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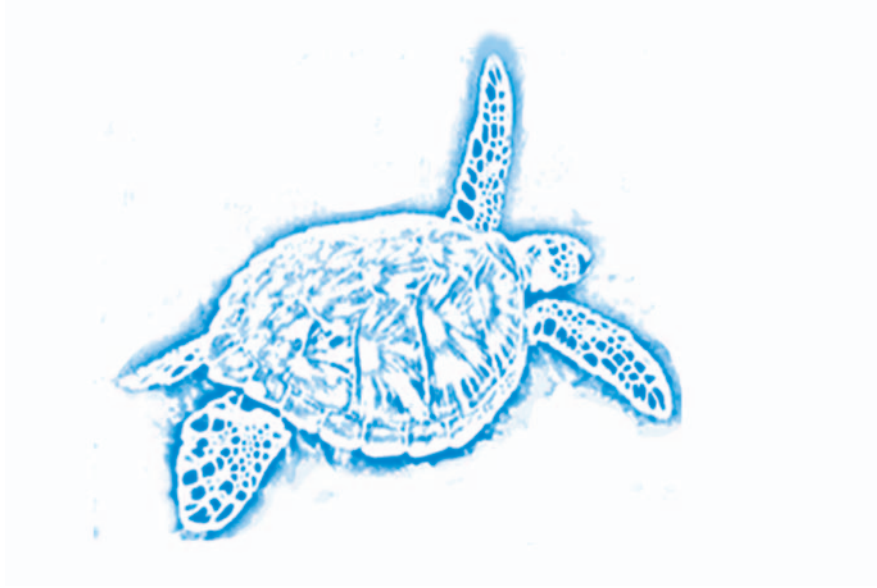

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RESULTS FROM GENETIC SCORING OF DEAD NESTLINGS OF *CARETTA CARETTA* FROM GREEK BEACHES

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INTRODUCTION

Population genetics is an indispensable source of information for our understanding of the past, present and, by extension, future state of any natural population (Avise 1994). This is particularly so for populations which are under threat or under exploitation by man. With regard to the loggerhead turtle *Caretta caretta*, several population genetics studies have already made significant advances in our understanding of the distribution, migration patterns and nesting habits, all of which are of fundamental importance in drawing a comprehensive and effective strategy for the protection and management of the natural populations of the species (Bowen et al. 1994, 1995, Schroth et al. 1996, Bolten et al. 1998, Laurent et al. 1998). The potential contribution of genetic tools to the understanding of the population biology of the species and its management and protection extend beyond delineating migration and homing patterns. It may provide information on the separate roles of males and females in the spatial genetic heterogeneity of populations, on effective population sizes (which may not parallel the consensus sizes, yet are more sensitive indicators of the species' long-term potential for survival) and on monitoring the effects of protective measures on the genetic wealth of the species. The results reported here address some of these questions.

MATERIAL AND METHODS

One or two dead nestlings were collected during post-hatch excavation of nests, routinely carried out by ARCHELON's monitoring projects, at six nesting beaches of Greece. Part of the tissue was stored in 90% alcohol and shipped to the Institute of Marine Biology of Crete. There has been no attempt to determine stage of development of the nestling at death. The number of sampled nests and the total number of nestlings obtained from each

shore are given in Tab. 1. Each nestling was scored at four microsatellite loci, which are parts of DNA that consist of tandemly repeated dinucleotides normally varying in the number of repeats. We used the primers published by FitzSimmons et al. (1995) and FitzSimmons (1998).

Nesting area	Code	Nests sampled	Total number of nestlings
Lakonikos Bay	LAK	30	59
Bay of Kyparissia	KYP	30	60
Zakynthos	ZAK	33	66
Bay of Chania	CHA	21	34
Rethymno	RET	29	54
Bay of Messara	MES	10	15
TOTAL		**153**	**288**

Tab. 1. Sampled sites, number of nests and total number of nestlings sampled.

RESULTS

1. Allele frequencies and genetic variation

Tab. 2 provides number of nestlings scored, number of observed alleles and observed as well as expected heterozygosities. One observation is that there is no systematic trend for the observed heterozygosity to be larger or smaller than the expected heterozygosity, which in turn means that there are no signs that natural populations deviate strongly from random mating.

