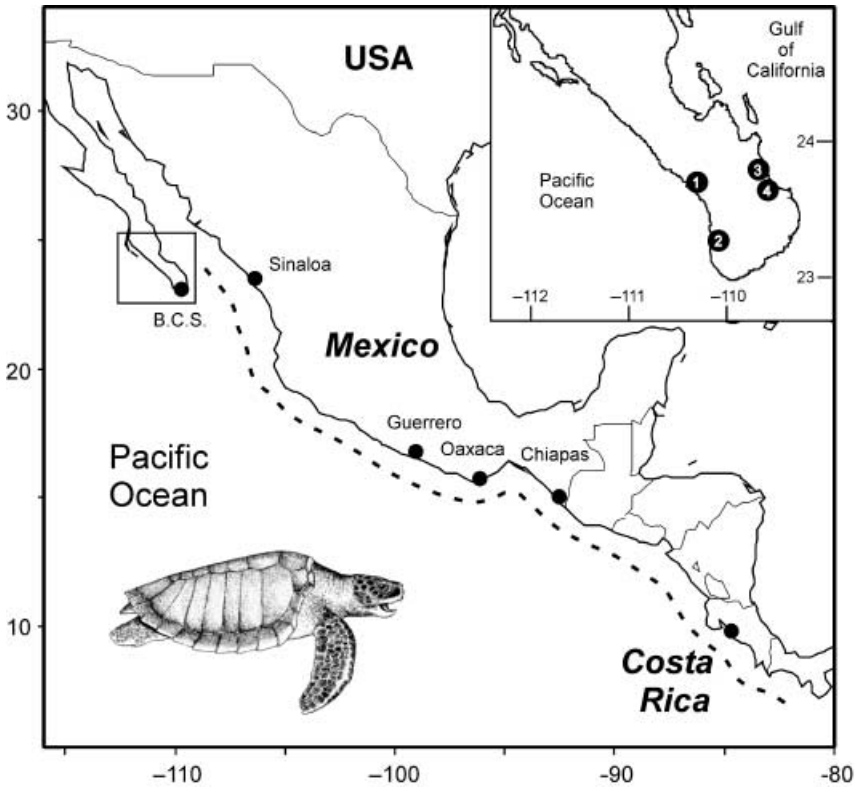


Fig. 1 Collecting nesting beaches (black dots) and reproductive range (hatched line) of *Lepidochelys olivacea* in the eastern Pacific. Insert. Detail of collecting sites in Baja California Sur: (1) Las Tinajas, (2) San Cristobal, (3) Punta Arena, and (4) Punta Colorada. Data from continental rookeries are from Briseño-Dueñas (1998).

compared to establish their identity. Haplotype (h) and nucleotide (π) diversities were calculated using ARLEQUIN version 2.001 (Schneider *et al*. 1999). Population structure was assessed with the same program by way of analysis of molecular variance (AMOVA, Excoffier *et al*. 1992), in which a pairwise matrix of the proportion of variable sites (p distance due to the small divergence) was used as molecular Euclidean distances among haplotypes. Pairwise molecular F_{ST} analogs (Φ_{ST}) were computed to test for differences between colonies and significance was adjusted for multiple tests using the sequential Bonferroni correction (Rice 1989). Maternal gene flow was estimated between pairs of colonies using the relationship $Nm = (1 - \Phi_{ST})/2\Phi_{ST}$ (Nei 1987; Schneider *et al*. 1999). We also estimated Nm using the Migration–Isolation (MI) model implemented in the program MDIV (Nielsen & Wakeley 2001). This method uses Bayesian inference and a Markov chain Monte Carlo (MCMC) simulation to compute posterior distributions of migration and divergence time. We used Markov chains of increasing length ranging from 5 to 10 million steps, as needed, to achieve convergence with 10% of the total number of cycles as burn-in time to avoid dependence on initial conditions. Because F_{ST}-based estimates of gene flow are unable to differentiate between population structure and historical population events such as colonization, fragmentation and expansion, a nested clade analysis (NCA) was conducted to assess the geographical association of the mitochondrial haplotypes and test if this association was due to the combined or separate effect of restricted gene flow or other historical population events (Templeton 1998). This method included constructing a gene network using the method proposed by Templeton *et al*. (1992) and implemented in the program TCS 1.18 (Clement *et al*. 2000). The nesting algorithm of Templeton *et al*. (1987) was subsequently applied and the resulting nested cladogram was used for contingency analysis with GEODIS 2.2 (Posada *et al*. 2000), in which haplotype frequencies and geographical coordinates of each colony were considered. Inferences from this analysis were based on the key of Templeton (1998) with subsequent modifications (http://darwin.uvigo.es/download/geodisKey_14Jul04.pdf). To elucidate relationships among haplotypes in the eastern Pacific, a neighbour-joining phylogram was constructed using p distances due to the small divergence and *Lepidochelys kempii* as an outgroup with MEGA 2.1 (Kumar *et al*. 2001) and node support was assessed with nonparametric bootstrap (500 replicates). Finally, the historical demography (colonization or bottlenecks) of peninsular and continental samples was analysed with mismatch distributions. This is possible because the shape of the distributions is affected by genetic signatures of population expansion and decline, which can be used to infer historical population dynamics using null models based on coalescence theory (Rogers & Harpending 1992; Rogers 1995). In general, populations that have

