Turk J Zool
24 (2000) 191–197

Genetic Structure of Mediterranean Sea Turtle Populations

Yakup KASKA
Pamukkale University, Faculty of Science and Literature, Department of Biology, Denizli-TURKEY

Received: 20.04.1999

Abstract: The mitochondrial DNA (mtDNA) control region sequences of two species (*Caretta caretta* and *Chelonia mydas*) of sea turtles from the Mediterranean were analysed using samples from Northern Cyprus. Only one single haplotype for each species was detected. These results were compared with other published work regarding the genetic structure of sea turtle populations. These results suggest that the Mediterranean population of sea turtles were separated from their Atlantic relatives in the recent past. In order to protect these endangered sea turtles and to preserve the genetic diversity of the sea turtle population in the Mediterranean, individual nesting sites must be protected. That genetic studies can be done on the systematics of vertebrates, especially taxonomic studies of Herpetofauna, has also been shown by other related literature.

Key Words: *Chelonia mydas, Caretta caretta,* genetic diversity, DNA sequence.

Akdeniz Deniz Kaplumbağa Populasyonlarının Genetik Yapısı

Özet: Akdeniz'deki iki deniz kaplumbağası (*Chelonia mydas* ve *Caretta caretta*) türlerinin mitokondriyel DNA kontrol bölgesi sekuens analizi, Kuzey Kıbrıs'tan toplanan örnekler kullanılarak yapılmıştır. Her tür için tek tip haplotip tesbit edilmiştir. Bu sonuçlar deniz kaplumbağa populasyonlarının genetik yapısı ile ilgili araştırmaların sonuçları ile karşılaştırılmıştır ve bu sonuçlar Akdeniz deniz kaplumbağalarının Atlantik akrabalarından yakın bir zaman önce ayrılmış olduklarını göstermiştir. Tehlike altındaki bu deniz kaplumbağalarını ve Akdeniz'deki mevcut genetik çeşitliliği korumak için her yuvalama bölgesinin de korunması gerekmektedir. Genetik çalışmaların omurgalılarda ve özellikle herpetofauna ile ilgili taksonomik çalışmalarda kullanılabileceği diğer literatürler ışığında gösterilmiştir.

Anahtar Sözcükler: *Chelonia mydas, Caretta caretta,* Genetik çeşitlilik, DNA sekuensi.

Introduction

The natal homing hypothesis for the reproductive migration of sea turtles suggests that females return to breed and nest at the same beach on which they hatched. Hatchlings and juveniles move through several habitats during development. Adults migrate between feeding and nesting grounds that are hundreds or thousands of kilometers apart, yet these movements are difficult to track in the marine environment (1). Much of what is known about the life history of sea turtles has come from tagging experiments on nesting females.

The herbivorous green turtle is distributed circumglobally in tropical and subtropical oceans. The carnivorous loggerhead turtle occurs in the Mediterranean, and in the Atlantic, Indian, and Pacific Oceans (2). The nesting habitats of loggerhead turtles and green turtles overlap in some areas, for example, the eastern Mediterranean. Only small populations of green turtles and loggerhead turtes nest in the Mediterranean: approximately 400 green turtle and 2000 loggerhead turtle females per season (3). One prediction of the natal homing hypothesis is that each nesting colony should comprise a group of isolated maternal lineages, as females assort themselves according to their natal beach (1). Hendrickson (4) proposed an alternative explanation for female nest site fidelity, called the social facilitation hypothesis. Under this hypothesis, first-time nesting females follow experienced breeders from the feeding grounds to a nesting beach, and use this site for all subsequent nestings. Both of these hypotheses have proven difficult to test directly, as no tag applied to a hatchling has been recovered from an adult. Natal site philopatry, however, generates a testable prediction about the genetic structures of populations. If females return faithfully to their natal beach, then each nesting population should be effectively isolated in terms of female transmitted traits (such as mitochondrial DNA). In contrast, social facilitation should result in high rates of female-mediated gene flow between beaches that share feeding grounds.