2. Genetic differences among nesting areas

Allele frequencies (not shown here in need of space) were used to obtain the degree of genetic relatedness among nesting areas. There was no obvious trend for a correlation between genetic similarity and geographic proximity of sites, for significant deviations from random mating within populations or for the population as a whole.

3. Multiple paternity

The existence of scores for two siblings from the same nest allows an answer to the question of whether the two nestlings have a common father. Detection of multiple paternity through the genotypes of the offspring of a female depends critically on two factors: the number of offspring examined and the amount of variation in the population of the genetic loci used. Out of 24 possible comparisons, in 18 cases the observed value was smaller than the one expected if the hatchlings were full sibs, and in 6 it was larger. Thus, there is reason to suspect that multiple paternity is common.

	Cc7	Cc117	Ei8	Cm84
LAK	45 (17x2+11)	51 (22x2+7)	53 (23x2+7)	46 (19x2+8)
	13	10	7	8
	0.885	0.803	0.789	0.778
	0.642	0.827	0.833	0.778
KYP	37 (12x2+13)	40 (16x2+8)	41 (14x2+13)	45 (18x2+9)
	10	13	9	10
	0.847	0.854	0.825	0.834
	0.920	0.854	0.796	0.722
ZAK	42 (14x2+14)	51 (19x2+13)	50 (21x2+8)	45 (15x2+15)
	10	7	8	9
	0.802	0.656	0.731	0.770
	0.785	0.625	0.793	0.783
CHA	23 (4x2+15)	20 (3x2+14)	28 (8x2+12)	21 (4x2+13)
	14	7	8	7
	0.897	0.712	0.810	0.739
	0.921	0.794	0.900	0.558
RET	28 (7x2+14)	35 (11x2+13)	33 (11x2+11)	36 (11x2+14)
	10	7	8	8
	0.815	0.665	0.761	0.682
	0.809	0.583	0.840	0.620
MES	10 (2x2+6)	13 (4x2+5)	11 (2x2+7)	7 (1x2+5)
	8	8	9	7
	0.849	0.799	0.797	0.819
	1	0.833	0.889	0.667

Tab. 2. Number of nestlings scored (first row), number of alleles observed (second row), expected heterozygosity (third row) and observed heterozygosity (fourth row) for each nesting area and microsatellite locus. The parenthesis in the first row breaks the number of nests scored in those with two scored nestlings (first number) and with one (third number).

4. Triploidy

In the process of scoring we came across nestling that had three alleles (trizygosity), which is incompatible with diploidy. For the whole collection of samples the minimum estimate of trizygosity was 6%.

DISCUSSION

The microsatellite data we obtained are compatible with the null hypothesis that nestlings found in a nesting area are a random sample of the combined nestling population. This is equivalent to saying that we cannot reject the hypothesis that females nesting in different beaches are drawn from the same random mating population. This is not equivalent to

saying that the data provide evidence for this hypothesis, simply that the data cannot reject the hypothesis.

The results are also useful in another context. The observation that a reasonably high amount of genetic variation was found among nestlings from the same beach, as well as in all beaches combined, and that observed heterozygosities did not trail behind expected heterozygosities is an indication that the breeding population of *C. caretta* from which the sampled females originated is large and does not show any signs of inbreeding. This agrees with a model of thorough mixing of adults at the feeding grounds, irrespective of the natal origin.

Multiple paternity may have several implications for the conservation of the species. An obvious difference between a nest with a multiple paternity and one with a single paternity is that the former contains a larger part of the population's genetic variation. This may mean that a higher number of hatchlings may survive from a multiple rather than from a single paternity nest, owing to an improved chance of the sibship to cope with a variety of adverse conditions. It may also mean that genetic competition among individuals may start very early in life, while still in the same nest.

The observation that 6% of the hatchlings appeared to possess three rather than two microsatellite alleles is unexpected. The observation of trizygosity in more than one locus makes triploidy the most likely explanation. This means that one of the gametes failed to undergo meiosis. Triploidy would lead to death at an early stage of development or at some later stage in life. The questions that arise are of several kinds: a) what percentage of deaths among hatchlings is due to trisomy? b) does it result from meiotic abnormalities in the female or male parent? c) does the frequency of these abnormalities relate to stress? These questions cannot be evaluated without specifically designed experiments and field studies.

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