remained in demographic equilibrium for a long time present chaotic and ragged distributions, while those that have experienced sudden demographic expansions, resulting from bottlenecks or founder effects, show smooth wave-like distributions (Harpending 1994). Mismatch distributions and parameters of the model of sudden expansion: $\tau = 2\mu t$, $\theta_0 = 2\,\mu N_0$ before the expansion, $\theta_1 = 2\,\mu N_1$ after de-expansion, where μ is the fragment specific mutation rate, t is the time since expansion in generations, and N is the effective population size (Rogers & Harpending 1992), and its goodness-of-fit test were computed using ARLEQUIN version 2.001 (Schneider *et al*. 1999). Effective population size (N_e) was estimated from $\theta = 2N_e\mu$, using a site-specific mutation rate of 2×10^{-8}/year.

Results

Genetic diversity

Twenty-six distinct *Lepidochelys olivacea* control region haplotypes were identified worldwide from the different data sources, of which 11 were distributed in the eastern Pacific and had been included in population genetic studies. A total of 60 samples were obtained in both nesting seasons from the four sampling areas: Punta Tinaja ($n = 4$, 2 amplified), San Cristóbal ($n = 42$, 36 amplified), Punta Colorada ($n = 4$, 2 amplified), and Punta Arena ($n = 10$, 8 amplified). Due to insufficient samples for within-BCS comparisons we subsequently pooled all samples. In addition, we analysed 89 specimens from the eastern Pacific for which population frequencies were available (Briseño-Dueñas 1998). Among the 48 peninsular sequences, we identified five haplotypes (L, N, O, U, and V) (GenBank Accession nos: AY920519–AY920523); all of which had been previously found in other eastern Pacific continental colonies (Bowen *et al*. 1998; Briseño-Dueñas 1998). Of these, only the most abundant haplotype (N, GenBank AF051776, AY920519) had been found outside of this region, since Shanker *et al*. (2004) reported it as rare on the eastern coast of India. No additional variable sites were found outside the 487 overlapping bp of our sequences with those previously generated with primers nested into ours (Bowen *et al*. 1998; Briseño-Dueñas 1998). In BCS, both haplotype ($h = 0.16$) and nucleotide ($\pi = 0.06\%$) diversities were very low, as a consequence of haplotype N dominating in 91.6% of the samples, and of the shallow divergence among the five haplotypes (see below). In contrast, the same haplotype was found in only 59.6% of continental turtles (Table 1).