In recent reports, analyses of metarnally transmitted mitochondrial DNA (mtDNA) have proven useful for resolving questions about nesting behaviour and population demography in sea turtles (i.e., 5, 6, 7, 8).

mtDNA has the virtues of a maternal, nonrecombining mode of inheritence, rapid pace of evolution, and extensive intraspecific polymorphism. It is tightly packed with genes for 13 messenger RNAs, 2 ribosomal RNAs, and 22 transfer RNAs. In addition to these 37 genes, an area known as the "D-loop", roughly 0.8 kilobases long, appears to exercise control over mtDNA replication and RNA transcription (9). Patterns of variation of mtDNA have been used extensively for the study of population genetic structure, phylogeographic relationships, and other aspects of the molecular ecology of various organisms (10). In general, genetic studies of population structure with conservation implications and taxonomic problems have been conducted on fishes (11, 12, 13, 14), amphibia (15, 16), reptiles (17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28), birds (29, 30) and mammals (31, 32, 33) (Table 1). Allozyme data, though not reflecting a truly unbiased sampling of the genome, can serve as an estimate of variability among structural loci. More recently, much attention has also been given to the application of mtDNA markers in the management of endangered or threatened species. In many cases, mtDNA studies have delineated the structure of populations, and thus have provided guidance for the level at which mangement priorities should be set for the protection of a particular species. In the case of sea turtles, mtDNA surveys of breeding colonies have focused primarily on the delineation of demographically independent population units with significance for conservation.

Allard *et al.* (34) and Lahanas *et al.* (35) applied analysis of mtDNA control region sequences to problems in green turtle biology, and more recently Encalada *et al.* (36) employed mtDNA control region sequences to assess the population genetic structure and phylogeography of green trutles in the Atlantic Ocean and Mediterranean Sea. Schroth *et al.* (37) also looked at the genetic diversity of the loggerhead turtle population in the Mediterranean. Their study showed that colonies of

Table 1. Some of the recent genetical literature on species from fish, amphibia, reptiles, birds and mammals.

Species	Methodology	Ref.
Salvelinus confluentus populations	Protein electrophoresis	11
Salmo trutta populations	mtDNA control region sequence	12
Oncorhynchus gilae populations	mtDNA control region sequence	13
Albula species	Protein electrophoresis	14
Salamandra salamandra and *S. atra*	mtDNA, Cytochrome-b sequencing	15
14 species from *Hyperoliidae* family	mt 12S rRNA, tRNA sequencing	16
Cerrophidion godmani populations	Protein electrophoresis	17
Brachylophus vitiensis and *b. fasciatus*	Electrophoresis, DNA sequencing	18
Ateuchosaurus chienensis, A. pellopleurus	Karyotype analyses	19
Lepidodactylus lugubris populations	Allozyme electrophoresis	20
5 species groups of *Sceloporus scalaris*	mtDNA sequencing	21
Ameiva exsul populations	RAPD-PCR	22
Emoia species	mtDNA sequencing	23
Urosaurus ornatus populations	mtDNA restiction site analyses	24
Malaclemys and *Graptemys* genera	mtDNA, Cytochrome-b sequences	25
3 populations of *Trachemys scripta*	Protein electrophoresis	26
Sternotherus odoratus subspecies	mtDNA sequencing	27
Pseudemys rubriventris populations	Enzyme gene loci anayses	28
Perca flavescens and *P. fluviatilis*	Protein electrophoresis	29
Fringilla spp. *Carduelis chloris*	mtDNA control region sequencing	30
Phoca vitulina	mtDNA sequencing	31
Zalophus californianus populations	mtDNA, cytochrome-b sequencing	32
Megaptera novaeangliae	mtDNA sequencing	33

turtles separated by only tens of kilometers are genetically distinct, and that female natal homing is precise with limited gene flow between turtle colonies being male-mediated.

The purpose of this study was to determine whether the Mediterranean populations of green turtles and loggerhead turtles are genetically distinct from their Atlantic relatives. Following other published work (36, 37) with the same aim, and other mtDNA control region sequences presented at Genbank, the additional aim of this work became a further exploration of the diversity present in the Mediterranean.

