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A review of the application of molecular genetics for fisheries management and conservation of sharks and rays

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Since the first investigation 25 years ago, the application of genetic tools to address ecological and evolutionary questions in elasmobranch studies has greatly expanded. Major developments in genetic theory as well as in the availability, cost effectiveness and resolution of genetic markers were instrumental for particularly rapid progress over the last 10 years. Genetic studies of elasmobranchs are of direct importance and have application to fisheries management and conservation issues such as the definition of management units and identification of species from fins. In the future, increased application of the most recent and emerging technologies will enable accelerated genetic data production and the development of new markers at reduced costs, paving the way for a paradigm shift from gene to genome-scale research, and more focus on adaptive rather than just neutral variation. Current literature is reviewed in six fields of elasmobranch molecular genetics relevant to fisheries and conservation management (species identification, phylogeography, philopatry, genetic effective population size, molecular evolutionary rate and emerging methods). Where possible, examples from the Indo-Pacific region, which has been underrepresented in previous reviews, are emphasized within a global perspective.

Key words: evolution; genetic effective population size; Indo-Pacific; philopatry; phylogeography.

GENERAL INTRODUCTION

Molecular genetic approaches provide a unique tool for the study of sharks, skates and rays (elasmobranchs). A great virtue of genetic approaches is that a small tissue sample collected from the living or deceased animal at any age contains its complete nuclear (bi-parentally inherited) and mitochondrial (matrilineal) genomic information. By comparing neutral or conserved variation within selected regions of the genome it is possible to investigate the genetic relationships at the individual level (*e.g.* genotyping for parenthood, kinship and mark–recapture analysis) through

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to populations (assessing stock structure, population size, philopatry and local adaptation), species (defining species distributions and identifying cryptic species) and higher orders to discern evolutionary relationships.

The application of genetic techniques has developed rapidly with the increasing affordability of new analyses and the pressing need to address critical conservation and fisheries management issues, such as stock structure and population demography in the context of very high rates of shark harvest (FAO, 1999, 2000, 2005). The first genetic-based study on elasmobranchs was published 25 years ago (Smith, 1986). This study used allozymes (Appendix) to investigate genetic diversity in smoothhound *Mustelus lenticulatus* Phillips 1932 and blue sharks *Prionace glauca* (L. 1758). Allozyme research dominated the elasmobranch literature for over a decade addressing questions about genetic diversity, population structure and phylogeny (MacDonald, 1988; Lavery & Shaklee, 1989; Heist *et al*., 1995; Gaida, 1997; Gardner & Ward, 1998, 2002). The advent of the polymerase chain reaction (PCR, Saiki *et al*., 1985) enabled deoxyribonucleic acid (DNA) sequences of specific regions to be examined directly in new and more powerful ways. Early DNA studies of elasmobranchs examined highly conserved gene regions, which were used in phylogenetic analyses to investigate the origins of vertebrates (Bernardi & Powers, 1992; Stock, 1992; Le *et al*., 1993). Studies on more variable gene regions were then used to resolve phylogenetic relationships within the elasmobranchs up to the level of order (Lavery, 1992; Naylor *et al*., 1997; Douady *et al*., 2003; Corrigan *et al*., 2008; Velez-Zuazo & Argnarsson, 2011) although higher-level relationships between extant members of the elasmobranch subclass continue to be debated (Douady *et al*., 2003; Naylor *et al*., 2005).

More recently, descriptions of regional and global phylogeographic patterns using both nuclear and mtDNA markers have broadened the understanding of the historical and contemporary processes driving elasmobranch distribution patterns. Population genetic analyses have also directly informed fisheries managers about the appropriate scale of management through the elucidation of stock structure (Keeney *et al*., 2003; Ovenden *et al*., 2010). Forensic applications using DNA identification of shark fins allow more accurate monitoring of the shark fin trade (Shivji *et al*., 2002; Holmes *et al*., 2009). PCR has also paved the way for new ways to survey more of the genome such as amplified fragment length polymorphisms (AFLP), random amplification of polymorphic DNAs (RAPD) and microsatellite analysis. By focusing on hyper-variable regions of the genome these techniques offer increased resolving power, enabling analyses of stock structure on a finer scale. Microsatellites in particular have been used for individual identification and have opened up research into areas of reproduction, kinship, relatedness, movement and philopatry.

Emerging genetic technologies are enabling the development of new markers and greater sequence data production at reduced costs, resulting in a shift away from studies on gene fragments to entire genomes. This transition has also caused a paradigm shift from neutral to adaptive variation. Many of the limitations of the molecular techniques in current use (*e.g.* proposed low genetic variation in elasmobranchs and challenges with microsatellite development) are likely to be overcome with these advances in molecular science.

Below is a comprehensive review of molecular techniques used to address the questions of ecological and evolutionary science in elasmobranchs, and their

application to fisheries management and conservation. The review takes a global perspective; however, the emphasis is placed on the Indo-Pacific region, which contains the greatest global biodiversity of elasmobranchs (Fowler *et al*., 2005), but remains under-represented in global genetic studies to date (Beheregaray, 2008).