Table 1 Genetic diversity indices of six nesting colonies of olive ridleys in the eastern Pacific. Data from continental rookeries are from Briseño-Dueñas (1998)

Colonies	n	No. haplotypes	H (SD)	π (%) (SD)
BCS	48	5	0.1613 (0.0715)	0.0595 (0.0712)
Sinaloa	15	4	0.6190 (0.1196)	0.2269 (0.1760)
Guerrero	18	4	0.5948 (0.1086)	0.3020 (0.2144)
Oaxaca	21	7	0.6143 (0.1164)	0.2210 (0.1693)
Chiapas	20	4	0.4895 (0.1167)	0.2043 (0.1605)
Costa Rica	15	4	0.7333 (0.0669)	0.2542 (0.1911)

Genetic structure

AMOVA showed that a significant fraction of the molecular variance was partitioned among all six nesting samples in the eastern Pacific ($\Phi_{ST} = 0.048$, $P = 0.006$). Pairwise Φ_{ST} revealed that BCS was the only significantly different location relative to Sinaloa, Guerrero, Chiapas and Costa Rica, albeit tablewide significance only remained for Guerrero and Costa Rica after sequential Bonferroni correction (Table 2). No significant differentiation was found among any of the continental colonies ($\Phi_{ST} = 0.013$, $P = 0.22$), resulting in high estimates gene flow (Table 3). Estimates of maternal gene flow between BCS and the continental colonies were moderate, the lowest was with Costa Rica, perhaps due to geographical distance, and the highest with Oaxaca, the most important nesting beach in the eastern Pacific (Table 3). The findings involving the continental rookeries were originally reported by Briseño-Dueñas (1998). Φ_{ST} and model-based Nm estimates were largely consistent, with smaller values associated to significant pairwise Φ_{ST}.

Table 2 Pairwise Φ_{ST} (lower matrix) and corresponding P values (upper matrix) of eastern Pacific olive ridley turtles. Data from continental rookeries are from Briseño-Dueñas (1998)

Colonies	BCS	Sinaloa	Guerrero	Oaxaca	Chiapas	Costa Rica
BCS	—	0.0314	0.0013*	0.0889	0.0109	0.00001*
Sinaloa	0.0668	—	0.3855	0.9633	0.7131	0.6149
Guerrero	0.1264	0.0022	—	0.2354	0.0384	0.0499
Oaxaca	0.0316	–0.0439	0.0211	—	0.9653	0.2674
Chiapas	0.0733	–0.0251	0.0694	–0.0331	—	0.2512
Costa Rica	0.2359	–0.0256	0.0911	0.0164	0.0199	—

*Significant ($P < 0.05$ tablewide) after sequencial Bonferroni correction (Rice 1989).

Table 3 Estimates of maternal gene flow *Nm* among nesting colonies of *Lepidochelys olivacea* as $(1 - \Phi_{ST})/2\,\Phi_{ST}$ (below diagonal) and from MCMC simulations of the Migration–Isolation model (above diagonal). Data from continental rookeries are from Briseño-Dueñas (1998)

Colonies	BCS	Sinaloa	Guerrero	Oaxaca	Chiapas	Costa Rica
BCS	—	20	2	*	8	2
Sinaloa	7	—	3	*	5	*
Guerrero	3	232	—	*	1	1
Oaxaca	15	∞	23	—	*	18
Chiapas	6	∞	7	∞	—	1
Costa Rica	2	∞	5	30	25	—

*Indicates *Nm* larger than 30, which was the upper limit that we could compute with this method.

Nevertheless, these estimates must be interpreted with caution for two reasons: (i) when Φ_{ST} is close to zero, as in this case, the order of magnitude of *Nm* cannot be accurately estimated (Templeton 1998); and (ii) recent colonization events cannot be distinguished from contemporary movements between colonies (Bowen *et al*. 1998).

Phylogeography

The low divergence of *L. olivacea* haplotypes in the eastern Pacific was evident in the phylogenetic analysis (Fig. 2). However, two monophyletic groups can be distinguished, one poorly supported lineage (45% bootstrap), grouping only haplotypes from Guerrero, Oaxaca, Chiapas, and Costa Rica; and another one moderately supported (63% bootstrap), including all haplotypes found in BCS and in other colonies. Apart from the monophyly of BCS haplotypes, no other phylogeographic pattern is evident. Nested clade analysis of the TCS network (Fig. 3A) grouped the haplotypes into five 'one-step' clades, three of them indicating genetic or geographical variation but none significant. 'One-step' clades grouped into two 'two-step' clades and one (clade 2-2) showed significant geographical association ($\chi^2 = 29.115$, $P = 0.001$). No geographical association was found at the level of the total cladogram, reflecting the low divergence among nesting colonies. Geographical distance analysis of clade 2-2 showed significant differences for clade (D_c) and nested clade (D_n) distances, which are inferred to be caused by restricted gene flow between BCS, Sinaloa, Guerrero, and Oaxaca, and with isolation by distance from Chiapas and Costa Rica (Fig. 3B).