Materials and Methods

Tissues were dissected from 17 green turtle and 10 loggerhead turtle hatchlings from west coast beaches of Northern Cyprus during the hatching season of 1995, from hatchlings sacrificed for sex determination purposes and from hatchlings which had been found dead in the nest column during nest excavation after hatching. One sample per nest was taken. Heart, liver and brain samples from the hatchlings were dissected and preserved in absolute alcohol and stored at room temperature. Total DNA isolations from the heart samples were conducted by digesting with proteinase K at 50 °C for 4 h. Contaminating proteins were removed by sequential extraction with equal volumes of phenol-chloroform and the DNA was recovered from the solution by ethanol precipitation in the presence of 1.25 M ammonium acetate and resuspended in TE buffer (10 mM Tris. Cl (pH: 8.0), 1 mM Ethylene Diamine Tetra Acetic Acid [EDTA]) (38).

These DNA samples were then amplified with a Polymerase Chain Reaction (PCR), which is an in vitro technique which allows the amplification of a specific DNA region that lies between two regions of known DNA sequence. PCR amplification of DNA can be achieved by using oligonucleotide primers (39). These are short, single-stranded DNA molecules which are complementary to the ends of a defined sequence of DNA template. The basic components of a PCR amplification are template DNA (1 µl), reaction buffer (2.5 µl), magnesium, dNTPs (2 µl each), 2 oligonucleotide primers (2 µl each) and DNA polymerase (0.2 µl), and optional additives such as Bovine Serum Albumin (BSA) (0.3 µl) and 15 µl distilled water make up the 25 µl reaction mixture. The reaction components are overlaid with mineral oil and the tube placed in a thermal cycler. This is basically to prevent evaporation in the majority of the thermal cyclers which do not heat the lids of the reaction tubes. The oil also helps prevent sample-to-sample contamination. The thermal cycler is an automatic instrument that takes the reaction through a series of different temperatures for varying amounts of time. This series of temperatures and time adjustments is referred to as one cycle of amplification. Each PCR cycle theoretically doubles the amount of targeted template sequence (amplicon) in the reaction. DNA synthesis always proceeds in the 5' to 3' direction since the polymerization is always from the 5' α-phosphate of the deoxynucleoside triphosphate to the 3' terminal hydroxyl group of the growing DNA strand. The number of cycles used was between 25 and 35. With increasing cycle numbers an increase in the amount of unwanted artificial products but no increase in the desired product is commonly observed. Four primers were used in this study: LTCM1 (5'-CCC AAA ACC GGA ATC CTAT-3'), LDCM1 (5'-AGT GAA ATG ACA TAG GAC ATA-3'), and HDCM1 (5'-ACT ACC GTA TGC CAG GTTA-3') developed by Allard *et al.* (34) and LTCM2 (5'-CGG TCC CCA AAA CCG GAA TCC TAT-3') and HDCM2 (5'-GCA AGT AAA ACT ACC GTA TGC CAG GTT A-3') developed by Encalada *et al.* (36). These primers were designed to target an area of 510 basepairs of the 5' end of the control region. It is necessary first to calculate the molar extinction coefficient of the primer at 260 nm. The molar extinction coefficient is equivalent to the absorbance at 260 nm (A260) of a 1 M solution of primer. Dividing the A260 of the primer stock solution by the molar extinction coefficient will give the molar concentration of the primer.

Electrophoresis of an aliquot of the PCR on an agarose gel (0.25 mg Agarose in 30 ml 1xTBE buffer) was carried out and visualized by staining with ethidium bromide, which is a fluorescent dye that intercalates into the DNA. After staining, ultraviolet transillumination allows visualisation of the DNA in the gel, which can be recorded photographically.

Two green turtle and one loggerhead turtle samples were sequenced by following the radiolabelling protocol, and other samples were sequenced with fluorescently labelled primers and analysed with an automatic DNA sequencer (Applied Biosystems model 373). Individual sequences were then aligned with the naked eye.

Dideoxy Sequencing is carried out using a DNA polymerase to extend a primer along a single-stranded template in the presence of the four dNTPs. Single-stranded DNA was used directly in the sequencing reaction. Amplified double-stranded mtDNA was purified with Amicon centricon centrifugal microconcentrators (Centricon-100 and 30). The sequencing reaction is terminated in a random fashion by the incorporation of a

dNTP analog, a dideoxynucleoside triphosphate (ddNTP), producing DNA chains of varying length that all terminate with the same 3' base. These are separated by high-resolution polyacrylamide gel electrophoresis.

