Sex-biased dispersal of great white sharks

In some respects, these sharks behave more like whales and dolphins than other fish.

Ecological information about populations of great white sharks (*Carcharodon carcharias*) has been difficult to acquire, not least because of the rarity and huge size of this fish. Here we use genetic methods to show that the dispersal of *C. carcharias* is sex-biased, with philopatric (non-roving) females and roving males. In conjunction with other shared life-history features, including low fecundity, long lifespan and late age at maturity, our findings indicate that the population biology of *C. carcharias* may be more similar to that of marine mammals[1,2] than to that of other fish.

Carcharodon carcharias (Fig. 1) is globally distributed in temperate waters off continental shelves[3] and investigations have been undertaken on several populations[4,5]. The analysis described here focuses on sharks collected off the coasts of South Africa (SA), Australia (AU) and New Zealand (NZ), where we were able to sample a relatively large number of individuals.

Pairwise comparison of complete sequences from the non-coding 'control' region of the maternally inherited mitochondrial genome reveals two divergent genetic lineages within *C. carcharias* which differ by about 4% in sequence (Fig. 2a). Estimates of population differentiation on the basis of the control-region sequences (using F_{ST} analysis) show that *C. carcharias* from AU and NZ coastal waters are not significantly different, but that individuals from these populations are distinct from SA sharks (F_{ST} values of 0.81 and 0.89 for pairwise estimates between SA and AU, and SA and NZ, respectively; $P < 0.0001$ for each). The estimated differentiation approaches the theoretical maximum of unity, which suggests long-term isolation of these populations.

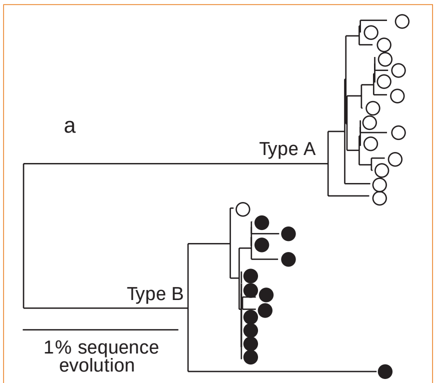
A survey of restriction-fragment-length polymorphisms in mitochondrial DNA from further individuals (Fig. 2b) supported this result, with only 1 of the 95 animals surveyed found to be out of place — a 3.5-metre male captured in Tasmania (AU) with a South African haplotype. These results suggest that female-mediated gene flow between ocean basins is rare.

In contrast, population genetic analysis of the sharks from AU and NZ ($n = 52$) and SA ($n = 43$) for five polymorphic nuclear-encoded microsatellite loci[6] reveals no significant differentiation (Table 1). The low differentiation between populations that was identified by using nuclear DNA markers suggests that male-mediated gene flow occurs at a level that is sufficient to homogenize allele frequencies, and that dispersal of individuals is more extensive than has been indicated by movements estimated from tagging data[5].

The contrast between the sequence differentiation revealed for the maternally inherited genetic marker and the lack of nuclear-gene differentiation indicates that female sharks are probably philopatric and that males may undertake transoceanic excursions. Although we have no direct evidence for this idea, sex-specific differences in habitat use in several shark species[7,8], including *C. carcharias*[5,9], and in migratory behaviour[10] have been reported. These and other similarities between sharks and marine mammals suggest that general theories of sex-biased dispersal in birds and mammals[11] may also be relevant to *C. carcharias* and other species of shark.

Carcharodon carcharias is categorized as 'vulnerable' on the World Conservation Union's 'red' list of threatened animals[3], so our findings may have implications for conservation strategies. Exploitation of a population in which immigration from surrounding stocks may be low and females are philopatric could lead to a rapid decline in stock size and future sustainability. The global dispersion of males may indicate that the demography of widely separated populations is linked, underscoring the need for international regulations to govern exploitation of great white sharks.

Figure 1 The great white shark (*Carcharodon carcharias*), which, although rare, inhabits temperate waters throughout the world.



b

Population	Type A	Type B
SW Pacific	48	1
SW Indian	0	39

Figure 2 Sequence and survey of restriction-fragment-length polymorphisms (RFLPs) of the mitochondrial-DNA control region from great white shark populations reveals two divergent mitochondrial DNA types (called A and B). **a,** Phylogram depicting the relationships between individuals for which complete control-region sequences were determined. Black circles, samples from South African population; white circles, samples from Australian/New Zealand populations. (GenBank accession numbers for the D-loop sequences are AY026196–AY026224). **b,** Summary of the distribution of haplotypes determined using RFLP analysis of control-region sequences amplified by polymerase chain reaction. The observed distribution is significantly different from random expectations; Fisher's exact test, 0.958; $P < 0.0001$. Full details of methods and further information are available at http://stripe.colorado.edu/~am/WhiteSharks.html