Demographic history

Despite of the small number of substitutions among haplotypes, mismatch distributions in four of the five continental colonies were multimodal, unlike the unimodal mismatch distribution from BCS. The distribution from Costa Rica was also unimodal but right shifted and centred on one difference (Fig. 4), indicating an older expansion in this nesting colony (Rogers & Harpending 1992). Sudden expansion model fitted all mismatch distributions (Table 4). Parameters θ_0 and θ_1 computed using the program ARLEQUIN 2.0 suggest that the expansion of continental colonies was fast, while in BCS expansion has been slow and not within the same order of magnitude (Table 4). Effective population sizes estimated from these parameters are suggestive of a considerable demographic expansion in Costa Rica and Chiapas ($N_1 > 900\,000$ organisms) subsequent to an initial suggested founder event ($N_0 = 0$) (Table 4).

Discussion

Sampling remarks

We consider very unlikely that the observed diversity in BCS may be a sampling artefact. We avoided double sampling adult females by tagging and nest samples were only accessed within the internesting period, thus preventing

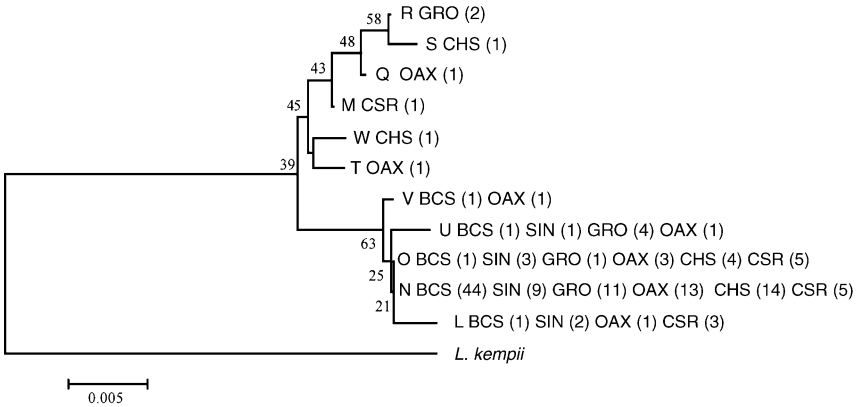
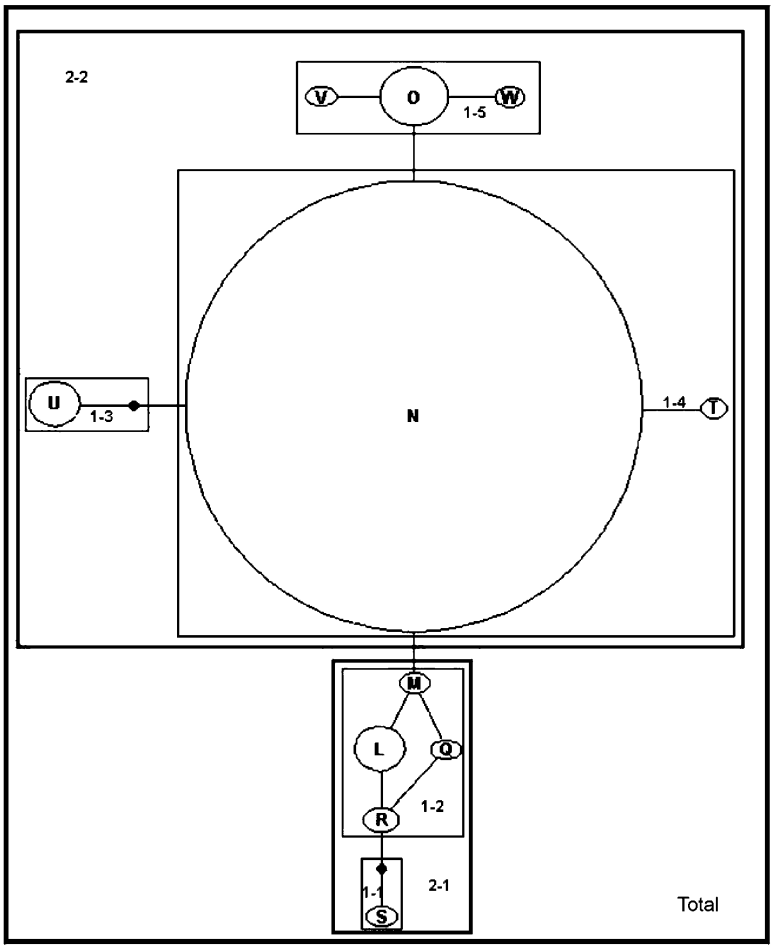