For radiolabelling sequencing, an eppendorf tube containing 6 µl single-stranded PCR product (6x concentrate), 1 µl primer (10 µM), using opposite primer to the one used in PCR, 1 µl DMSO (Dimethyl sulfoxide), and 2 µl 5x sequenase buffer was prepared for each DNA sample to be sequenced. These samples were placed in a heating block which was heated >80 °C and allowed to cool slowly to room temperature. The labelling mix [1 µl DDT, 2 µl G-mix or I-mix, 2 µl enzyme mix (1.625 µl enzyme dilution buffer, 0.25 µl Sequenase and 0.125 µl Ppase), and 0.25 µl ^{35}S-dATP added last] was prepared for each sample. 2.5 µl of each ddNTP was dispensed into a 96-well plate, as one set of four for each sample for both the G-mix and I-mix. A plastic adhesive film was placed over the wells. 20 ml of stopping dye was put in a nearby well and stored at -20 °C until needed. 5.25 µl labelling mix was added to each sample tube and spun down briefly (5 sec.) in a microfuge, then left to sit for 3 minutes at room temperature. The 96-well plate of ddNTPs was placed onto the platform in a water bath which was preheated to 42 °C. 3.5 µl of labelling mix was dispensed into the four wells containing ddNTPs and covered with adhesive plastic and lead brick, and incubated at 42 °C for 3 min. 4 µl of stopping dye was dispensed into each well, from the local stocks already established, covered and labelled with Radioactive tape and then stored at -20 °C. Acrylamide gel was prepared by using 40 g Urea, 28 ml dH_2O, 12 ml Acrylamide, 8 ml 10xTBE and then 320 µl of 25% APS and 40 µl TEMED were added, and the gel was poured, the comb inserted backwards and clamped to the top, and left overnight or for a minimum of 3 hrs. It was incubated for 3 minutes in the water bath and 3 minutes on ice and then 3 µl of the samples was loaded when the gel was ready (50 °C) and the water bath was at 90 °C. Some stopping dye was added to every second well and omitted from the linking wells and the loading order was noted on the glass. The gel was run at 90 Watts until the gel temperature reached 50 °C, and after loading was run at 60 Watts. After a 3- or 7-hour run, the gel was covered with plastic and dried for 2 hrs in a vacuum dryer. After drying, the gel was placed in a tray with a film under red light, left for 3 days, and the film was then developed in a developing machine.

For sequencing by fluorescent labelling, the 6-times concentrate double-stranded PCR was prepared by Centricon 100, 2.8 µl distilled water, 3.2 µl primer (1 µM), 6 µl PCR product and 8 µl terminator ready reaction mix was added. One drop of mineral oil was added and then it was run for 24 cycles PCR. Tubes were prepared by adding 2.0 µl 3M Sodium acetate and 50 µl 95% ethanol. The entire PCR product was transferred into these tubes and then left on ice for 10 minutes. Then it was centrifuged for 15 minutes at maximum speed in a microcentrifuge. The ethanol was aspirated and the pellet was rinsed by adding 250 µl 70% ethanol and the aspirated again. The pellet was dried in a vacuum centrifuge for 8 minutes. The pellet was resuspended with just enough loading solution (5:1 deionised formamide and 50 mM EDTA) to bring the sample volume up to a final volume of 4–5 µl and then it was centrifuged briefly. The samples were heated at 90 °C for 2 min. and incubated on ice until loading on a 4.5% acrylamide gel and then analysed with an automated DNA sequencer.

Results and Discussion

The sequences that were obtained for each species can be seen in Table 2. All the samples belonging to one species showed the same pattern of sequence with both types of sequencing method used. The samples were aligned for 487 bases from the 5' end of the control region. There were no polymorphisms in the 17 green turtle samples. This finding lowers the estimated genetic diversity of the green turtle population nesting on Cyprus and shows no genetic exchange with the Atlantic population. Encalada *et al.* (36), in a study of the phylogeny and genetic structure of Atlantic and Mediterranean green turtle populations, found 18 haplotypes (accession numbers in Genbank, Z50124–Z50140) in 147 individuals from nine nesting populations by sequencing the mtDNA control region. All the green turtle samples in the present study exhibited haplotype XIII from Encalada *et al.* (36). It would be interesting to look at samples from green turtles nesting on Turkish beaches in order to look for other possible hapotypes in the Mediterranean, since both the samples in the present study and those in Encalada *et al.* (36) were from Cyprus.

