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# Isolation and genetic diversity of endangered grey nurse shark (*Carcharias taurus*) populations

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Anthropogenic impacts are believed to be the primary threats to the eastern Australian population of grey nurse sharks (*Carcharias taurus*), which is listed as critically endangered, and the most threatened population globally. Analyses of 235 polymorphic amplified fragment length polymorphisms (AFLP) loci and 700 base pairs of mitochondrial DNA control region provide the first account of genetic variation and geographical partitioning (east and west coasts of Australia, South Africa) in *C. taurus*. Assignment tests, analysis of relatedness and *Fst* values all indicate that the Australian populations are isolated from South Africa, with negligible migration between the east and west Australian coasts. There are significant differences in levels of genetic variation among regions. Australian *C. taurus*, particularly the eastern population, has significantly less AFLP variation than the other sampling localities. Further, the eastern Australian sharks possess only a single mitochondrial haplotype, also suggesting a small number of founding individuals. Therefore, historical, rather than anthropogenic processes most likely account for their depauperate genetic variation. These findings have implications for the viability of the eastern Australian population of grey nurse sharks.

**Keywords:** *Carcharias taurus*; low genetic variation; migration

## 1. INTRODUCTION

Recent global declines of many shark populations have drawn attention to the devastating effect of over-exploitation on marine ecosystems (Baum *et al.* 2003). Interpretation, and possible remediation, of these declines requires both demographic and genetic knowledge. Levels of genetic variation may provide good predictors of population persistence, as average heterozygosities are 35% lower in threatened taxa, than in related, non-threatened taxa (Spielman *et al.* 2004). Isolation and reduction in population size

accelerate the erosion of evolutionary potential and escalate the risk of extinction through inbreeding depression (Frankham *et al.* 2002; Taylor 2003). However, while reduced genetic variation is frequently symptomatic of recent reductions in population size, it may also reflect more ancient events, pre-dating human influences (Menotti-Raymond & O'Brien 1993). Further, low genetic diversity does not necessarily imply loss, as low mutation rate might also be a factor (Martin 1995).

The grey nurse shark (*Carcharias taurus*) is a large, coastal shark with a widespread but disjunct distribution (Compagno 1984; figure 1). It is listed as globally vulnerable by the World Conservation Union and as critically endangered in eastern Australian waters (Cavanagh *et al.* 2003). Recreational and commercial fishing and netting have recently severely reduced the eastern Australian population. Quasi-extinction risk is very high unless the population experiences significant immigration (Otway 2004; Otway *et al.* 2004). Therefore, we have assessed whether eastern Australian grey nurse sharks are genetically isolated, by estimating genetic partitioning and inferred dispersal among populations from the Pacific and Indian Oceans. Intrapopulation estimates of genetic variability from each of these populations provide baseline information for assessing extinction risk.

## 2. MATERIAL AND METHODS

Genomic DNA was extracted from 25 mg of tissue, collected from 67 *C. taurus* from the eastern and western Australian coasts and South Africa (figure 1), using a QIAmp Tissue Kit (Qiagen Inc., Valencia, CA). AFLP typing of 65 individuals (table 1) involved digestion of 200–400 ng of genomic DNA with the restriction enzymes *MseI* and *EcoRI* and generation of profiles using 12 different primer pairs in the final (selective) polymerase chain reaction (PCR). Each primer pair combination had a total of three selective nucleotides on the *EcoRI* primer (GAC TGC GTA CCA ATT C+ACT, AGT, ATC or AAC) and four selective nucleotides on the *MseI* primer (GAT GAG TCC TGA GTA A+CAAC, CTGC, CAGC or CTTC). A total of 235 polymorphic AFLP loci were scored. Allele frequencies were estimated with a Bayesian method, assuming a non-uniform prior distribution of allele frequencies (AFLP-SURV v. 1.0; Vekemans 2002). The same software calculated expected heterozygosity (*He*), pairwise *Fst* (significance assessed by 1000 permutations) and pairwise relatedness (*r*). The distance measure, (1–*r*), was used to generate a neighbourhood joining tree using MEGA 3.0 (Kumar *et al.* 2004). Genetic subdivision among South African, east and west Australian samples was assessed using STRUCTURE 2.1 (Pritchard *et al.* 2000; Evanno *et al.* 2005), assuming no prior information, the admixture model with correlated alleles, and a burn-in phase of 10 000 iterations followed by a run phase of 100 000 iterations. The number of populations (*K*) ranging from 1 to 3 was tested in three independent runs to establish consistency. The posterior probability was then calculated for each value of *K* to choose the most likely *K*. We also used a model with essentially the same parameters as earlier, but providing prior information of population membership, to identify immigrants, or individuals who have recent immigrant ancestry (testing for immigrants over the last two generations; Pritchard *et al.* 2000).