Fig. 2 Neighbour-joining tree of 11 olive ridley mtDNA control region haplotypes. Branch numbers represent bootstrap support values. Next to the haplotype letter is their geographic distribution and frequency. SIN, Sinaloa; GRO, Guerrero; OAX, Oaxaca; CHS, Chiapas; CSR, Costa Rica; BCS, Baja California Sur.

A



B

Haplotypes			1-step clades			2-step clades		
No	§Dc	£Dn	No	Dc	Dn	No	Dc	Dn
R^I	0	617.58						
L^I	1165.68	1164.684						
M^I	0	1168.00						
Q^I	0	133.85						
I-T	-	-						
H_0 not rejected			$1\text{-}2^I$	989.85	991.57			
S^T	0	0	$1\text{-}1^T$	0.00	153.15			
			I-T	989.85	838.42			
			H_0 not rejected			$2\text{-}1^T$	929.66	992.72
T^T	0	482.64						
N^I	813.96	813.56						
I-T	813.96	330.92						
H_0 not rejected			$1\text{-}4^I$	809.51	809.08			
U^T	0	0	$1\text{-}3^T$	382.67^S	408.03^S			
V^T	672.43	623.31						
O^I	833.72	838.45						
W^T	0	149.56						
I-T	385.43	373.05						
H_0 not rejected			$1\text{-}5^T$	792.54	966.39			
			I-T	123.23	-12.5516			
			1-2-3-4:NO (RGF-ID)			$2\text{-}2^I$	813.81	812.85
						I-T	-115.85	-179.87
						H_0 not rejected		

Fig. 3 Nested clade analysis of *Lepidochelys olivacea* mtDNA haplotypes in the eastern Pacific. (A) Nested cladogram. Size of ovals is proportional to haplotype frequency; lines between them represent one mutational step, black circles are hypothetic haplotypes not sampled. Thin lines indicate 1-step clades, thick lines 2-step clades, and the thicker line indicates the total cladogram. (B) Table of within-clade and nested clade distances generated with GEODIS version 2.0 according to the nested cladogram presented above. §D_c, within-clade distance; £D_n, nested clade distance; I-T, interior (I)-tip (T) contrasts; S, significantly small distances. The chain of inferences (Templeton, 1998) is showed below the I-T values of those clades with significant χ^2 values ($P < 0.05$). If not the case, the null hypothesis (H_0) of no geographical association was not rejected. Following this chain, is the biological inference key where RGF-ID is restricted gene flow with isolation by distance.