Schroth *et al.* (3), in their study of DNA sequence analyses of the mitochondrial region of loggerhead turtles in the Eastern Mediterranean, found 11 haplotypes (GenBank accession number U72747) by sequencing 518 base pairs from 30 individuals, including some from Cyprus. They also reported briefly that there are haplotypes which are limited to geographically well-defined areas, but they did not report the haplotype distribution within the Mediterrenaean. Their study also

Table 2. The mitochondiral DNA control region sequences of the samples from Northern Cyprus. (*Bold letters indicate the polymorphic sites* found in the literature; Hyphens ("–") within the sequences represent "gaps", which are added in order to align and allow for the presence of base "insertions" in other sequences at corresponding positions).

Chelonia mydas						
1	TACCTTTAC	ACAGGGAATA	AAAGTGTCCA	CACAAACTAA	CTACCTAAAT	TCTCTGCCGT
61	GCCCAACAGA	ACAATACCCG	CAATACCTAT	CTATGTATTA	T**C**GT**A**CATCT	ACTTATTTAC
12	CAATAGCATA	TGACCAGTAA	T**G**TTAACAGT	TGATTTG**A**CC	CTAAACATAA	AAAATCATT**G**
181	**A**ATTTACATA	AA**T**ATTTTAA	CAACATGAAT	ATTAAGCAGA	GGATTAAAAG	TGAAATGACA
241	TAGGACATAA	AATTAAAC**C**A	TTATACTCAA	CCATGAATAT	**C**GTCACAGTA	AT**T**GGTTATT
301	TC**C**TAAATAG	CTATTCACGA	GAAATAAGCA	ACCCTTGTTA	GTAAGATACA	ACATTACCAG
361	TTTCAAGCCC	ATT**C**A**G**TCT**G**	TGGCGTACAT	AATTTGATCT	ATTCTGGCCT	CTGGTTAG**T**T
421	TTTCAGGCAC	ATACAAGTA**A**	C**G**ACGTTCAT	TCGTTCCCCT	TTAAAAG - - -	- - - - - - -GCC
481	TTGGGTTGAA	TGAG**T**TCTAT	ACATTAAATT			
Caretta caretta						
1	CCAATTAAAC	TACCCTTTGA	CGCAAAAGAA	GCGCCAACAT	GTAAATTTAC	CTATATTCTC
61	TGCCGT**G**CCC	AACAGAA**T**AA	TATCC**AT**AAT	ACCTATCTAT	GTATTAT**C**GT	ACATC**A**ACTT
12	ATTTACCACT	AGCATATGAT	CAGTAATGTT	GTCGATTAAT	**C**TG**A**C**C**TTAA	ACATAAAAA**C**
181	T–ATTAATTT	T**G**CATAAACT	GTTTTAG**T**TA	CATGA**C**TATT	ATAC**AG**GTAA	TA**GG**AATGAA
241	ATGATAT**A**GG	ACATAAAATT	AAACC**A**TTAT	TCTCAACCAT	GAATATCGT**T**	**A**CAGTAATAG
301	GTTATTTCTT	AGTTCA**G**CTC	ATCACGAGAA	ATAAGCAA**T**C	CTTGTTAGTA	AGATACA**AT**A
361	T**T**ACCAGTTT	CA**A**G**T**CCATT	AAGTCAT**G**TC	GT**A**CATAACT	GA**T**CTATTCT	GGCCTCTGGT
421	TGG**T**TTTTTC	AGGCACATTA	**A**G**GC**A**G**TAA**A**	GTTCA**T**TCGT	TCCTCTTTAA	AAGGCCTC**T**G
481	GTT**GCAAGT**A	AATGAGTTCT	ATA**C**ATTAAA	TTTATAACCT	GGCATACGGT	GGTTTTAC

suggested that there are genetic differences not only between different coastal areas (for example, Greece and Turkey), but also between colonies from adjacent nesting beaches. The sequences of 10 samples in this study also revealed a single haplotype. The results of this study match the Haplotype B (Genbank No AJ001075) and Haplotype III of Schroth *et al.* (37) reported with accession number U72747, except with the variation at locus 297 (317 in Table 2) with "G". The accession number (U72747) of Schroth *et al.* (37) matches the haplotype "C", except for the length of the sequences. Variations with the other reported loggerhead turtle mtDNA control region sequences (Genbank Nos; U22261, U40435, L35254, L35255) (40, 41) are also shown in bold letters in the sequences (Table 2).