IDENTIFICATION OF SHARKS AND RAYS USING GENETIC MARKERS

Accurate identification of elasmobranch species is the basis for addressing many ecological and evolutionary questions, and is fundamental to management and conservation. In 1973, a new scheme was proposed for classifying extant elasmobranchs based on extensive comparative morphological studies (Compagno, 1973). Genetic studies have supported many of the groupings proposed by Compagno (1973), but have also repositioned a number of lineages (Velez-Zuazo & Argnarsson, 2011). While morphology is often the fastest and cheapest approach to identification there are many circumstances where DNA-based techniques may be particularly useful such as distinguishing shark species from morphologically similar congeners (*e.g.* within *Carcharhinus*) and identifying cryptic species, immature specimens, shark fins or body parts and hybrids.

DNA BARCODING APPROACHES

DNA barcoding is a taxonomic tool that uses a short standardized piece of DNA sequence to identify an organism as belonging to a particular species (Hebert *et al*., 2003). Nucleotide sequence for the selected marker is obtained and compared to a library of reference sequences. The accuracy of DNA barcoding relies wholly on the correct taxonomic identification of the type of specimens used to generate the reference library and on the discriminatory power of the genetic markers used. The DNA marker commonly used for species identification in barcoding fishes is a 648 bp region of the mitochondrial (mt)DNA called cytochrome c oxidase subunit I (*coI*). Barcoding reference data is stored and managed in online global databases (*e.g.* http://www.boldsystems.org). Ward *et al*. (2005) published *coI* sequences from 143 teleost species and 61 species of sharks and rays in Australia, subsequently expanding the number of elasmobranch species to 210 (Ward *et al*., 2008). The application of *coI* barcoding has also been successfully used to identify unknown samples in a range of fisheries including the Australian shark fin (Salini *et al*., 2007; Holmes *et al.,* 2009), Alaskan (Spies *et al*., 2006) and Canadian skate (Coulson *et al.,* 2011) fisheries.

The DNA barcoding technique is not limited to the *coI* gene. Hoelzel (2001) designed universal primers targeting small regions (155 or 188 bp) of the mtDNA *cytochrome b* (*cytb*) and nicotinamide adenine dinucleotide (NADH)-ubiquinone oxidoreductase chain 2 (*nd2*) gene. Direct sequencing of the products differentiated 16 lamniform species. Blanco *et al*. (2008) differentiated 23 species with a longer region of cytb (423 bp) in a technique they named Forensically Informative Nucleotide Sequencing (FINS). One of the first papers to develop molecular markers to specifically improve Batoidea species-specific monitoring was published by Tinti *et al*. (2003). Their focus was differentiating skate species from the north-east Atlantic Ocean and the Mediterranean Sea with mtDNA 16S ribosomal DNA (rDNA). Rodrigues-Filho *et al*. (2009) also used mtDNA 16S in addition to mtDNA

12S to identify 11 species from 122 tissue samples collected at a fish market in Brazil.

To distinguish between elasmobranch species, direct sequence analysis is more accurate but is also more expensive than indirect techniques such as restriction fragment length polymorphism (RFLP). Martin *et al*. (1992) purified mtDNA genomes from 16 species of *Carcharhinus* and then used RFLPs of the entire genomes to distinguish the species. Heist & Gold (1999*a*) proposed a polymerase chain reaction (PCR)-RFLP analysis of an amplified section of a short fragment (396 bp) of mtDNA to assist in the challenge of species identification from processed tissue in the control of trade of endangered species. To date the method has been successfully used for distinguishing between the Brazilian sharpnose shark *Rhizoprionodon lalandii* (Müller & Henle 1839) and the Caribbean sharpnose shark *Rhizoprionodon porosus* (Poey 1861) on the Atlantic coast (Mendonça *et al*., 2009*a*).

More recently, AFLPs have been used to identify species. This technology has the power to detect multiple nucleotide differences that are widely spread over the genome. Zenger *et al*. (2006) tested 12 primer pairs against 15 divergent shark species within the superorder Galeomorphii and found high numbers of polymorphic loci and high levels of allelic diversity. Corrigan *et al*. (2008) conducted large-scale, population-level analysis of both mtDNA and nuclear AFLP markers for the banded wobbegong *Orectolobus halei* Whitley 1940, ornate wobbegong *Orectolobus ornatus* (De Vis 1883) and spotted wobbegong *Orectolobus maculatus* (Bonnaterre 1788). Although the AFLP technique has many potential applications, it has not featured commonly in elasmobranch species diagnostics to date. The slow uptake of the technique may reflect a potential problem with the technology as it is based on DNA fragment size differences from the large and complicated nuclear genome which makes it difficult to infer DNA sequence variation.

Routine genetic monitoring of species composition of catches in fisheries taking morphologically similar species, or product such as shark fin, is made feasible using species-specific assays for targeted species detection. Shark identification tools have been developed using multiplex PCR reactions incorporating multiple species-specific primers targeting the nuclear ribosomal RNA internal transcribed spacer 2 (rRNA ITS2) and mtDNA *cytb*. Assays are currently available for 28 species, and 21 of these species can be identified in a single multiplex reaction (Pank *et al*., 2001; Shivji *et al*., 2002; Chapman *et al*., 2003, 2009*a*; Abercrombie, 2004; Nielsen *et al*., 2004; Abercrombie *et al*., 2005; Clarke *et al*., 2006; Doukakis *et al*., 2011). Multiplex PCR assays for shark identification have recently been developed for the mtDNA *coI* region (Caballero *et al*., 2012).