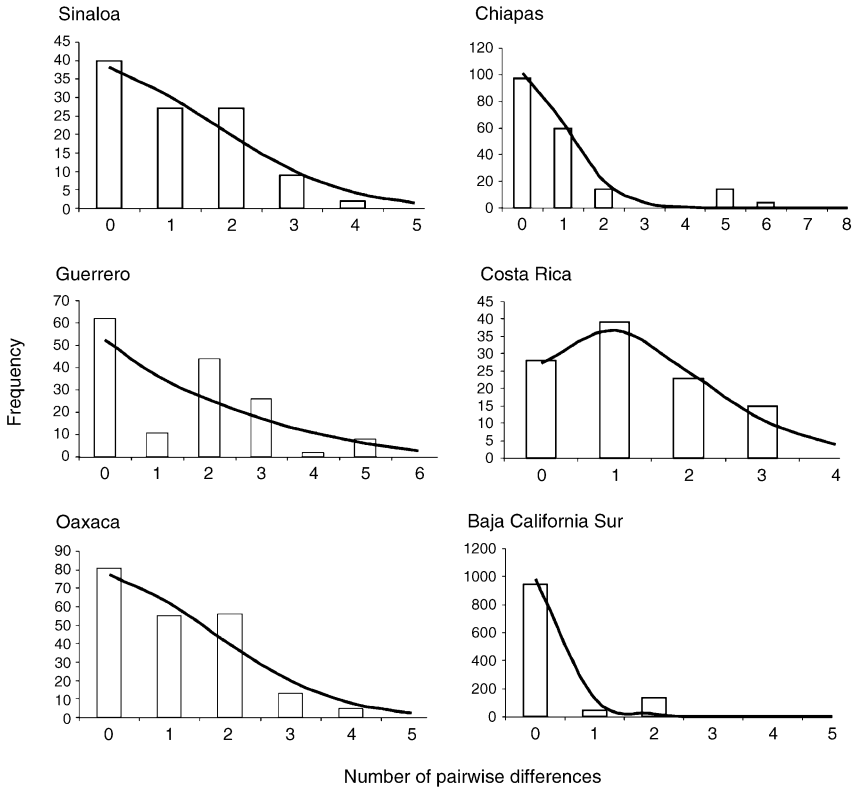


Fig. 4 Mismatch distributions of mtDNA control region sequences of olive ridley in six colonies of the eastern Pacific. Lines represent expected distributions according to the sudden expansion models and its parameters.

Table 4 Historical demography analyses of six colonies of eastern Pacific olive ridleys: parameters of Roger's sudden expansion model and corresponding goodness of fit test. Data from continental rookeries are from Briseño-Dueñas (1998).

	Colonies					
	BCS	Sinaloa	Guerrero	Oaxaca	Chiapas	Costa Rica
Parameters						
n	5	4	4	7	4	4
τ	3.000	1.811	3.256	1.705	0.643	1.352
t (10^6 years)	3.08	1.86	3.34	1.75	0.66	1.39
θ_0	0.102	0.002	0.004	0.001	0.000	0.000
(N_0)	(5236)	(103)	(205)	(51)	(0)	(0)
θ_1	0.148	2.169	1.968	2.213	18.398	223.750
(N_1)	(7597)	(111 345)	(101 027)	(113 604)	(944 456)	(11 486 140)
Goodness-of-fit test						
SSD	0.01681	0.00651	0.05253	0.00863	0.00825	0.00215
P	0.12000	0.73000	0.23999	0.58999	0.25999	0.75999

n is number of haplotypes, $\tau = 2\mu t$, where μ is the fragment specific mutation rate and t is the time since expansion, $\theta_0 = 2\ \mu N_0$ before the expansion, $\theta_1 = 2\ \mu N_1$ after de-expansion, where μ is as above and N is the effective population size. SSD is sum of squared deviations, P is significance.

resampling clutches from the same turtle. Admittedly, it is not entirely certain that nest samples taken in both nesting seasons corresponded to different females; however, 72% of analysed samples are from a single nesting season (2002), from which the possibility of sample duplication is close to nil. With regard to intra-BCS nesting beach variability, most of the samples were taken from a single beach and even though haplotypic frequency may prove to be similar in all beaches, it is possible that a larger geographical sampling may reveal additional haplotypes and population structure. The very large contrast in mitochondrial genetic diversity between peninsular and continental samples is likely to persist or increase after additional samples from both regions are collected to provide more accurate estimates

of molecular diversity. To address the issue of unbalanced sampling of peninsular and continental nesting beaches we computed molecular diversity indices and levels of genetic differentiation in balanced pseudosamples ($n = 40$ and $n = 15$) bootstrapped from peninsular and continental haplotypes (details not shown), corroborating that the main conclusions presented below are not affected by the unbalanced sample sizes.