Table 1 Nuclear-encoded microsatellite data from great white sharks

*Locus**	*No. of alleles*			*Observed heterozygosity*		*Θ†*	*ρ‡*
	AU/NZ	*SA*	*All*	*AU/NZ*	*SA*		
Ccar1	6	5	7	0.686	0.643	NS	NS
Ccar6	4	5	5	0.647	0.595	NS	NS
Ccar9	15	12	15	0.882	0.857	NS	NS
Ccar13	9	7	9	0.804	0.786	NS	NS
Ccar19	4	3	4	0.451	0.452	NS	NS
All	38	32	40	0.694	0.667	NS	NS

Two populations of great white sharks were surveyed: Australian–New Zealand (AU/NZ) and South African (SA). The results indicate that there is a lack of significant differentiation between the two populations. NS, not significant. Further information is available at: http://stripe.colorado.edu/~am/WhiteSharks.html

*GenBank accession number is AF184085 for microsatellite *Ccar6*; for information about the other loci, see ref. 6.

†This statistic is an analogue of F_{ST} (ref. 12), a measure of population differentiation.

‡Unbiased estimator of Slatkin's R_{ST} (ref. 13), another measure of population differentiation.

Amanda T. Pardini*, Catherine S. Jones*, Leslie R. Noble*, Brian Kreiser†‡, Hamish Malcolm§, Barry D. Bruce§, John D. Stevens§, Geremy Cliff||, Michael C. Scholl¶, Malcolm Francis#, Clinton A.J. Duffy✩, Andrew P. Martin‡

*Department of Zoology, University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, UK

†Department of Biological Sciences, University of Southern Mississippi, Hattiesburg, Mississippi 39406, USA

‡Department of Environmental, Population and Organismic Biology, University of Colorado, Boulder, Colorado 80309, USA

e-mail: am@stripe.colorado.edu

§CSIRO Marine Research Laboratories, Castray Esplanade, Hobart, Tasmania 7000, Australia

||Natal Sharks Board, Private Bag 2, Umhlanga Rocks 4320, South Africa

¶PO Box 1258, Gansbaai 7220, Western Cape, South Africa

#National Institute of Water and Atmospheric Research, PO Box 14-901, Kilbirnie, Wellington, New Zealand

✩Scientific & Research Unit, Department of Conservation, PO Box 112, Hamilton, New Zealand

1. Escorza-Trevino, S. & Dizon, A. E. *Mol. Ecol.* **9,** 1049–1060 (2000).
2. Lyrholm, T., Leimar, O., Johanneson, B. & Gyllensten, U. *Proc. R. Soc. Lond. B* **266,** 347–354 (1999).
3. Compagno, L. J. V., Marks, M. A. & Fergusson, I. K. *Environ. Biol. Fish.* **50,** 61–62 (1997).
4. Strong, W. R. Jr, Bruce, B. D., Nelson, D. R. & Murphy, R. D. in *Great White Sharks: the Biology of* Carcharodon carcharias (eds Klimley, A. P. & Ainley, D. G.) 401–414 (Academic, San Diego, 1996).
5. Cliff, G., Van der Elst, R. P., Govender, A.,Witthuhn, T. K. & Bullen, E. M. in *Great White Sharks: the Biology of* Carcharodon carcharias (eds Klimley, A. P. & Ainley, D. G.) 393–400 (Academic, San Diego, 1996).
6. Pardini, A. T., Jones, C. S., Scholl, M. C. & Noble, L. R. *Mol. Ecol.* **9,** 1171–1193 (2000).
7. Economakis, A. E. & Lobel, P. S. *Environ. Biol. Fish.* **51,** 129–139 (1998).
8. Klimley A. P. *Environ. Biol. Fish.* **18,** 27–40 (1986).
9. Klimley, A. P. *South. Calif. Acad. Sci. Mem.* **9,** 15–40 (1985).
10. Bass, J. A. in *Sensory Biology of Sharks, Skates and Rays* (eds Hodgson, E. S. & Mathewson, R. F.) 545–594 (Office of Naval Research, Arlington, Virginia, 1978).
11. Greenwood, P. J. *Anim. Behav.* **28,** 1140–1162 (1980).
12. Weir, B. S. & Cockerham, C. C. *Evolution* **38,** 1358–1370 (1984).
13. Goodman, S. J. *Mol. Ecol.* **6,** 881–885 (1997).

Flame retardants

Persistent pollutants in land-applied sludges

Disposal of sewage sludge by application to agricultural and other land is widely practised and is presumed to be environmentally beneficial, but we have found high concentrations of an environmentally persistent class of organic pollutants, brominated diphenyl ethers (BDEs), in 'biosolids' from four different regions of the United States. These compounds are widely used as flame retardants, and their presence suggests that the environmental consequences of land application of biosolids need further investigation. We also frequently detected BDEs in wild-caught fish, indicating another pathway for human exposure.