For 61 individuals, approximately 700 base pairs of mitochondrial DNA (mtDNA) control region was PCR amplified using primers designed from conserved chondrichthyan sequences (forward primer MtGNf (5'-AAY CTG RCA TCT GAT TAA TGC) and reverse primer MtGNr (5'-CATYTTAGCATCTT-CAGTGC)). Amplifications (in an MJ Research PTC100 thermocycler) were conducted in 50 µl reactions with initial denaturation for 3 min at 94 °C, followed by six 'touchdown' cycles of 94 °C, denaturation for 30 s, annealing temperatures (62, 60, 59, 58, 57 and 55 °C) for 45 s and an extension step of 72 °C for 1 min. On completion of the last touchdown cycle, a further 33 cycles were conducted at 55 °C annealing temperature with a final 72 °C for 3 min. Nucleotide composition was assessed using single-stranded

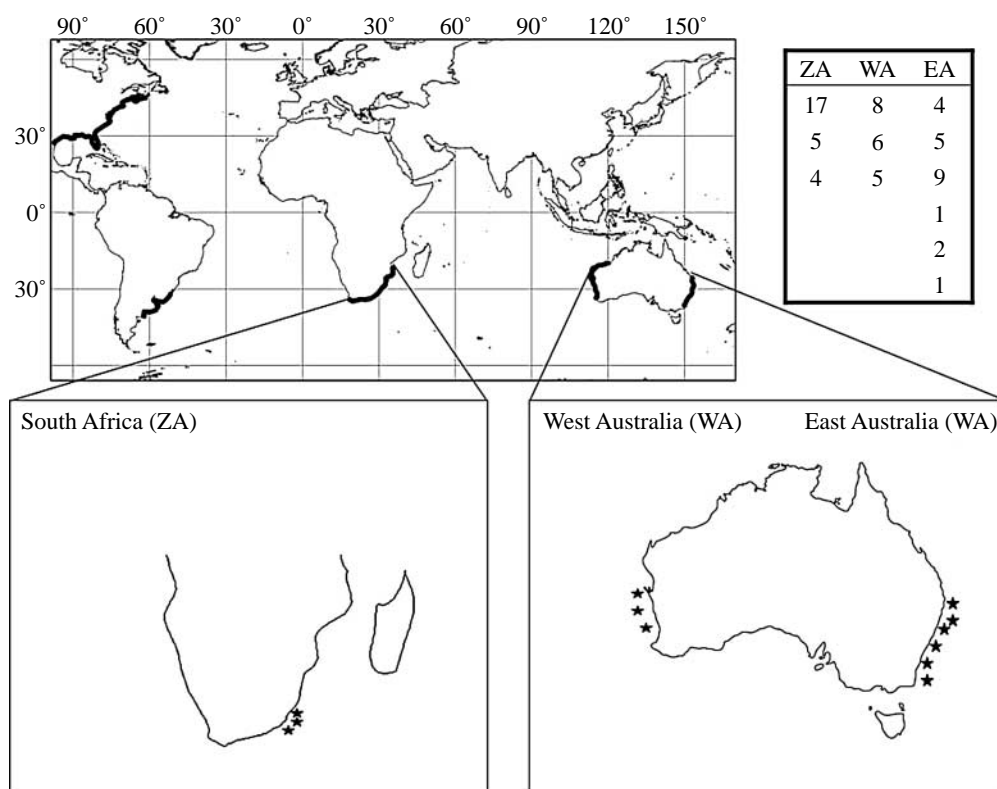


Figure 1. Distribution of *C. taurus* (marked black), showing sampling locations (marked with star). Number of individuals sampled at each location from Australian and South African waters (table 1) are arranged in a north–south order.

Table 1. Genetic diversity at AFLP and mitochondrial markers in *C. taurus* sampled from east and west Australia and South Africa. (AFLP diversity is estimated by expected heterozygosity ( $H_e$ ) and the number of polymorphic loci ( $PL$ ). Diversity at the mitochondrial control region is described by the number of haplotypes ( $H$ ), haplotype diversity ( $H_d$ ) and nucleotide diversity ( $\pi$ ).  $N$ , number of individuals from which AFLP and mitochondrial data were obtained.)

sampling region	AFLP diversity			mitochondrial diversity			
	$N$	$PL$	$H_e (\pm s.e.)$	$N$	$H$ type <sup>a,b</sup>	$H_d (\pm s.e.)$	$\pi (\pm s.e.)$
eastern Australia	22	59	0.103 $\pm$ 0.011	19	C <sup>19</sup>	0	0
western Australia	19	72	0.115 $\pm$ 0.011	16	C <sup>11</sup> E <sup>5</sup>	0.458 $\pm$ 0.024	0.0031 $\pm$ 0.0001
South Africa	24	167	0.145 $\pm$ 0.010	26	A <sup>3</sup> B <sup>8</sup> C <sup>11</sup> D <sup>4</sup>	0.717 $\pm$ 0.010	0.0030 $\pm$ 0.0001

<sup>a</sup> Haplotypes are coded A–E.