Patterns and processes of genetic diversity and structure in the eastern Pacific

Molecular diversity in BCS ridleys ($h = 0.16$, $\pi = 0.06\%$) was significantly lower than previously reported in eastern Pacific continental colonies (average, $h = 0.60$, $\pi = 0.26\%$), and elsewhere as in Australia ($h = 0.54$, $\pi = 0.34\%$), or Sri Lanka ($h = 0.72$, $\pi = 2.07\%$). This low diversity is caused by the predominance of one haplotype in 91.6% of individual turtles and the close relationship among the haplotypes found in peninsular nesting beaches. Similar but not as extreme results have been found in Orissa beach, India ($h = 0.27$, $\pi = 0.3\%$), where eight haplotypes were found and one was present in 85.2% of the samples (Shanker *et al*. 2004), whereas a similar nucleotide diversity has only been found in Surinam ($\pi = 0.06\%$), where it has been attributed to a founder effect and recent colonization (Bowen *et al*. 1998). In BCS, the almost unprecedented low levels of genetic diversity in olive ridleys may result from different factors including a strong genetic drift associated with a founder effect and recent colonization of the peninsula; a population bottleneck produced by intensive exploitation and poaching; or purifying selection in response to the harsh nesting environment of peninsular beaches at this northern limit of the species' reproductive range. What is more certain is that the very biased genetic composition of peninsular ridleys produces a molecular fixation index significantly different from zero ($\Phi_{ST} = 0.048$, $P = 0.006$), unlike the one computed from widespread continental beaches, $\Phi_{ST} = 0.013$, $P = 0.22$ (Briseño-Dueñas 1998), indicating reproductive isolation and genetic differentiation of BCS turtles. This level of differentiation is only a fraction of that observed at global geographical scale among olive ridleys, $\Phi_{ST} = 0.58$ (Bowen *et al*. 1998) or in other marine turtles such as *Caretta caretta* $\Phi_{ST} = 0.64$ (Encalada *et al*. 1998). In the case of the eastern Pacific colonies, where proximity favours gene flow, BCS was found to be genetically different from most continental samples; we consider these differences to be biologically relevant even though only Guerrero and Costa Rica resulted in statistically significant pairwise Φ_{ST} after conservatively correcting for multiple tests (Table 2). Even though Φ_{ST} based estimates of *Nm* must be interpreted with caution, the very large values obtained in several pairwise comparisons confirm panmixia among continental colonies. These trends were largely confirmed by model-based estimates of gene flow using MCMC simulations.

Phylogeography, historical dispersion and demographic signatures

Nested clade analysis suggests that the pattern of geographical variation observed in some mtDNA haplotypes was caused by restricted genetic flow with isolation by distance. Nevertheless, distance between the colonies does not seem to be the only cause of differentiation, since BCS was not significantly different from Oaxaca, one of the most distant colonies. Moreover, *Lepidochelys olivacea* is known to be highly vagile (Plotkin *et al*. 1995), hence other factors such as nest site fidelity and natural selection are perhaps involved in this differentiation. Testing these hypothetical factors will require additional efforts such as tag and recapture of nesting females, as well as the use of other molecular markers (nuclear DNA) to provide evidence of nest site fidelity. The absence of more consistent levels of differentiation of BCS with all continental colonies may reflect the recent evolutionary history of *L. olivacea* in the region. Using a molecular clock proposed for the mtDNA control region of Testudines (Encalada *et al*. 1998), the time of colonization of the eastern Pacific by *Lepidochelys* has been estimated to be 0.3 million years ago (Ma) (Bowen *et al*. 1998; Shanker *et al*. 2004). In addition, other historical factors may have contributed to 'dilute' genetic differentiation; in the last 0.3 Ma, at least three glacial periods have been recorded. Consequently, the species range might have repeatedly contracted and expanded because of latitudinal isothermal movement in which abrupt changes as large as 10 °C occurred (Lea *et al*. 2000), i.e. in the eastern tropical Pacific sea surface temperature was 6 °C lower than the actual average during the most recent glacial maximum (Herbert *et al*. 2001). Thermal physiological constraints of Chelonidae (Ackerman 1997; Spotila *et al*. 1997) might have forced them to nest in warmer shores closer to the equator until temperature increased during interglacial periods, allowing the colonization (or recolonization) of northern areas. Demographic histories inferred from mismatch distributions support that colonies at low latitudes might have been the centre of dispersion of *L. olivacea*. Similarity of continental colonies distributions is consistent with the fact that they most likely share the same demographic history. Costa Rica, however, presents a right-shifted unimodal mismatch distribution suggesting that this colony may be older, which is consistent with its high haplotypic diversity (Table 1). BCS L-shaped mismatch distribution differs considerably from the continental distributions presenting a single mode at zero differences, characteristic of an expanding population after a recent bottleneck or founder event. Moreover, parameters of the sudden expansion model (θ_1 and θ_2) indicate a smaller and slower population