Over half of the sewage sludge produced annually in the United States is applied to land, amounting to roughly 4 million tons in 1998 (ref. 1). Sludges are treated before application to reduce odour and pathogen content and their metal burden is regulated. But attention has focused less on persistent organic pollutants since usage of the most notorious (for example, polychlorinated biphenyls) has decreased and pretreatment of industrial waste water has improved[1,2].

We analysed 11 biosolid samples before land application from Virginia, Maryland, New York state and California, and found that they all contained high concentrations of BDEs. These flame-retardant polymers are structurally similar to polybrominated biphenyls, the use of which was curtailed after a significant contamination incident in 1973 involving livestock feed in Michigan[3].

However, global consumption of BDEs continues to increase, reaching 67,125 metric tonnes in 1999 (refs 4, 5). The most bio-accumulative and toxic BDEs (those containing 4–6 bromine atoms) are being increasingly detected in humans and wildlife from both developed and remote areas[5–7]. These were present in significant amounts in the biosolids we examined and their relative contributions matched those in 'Penta', the commercial formulation used as a flame retardant in polyurethane foam (Fig. 1). North America accounts for about 98% of global demand for Penta, estimated at 8,290 tonnes in 1999 (ref. 4).

How BDEs are released from polymers has been uncertain, as these applications are considered to be non-dispersive[7]. However, breakdown of discarded polyurethane foam, which may contain up to 30% Penta by weight[5], may contribute to this. We found that the surface of foam became brittle and sloughed off after 4 weeks of exposure to ambient summer conditions. The particles generated are easily transported and the polymer matrix preserves the formulation's original BDE composition.

The total concentration of Penta-like BDEs in these biosolids was 1,100–2,290 µg per kg dry weight, suggesting that input was high and consistent, regardless of the region of origin and irrespective of pre-application treatment (see supplementary information). Concentrations exceed those in European sludges by 10- to 100-fold[8], which is commensurate with the greater demand for Penta in the United States. The European Commission recently proposed a ban on the use of Penta, on the basis of its reported exponential increase in human breast milk and perceived health risks[9].

The fully brominated Deca product constitutes 82% of the total global BDE market[4]. It is rarely reported in wildlife, perhaps because of its low bioavailability. Deca consists principally of a single BDE (BDE-209) and is used to curtail fires in textiles and in relatively stable, rigid polymers, such as those used in television and computer casings[5]. Unlike those of Penta constituents, BDE-209 concentrations varied widely among the biosolids we analysed (84.8–4,890 µg kg^{-1}; see supplementary information). Although there is little evidence for the degradation of Deca to Penta-like compounds, some photolysis of Deca to less brominated diphenyl ethers is possible[5,7].

We also detected BDEs in 87% of fish sampled from Virginia waters (quantification limit in fillets, 5 µg per kg lipid; $n = 334$). The principal Penta constituents (BDE-47, -100 and -99) predominated in these samples (Fig. 1). This finding indicates that significant environmental release of these pollutants is occurring in the United States and that humans may be exposed to them through their diet. Carp from one Virginia stream contained 47,900 µg kg^{-1} of total BDEs, rivalling the highest fillet

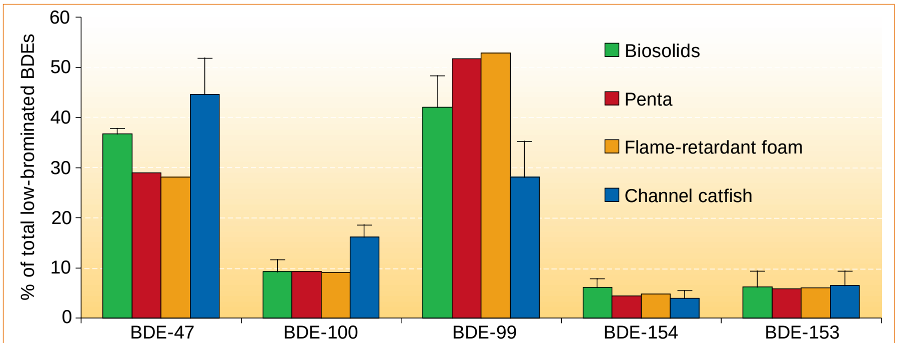


Figure 1 Brominated diphenyl ethers (BDEs) are produced commercially and occur in the environment as mixtures of compounds of varying bromination. Relative contributions of tetra- (BDE-47), penta- (BDE-100 and -99) and hexa- (BDE-154 and -53) brominated versions were similar in 11 biosolids obtained from four different regions of the United States, in the Penta commercial product (used as a flame retardant in polyurethane foam), in treated foam and in wild-caught fish (data shown are for 15 composite samples of channel catfish, *Ictalurus punctatus,* an omnivorous bottom-dwelling species) collected from Virginia lakes and rivers (error bars represent standard deviation). BDE-209 was not detected in fish but was present in biosolids.