<sup>b</sup> In each region, the number of each of the haplotypes identified (superscript).

conformational polymorphism analysis (following Sunnucks *et al.* 2000) and sequencing, which used the forward primer and BigDye termination (Perkin–Elmer Applied Biosystems) and was resolved on an ABI 377 sequencer. The 61 sequences were aligned and edited by eye, leaving 518 sites, of which only five were informative (GeneBank accession numbers: DQ250809–DQ250813). Haplotype diversity ( $H_d$ ), nucleotide diversity ( $\pi$ ) and theta per DNA sequence ( $\theta$ ) was calculated using the software DnaSP v. 4.10.2 (Rozas *et al.* 2003).

### 3. RESULTS

Strong genetic structuring of AFLP's among regions inferred low levels of dispersal. This subdivision was pronounced ( $F_{st}$ : 0.295;  $p < 0.001$ ) with highly significant ( $p < 0.001$ )  $F_{st}$  values for each pairwise comparison ( $F_{st}$  value between EA and WA = 0.243, WA and ZA = 0.345 and EA and ZA = 0.545). Population subdivision between South Africa and Australia was also strongly supported by analysis with STRUCTURE. However, discrimination between  $K=2$  and 3 was ambiguous (see Pritchard *et al.* 2000; Evanno *et al.* 2005 for discussion). Further STRUCTURE analysis,

using only Australian samples, clearly resolved two separate populations (eastern and western) thereby supporting  $K=3$ . STRUCTURE identified two putative immigrants, or individuals with migrant ancestors (within two generations), within the South African sample (probability of belonging less than 0.050). No immigration was detected within the Australian samples (probability of belonging for each individual greater than 0.999). Clustering of individuals into each sampling region is illustrated by the neighbourhood joining tree of genetic distance (figure 2).

Lower levels of genetic variation in Australian, as compared to South African, populations were supported by both AFLP and mitochondrial data, with the lowest level present in the east (table 1). Comparisons of the number of polymorphic loci present for each of the 12 primer pair combinations further supported this conclusion. The mean number of polymorphic loci ( $\pm$ s.d.) for both the east ( $4.92 \pm 2.82$ ) and west ( $6.00 \pm 2.98$ ) Australian populations