An inverse relationship between nesting population size and mtDNA diversity is apparent in other populations (35, 36). However, some small populations with very low diversity have been observed (e.g. Aves Island, Guinea Bissau). These results help to confirm that the Mediterranean populations of green turtles and loggerhead turtles were estabished recently by the migration of a very small number of females from the Atlantic. This may have occurred after the last glacial period (41). However, there is also evidence of the diversity of loggerhead populations within the Mediterranean and this may be because of the short post-glacial history of Mediterranean loggerhead colonies (37). Since post-glacial immigration, natal homing behaviour must have caused demographic isolation between different populations within the Mediterranean, leading to the genetic differentiation in the nuclear and mitochondrial genomes (37). All the loggerhead samples from Cyprus showed the haplotype "B". In order to detect other haplotypes in the Mediterranean, many more samples from a wider variety of locations are required.

The green turtle population nests only on the beaches of Cyprus and southeastern beaches of Turkey (3, 42). The coasts of Cyprus and Turkey represent the only significant remaining nesting habitat for green turtles in the Mediterranean Sea. Extinction here would nearly extirpate the green turtle population from an entire sea basin, and this is reason enough to merit a very high conservation priority. This nesting population is

threatened with imminent extinction by habitat degredation, incidental fishery mortality, and development in the tourist industry.

Perhaps the primary conservation value of these data lies in the appreciation of the need for thorough natural history studies in wildlife management. The literature suggests that conservation initiatives based on incomplete natural history information can be disastrous (41, 43), and seemingly esoteric aspects of organismal biology or ecology (such as temperature-dependent sex determination) make the difference between success and failure in wildlife management programmes. Preprogrammed female reproductive behaviour makes it unlikely that the loss of breeding habitats can be compensated for by emigration to other colonies; that is, the loss of nesting sites is accompanied by the loss of specific genotypes (37). Thus, to preserve the genetic diversity of the sea turtle population in the Mediterranean individual nesting sites must be protected.

Phenotypic variation among the populations of reptiles and amphibia of Turkey has been quantified extensively using morphological characters (i.e., 44). Unfortunately, genetic diversity at the intraspecific level is not available for any species in Turkey. Sequencing DNA and in particularly the mtDNA may help to solve the taxonomic problems present in the herpetofauna of Turkey.

Acknowledgements

I am greatful to Drs. J. Sheps, B. Cohen and R.W. Furness (University of Glasgow, UK) for their help during this work and for their comments on the initial version of this manuscript.