increase in BCS relative to continental colonies, particularly Chiapas and Costa Rica where the genetic signature of population growth is four orders of magnitude larger. These results are in agreement with the NCA and are consistent with the idea that colonies in this area may have been the source of expansion to the rest of the eastern Pacific. In the event of a founder effect in BCS suggested by the genetic data, it remains to be explained why, in the absence of the high competition for nesting sites typical of continental rookeries, the population of BCS has not grown faster. A possible cause is that the slow increment is a response to more rigorous conditions for nesting success, such as the low humidity in the beaches (López-Castro *et al*. 2004), but the influence of a more recent but deadlier human-induced decline due to historical exploitation (see below) cannot be ruled out. It remains to be shown experimentally and with additional genetic loci whether successfully reproductive BCS turtles, with the ability to compensate for low humidity, represent a singular biological and genetic unit as suggested by mtDNA, or if any random sample of continental turtles would respond behaviourally and phenotypically in the same way. One piece of evidence, in favour of the distinction of BCS turtles, is that while in most demographically important continental nesting beaches of the eastern Pacific olive ridleys nest in arribadas, nesting in Baja California is of solitary type, in which fewer nesting females maintain a considerably smaller effective population size.

Management and conservation implications

The prevailing notion that olive ridleys in the eastern Pacific constitute a single panmictic population has led all conservation efforts to focus on those colonies with higher abundance where, evidently, a greater proportion of reproductive output and genetic diversity is protected. This approach has been acceptable due to the difficulty of protecting each and every reproductive colony of the species, a problem shared with most marine resources (Palumbi 2003). However, our genetic analyses and previous reproductive studies strongly indicate that BCS may be reproductively isolated, thereby challenging the prevailing paradigm of a single panmictic evolutionary and conservation unit of olive ridleys in the eastern Pacific. Failure to recognize the individuality of the BCS population in the management plan of the species may lead, and has most likely already led, to negative impacts on the BCS nesting colony, its genetic variability, and its future success. The significant differences found in haplotypic frequencies between BCS and continental colonies are sufficient to classify the BCS ridleys as a distinct management unit (Moritz 1994); therefore protection and conservation of peninsular nesting areas must be a priority in Mexico. In spite of signs of recovery of eastern Pacific ridleys (US National Marine Fisheries Service & US Fish and Wildlife Service 1998), age-old problems affecting the viability of *L. olivacea* in BCS have not gone away. Those of major importance are poaching and illegal marketing of eggs, destruction of nesting areas by motor vehicles, and insufficient surveillance and law enforcement. In order to identify key areas for conservation, it is essential to make continuous surveys of the nesting areas during reproductive seasons in order to obtain better estimates of basic information such as population density, reproductive success, conditions in which nesting occurs, as well as continued genetic studies. Marine turtle conservation is complex and riddled with conflicting interests in the face of much uncertainty regarding the many social, economical and biological facets. If conservation is to be successful, specific all-inclusive plans need to be implemented to resolve these issues providing alternative and legal means of economical and social well-being to involved parties in concert with more stringent enforcement of management and conservation laws.

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