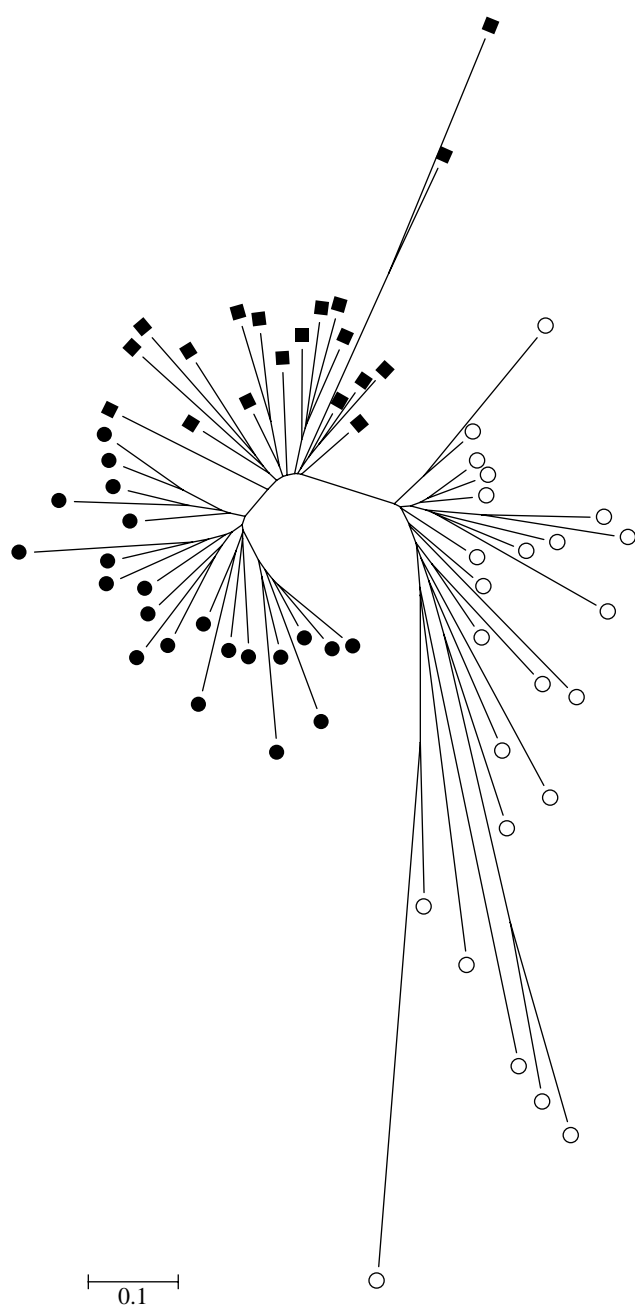


Figure 2. A neighbour joining tree of genetic distance ( $1-r$ ), clustering individuals sampled from east and west Australia (filled circles and filled squares, respectively) and South Africa (open circles). Note the longer branch lengths (i.e. greater genetic divergence) in the South African samples.

were significantly less (Wilcoxon sign;  $p < 0.005$ ) than for the South African sample ( $13.92 \pm 4.87$ ).

#### 4. DISCUSSION

Critically endangered east Australian *C. taurus* are characterized by less genetic variation than any other population sampled. Genetic variation is highest in the South African population, declining progressively to the east (to western and then eastern Australia; table 1). Limited historical dispersal with 'sequential founder' effects, rather than a recent anthropogenic 'bottleneck', best explain this pattern. Individuals sampled off Australia are highly divergent from those

sampled from South Africa. Further, major differences in genetic structure are evident between eastern and western Australian *C. taurus* ( $F_{st}$ : 0.243;  $p < 0.001$ ), inferring limited or zero dispersal between these populations. The isolation of Australian *C. taurus* has important implications for conservation management, since replenishment of the critically endangered eastern and vulnerable western Australian population is unlikely to be achieved via natural migration from more numerous populations elsewhere.

As a species, *C. taurus* appears to exhibit generally low levels of mitochondrial variation. Although the control region is the most rapidly evolving segment of the mitochondrial genome, little variation was detected (table 1). This level of mtDNA variation in *C. taurus* may be accounted for by a low rate of molecular evolution in sharks (Martin 1995). Overall, mitochondrial variation detected at the control region in *C. taurus* ( $\pi = 0.0025$ ) was lower than reported for some sharks yet similar to others. For example, substantially higher  $\pi$  of 0.0203 was estimated from 95 great white shark (*Carcharodon carcharias*) samples from Australian and South African waters (calculated from Pardini *et al.* 2001), while a comparable level of variation was detected in a sample of 323 blacktip sharks (*Carcharhinus limbatus*),  $\pi = 0.0021$  (Keeney *et al.* 2005). However, lack of any variation in east Australian *C. taurus* is notable. Further sampling of the east Australian population is unlikely to yield additional haplotypes ( $p \sim 0.1$ ), solved recursively (Hudson 1990) with  $\theta = 1.162$  calculated from South African and west Australian samples. Genetic variation at nuclear DNA was also lower in east than west Australian *C. taurus*; however, the magnitude of the difference was greater at the control region. Because effective sizes at mtDNA are approximately one-quarter that of nuclear DNA, differing patterns of variation at nuclear and mtDNA can be expected following population reductions (Fay & Wu 1999).

Low variability in east Australian *C. taurus* most likely reflects older and slower processes rather than a recent, human-induced population bottleneck. Indeed, human actions leading to the dramatic population size reduction of *C. taurus* off eastern Australia (particularly over the past 40 years) have not been of sufficient duration to expect substantial erosion of genetic variability, given the species' longevity (25+ years) and the 9–10 years required to reach maturity (Otway 2004). With current population estimates for the Australian east coast of approximately 500 animals (Otway 2004), the effective population size ( $N_e$ ) is about 50 (Frankham *et al.* 2002). Even at high rates of decline, for example, if  $N_e$  decreased over four generations as follows: 1000, 500, 100 and 50, the expected loss in heterozygosity, as described by  $H[1 - 1/(2N_e)]$  is only approximately 3%. In addition, even this negligible loss would be masked to an extent by mainly sampling adults whose genetic constitution was derived from the gene pool available prior to the loss of much diversity.

Here, we provide the first account of genetic structuring in *C. taurus*, a species in decline throughout much of its distribution, and designated as

vulnerable and critically endangered on the west and east coasts of Australia, respectively. Given the ongoing population declines of *C. taurus* in eastern Australia (Otway 2004; Otway *et al.* 2004) and negligible migration among Australian populations, extinction is imminent in east Australian waters without urgent conservation efforts.

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