References

1. Carr, A. Some problems of sea turtle ecology. Amer. Zool., 20: 489-498, 1980.
2. Dodd, C.K., Jr., Synopsis of the biological data on the loggerhead turtle Caretta caretta (Linnaeus 1758). U.S. Fish and Wildlife Service Biological Report, 88: 1-110, 1988.
3. Groombridge, B., Marine turtles in the Mediterranean; Distribution, population status, conservation: A report to the Council of Europe, World Conservation Monitoring Centre, Cambridge, K. 72 p., 1990.
4. Hendrickson, J.R., The green sea turtle, Chelonia mydas (Linn.), in Malaya and Sarawak. Proc. Zool. Soc., 130: 455-535, 1958.
5. Meylan, A.B., Bowen, B.W., and Avise, J.C., A genetic test of the natal homing versus social facilitation models for green turtle migration. Science, 248: 724-727, 1990.
6. Karl, S.A., Bowen, B.W., and Avise, J.C., Global population genetic structure and male-mediated gene flow in the green turtle (Chelonia mydas): RFLP analyses of anonymous nuclear loci. Genetics, 131: 163-173, 1992.
7. Bowen, B.W., Abreu-Grobois, F.A., Balazs, G.H., Kamezaki, N., Limpus, C.L., and Ferl, R. J., Trans-Pacific migrations of the loggerhead sea turtle demonstrated with mitochondrial DNA markers. Proc. Natl. Acad. Sci. USA. 92: 3731-3734, 1995.
8. Bowen, B.W., and Witzell, W.N., Proceedings of the international symposium on sea turtle conservation genetics. NOAA Technical Memorandum NMFS-SEFSC-396, 173 pp., 1996.
9. Avise, J.C., Bowen, B.W., Lamb, T., Meylan, A.B., and Bermingham, E., Mitochondria; DNA evolution at a turtle's pace: Evidence for low genetic variability and reduced microevolutionary rate in the Testudines. Mol. Biol. Evol., 9: 457-473, 1992.
10. Moritz, C., Dowling, T.E., and Brown, W.M., Evolution of animal mitochondrial DNA relevance for population biology and systematics. Ann. Rev. Ecol. Syst., 18: 269-292, 1987.
11. Leary, R.F., Allendorf, F.W., and Forbess, S.H., Conservation genetic of Bull trout in the Columbia and Klamath river drainages. Cons. Biol., 7 (4): 856-865, 1993.
12. Bernatchez, L., Guyomard, R., and Bonhomme, F., DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout Salmo trutto populations. Mol. Ecol., 1: 161-173, 1992.
13. Riddle, B.R., Propst, D.L., and Yates, T.L., Mitochondrial DNA variation in Gila trout Onocorhynchus gilae; implications for management of an endangered species. Copeia, 1998 (1): 31-39, 1998.
14. Pfeiler, E., Allozyme differences in Carribean and Gulf of California populations of bonefishes (Albula). Copeia, 1996 (1): 181-183, 1996.
15. Garcia-Paris, M., Alcobendas, M., and Alberch, P., Influence of the Guadalquvir river basin on mitochondrial DNA evolution of Salamandra salamandra (Caudata: Salamandridae) from southern Spain. Copeia, 1998 (1): 173-176, 1998.

16. Richards, C.M., and Moore, W.S., A phylogeny for the African treefrog family Hyperoliidae based on mitochondrial DNA. Mol. Phyl & Evol., 5 (3): 522-532, 1996.

17. Sasa, M., Cerrophidion godmani in Costa Rica: A case of extremely low allozyme variation. J. Herpetol., 31 (4): 569-572, 1997.

18. Colgan, D.J., and Da Costa, P., Genetic discrimination between the iguanas Brachylophus vitiensis and Brachylophus fasciatus. J. Herpetol., 31 (4): 589-591, 1997.

19. Ota, H., Lin, J., Bogadek, A., and Lau, M.W., Karyotype of the Lygosomine genus Ateuchosaurus from East Asia. J. Herpetol., 31 (4): 604-607, 1997.

20. Boissinot, S., Ineich, I., Thaler, L., and Guillaume, C., Hybrid origin and clonal diversity in the partenogenetic gecko, Lepidodactylus lugubris in French Polynesia. J. Herpetol., 31 (2): 295-298, 1997.

21. Creer, D.A., Kjer, K.M., Simmons, D.L., and Sites Jr, J.W., Phylogenetic relationship of the Sceloporus scalaris species group (Squamata). J. Herpetol., 31 (3): 353-364, 1997.

22. Tirado, G., and Lewis, A.R., Use of RAPD-PCR for parentage analysis in the teidd lizard Ameiva exsul. J. Herpetol., 31 (3): 436-440, 1997.

23. Bruna, E.M., Fisher, R.N., and Case, T.J., Cryptic species of pacific skinks (Emoia): further support from mitochondrial DNA sequences. Copeia, 1995 (4): 981-983, 1995.

24. Haenel, G.J., Mitochondrial DNA variation in populations of the tree lizard, Urosaurus ornatus. Copeia, 1997 (1): 174-178, 1997.

25. Lamb, T., and Osentoski, M.F., On the paraphyly of Malaclemys: a molecular genetic assesment. J. Herpetol., 31 (2): 258-265, 1997.

26. Scribner, K.T., Morreale, S.J., Smith, M.H., and Gibbons, J.W., Factors contributing to temporal and age-specific genetic variation in the freshwater turtle, Trachemys scripta. Copeia, 1995 (4): 970-977, 1995.

27. Walker, D., Nelson, W.S., Buhlmann, K.A., and Avise, J.C., Mitochondrial DNA phylogeography and subspecies issues in the monotypic freshwater turtle Sternotherus odoratus. Copeia, 1997 (1): 16-21, 1997.

28. Browne, R.A., Haskell, N.A., Griffin, C.R., and Ridgeway, J.W., Genetic variation among populations of the redbelly turtle (Pseudemys rubriventris). Copeia, 1996 (1): 192-195, 1996.

29. Marsden, J.E., Kassler, T., and Philipp, D., Allozyme confirmation that North American yellow perch (Perca flavescens) and Eurasian yellow perch (Perca fluviatilis) are separate spcies. Copeia, 1995 (4): 977-981, 1995.

30. Marshall, H.D., and Baker, A.J., Structural conservation and variation in the mitochondrial control region of Fringilline finches (Fringilla spp.) and the Greenfinch (Carduelis chloris). Mol. Biol. Evol., 14 (2): 173-184, 1997.

31. Arnason, U., nd Johnsson, E., The complete mitochondrial DNA sequence of the harbor seal, Phoca vitulina. J. Mol. Evol., 34: 493-505, 1992.

32. Maldonado, J.E. Davila, F.O., Stewart, B.S., Geffen, E., and Wayne, R.K., Intraspecific genetic differentiation in California sea lions (Zalophus californianus) from southern California and the Gulf of California. Mar. Mam. Sci., 11 (1): 46-58, 1995.

33. Baker, C.S., Plaumbi, S.R., Lambertsen, R.H., Weinrich, M.T., Calamboikidis, J., and O'Brien, S.J., Influence of seasonal variation on geographic distribution of mitochondrial DNA haplotypes in humpback whales. Nature, 344: 238-240, 1990.

34. Allard, M.W., Miyamoto, M.M., Bjorndal, K.A., Bolten, A.B., and Bowen, B.W., Support for natal homing in green turtles from mitochondrial DNA sequences. Copeia, 1994 (1): 34-41, 1994.

35. Lahanas, P.N., Miyamoto, M.M., Bjorndal, K.A., and Bolten, A.B., Molecular evolution and population genetics of Greater Caribbean green turtles (Chelonia mydas) as inferred from mitochondrial DNA control region sequences. Genetica, 94: 57-67, 1994.

36. Encalada, S.E., Lahanas, P.N., Bjorndal, K.A., Bolten, A.B., Miyamoto, M.M. and Bowen B.W., Phylogeoraphy and population structure of the Atlantic and Mediterranean green turtle Chelonia mydas: a mitochondrial DNA control regions sequence assessment. Mol. Ecol., 5: 473-83, 1996.

37. Schroth, W., Streit, B., and Schierwater, B., Evolutionary handicap for turtles. Nature, 384: 521-522, 1996.

38. Sambrook, J., Fritsch, E.F., and Maniatis, T., Molecular Cloning, A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989.

39. Newton, C.R., and Graham, A., PCR; Introduction to Biotechniques. BIOS Scientific Publishers Limited, Oxford, 1994.

40. Dutton, P.H., Davis, S.K., Guerra, T., and Owens, D., Molecular phylogeny for marine turtles based on sequences of ND4- leucine tRNA and control regions of mitochondrial DNA. Mol. Phyl. Evol., 5: 511-521, 1996.

41. Bowen, B.W., Kamezaki, N., Limpus, C.L., Hughes, G.H., Meylan, A.B., and Avise, J.C., Global phylogeography of the loggerhead turtle (Caretta caretta) as indicated by mitochondrial DNA haplotypes. Evolution, 48: 1820-1828, 1994.

42. Baran, I., and Kasparek, M., Marine Turtles in Turkey. Status survey 1988 and recommendation for conservation and management. Hiedelberg, 123pp, 1989.

43. Frazer, N.B., Sea turtle conservation and halfway technology. Cons. Biol., 6: 180-184, 1992.

44. Arikan, H., Olgun, K., Çevik, I.E., and Tok, C.V., A Taxonomical study on the Rana ridibunda PALLAS, 1771 (Anura: Ranidae) population from Ivriz-Ereğli (Konya). Tr. J. of Zoology, 22: 181-184, 1998.