PRECAUTIONS WHEN USING DNA ALONE FOR SPECIES IDENTIFICATION

The integrity of DNA methodologies depends upon knowledge of the taxon-specific limitations of the DNA-based reference library. For species-specific primer assays, congeneric and non-target species need to be tested as part of development. Assays designed for one fishery (*i.e*. group of shark species) that are applied to a different fishery may give unexpected results due to the presence of species absent from the original reference library. In this instance, secondary testing of positive samples should be made for validation. Cross-amplification of closely related species is likely

to occur in genera with many closely related congeners, such as that found within the genus *Carcharhinus* (Clarke *et al*., 2006). DNA-based techniques are typically most powerful when used alongside the traditional taxonomic methods.

Hybridization and cryptic species may also complicate identification. Hybrid offspring have been reported among river stingrays in the genus *Potamotrygon* (Toffoli *et al*., 2008; American Elasmobranch Society 2008 International Captive Elasmobranch Census http://elasmo.org/census.php), although this has not yet been validated using DNA markers. The first naturally occurring shark hybrid was identified due to a mismatch between morphology and species-diagnostic mtDNA sequences in the genus *Carcharhinus* (Morgan *et al*., 2012). Evidence of elasmobranch hybridization highlights the need for a holistic approach to species identification using both morphology and genetics, as hybrid animals cannot be identified from maternally inherited mtDNA alone. More information about hybrids can be obtained if mtDNA markers are used in conjunction with nuclear DNA markers. A further confounding factor is that mtDNA genes are sometimes found in the nuclear genome (Lopez *et al*., 1994), which can interfere with the use of mtDNA for species identification. To date, there are no reports of this phenomenon for elasmobranchs. Given the ubiquity of nuclear DNA in a range of taxa (Hazkani-Covo *et al*., 2010), however, researchers should be aware of their potential existence.

Increasing numbers of genetically cryptic elasmobranch species and lineages are being identified; *e.g.* shovelnose guitarfish *Rhinobatos productus* Ayres 1854 (Sandoval-Castillo *et al*., 2004), scalloped hammerhead *Sphyrna lewini* (Griffith & Smith 1834) (Abercrombie *et al*., 2005; Duncan *et al*., 2006; Quattro *et al*., 2006), Pacific cownose ray *Rhinoptera steindachneri* Evermann & Jenkins 1891 (Sandoval-Castillo & Rocha-Olivares, 2011) and the eagle ray *Aetobatus narinari* (Euphrasen 1790) (Richards *et al*., 2009; Schluessel *et al*., 2010). Cryptic complexes appear to be particularly pronounced in small demersal or benthopelagic species such as members of the families Triakidae, Hemigaleidae and Scyliorihinidae (Gardner & Ward, 1998, 2002; Yamaguchi *et al*., 2000; López *et al*., 2006; White & Last, 2006). Furthermore, increasing numbers of cryptic species are being identified from the Indo-Pacific Ocean region. One of the largest genera of sharks, *Mustelus,* is also one of the most systematically troublesome groups (Compagno, 1988). The gummy shark *Mustelus* spp. in Australia have been studied intensively across multiple fields of research (genetics, morphology, demographic stock assessment and tagging), and there are currently four species described: *Mustelus antarcticus* Günther 1870 (southern Australia), *Mustelus ravidus* (White & Last, 2006) (western Australia), *Mustelus stevensi* (White & Last, 2008) (western Australia) and *Mustelus walkeri* (White & Last, 2008) (north-eastern Australia) (White & Last, 2006, 2008). The spadenose shark genus *Scoliodon* was previously considered to be monotypic but mtDNA *nd2* and morphological analyses showed three species should be recognized (White *et al*., 2010*a*). Recent Southern Hemisphere research revealed cryptic diversity in lanternsharks *Etmopterus granulosus* (Günther 1880) and *Etmopterus baxteri* Garrick 1957 (Straube *et al*., 2011) and morphological and molecular investigations resulted in the resurrection of the maskray genus *Neotrygon* for a group of Indo-Pacific dasyatids which display considerable mtDNA genetic diversity (Last & White, 2008; Ward *et al*., 2008; Last *et al*., 2010).

DNA-based identification of shark catch can potentially improve misidentifications arising under fishery field conditions, and further overcome the limitations of broadly

categorized logbook entries which obscure the biological differences between species (Tillett *et al*., In press). For example, a single set of management arrangements is used for an assemblage of sharks in the genus *Carcharhinus* in the inshore fishery in Queensland, Australia, due in part to a lack of accurate species identification tools (DEEDI, 2009). The 1998 meeting of the Food and Agriculture Organization of the United Nations (FAO) technical working group on the conservation and management of sharks highlighted the need to promote greater accuracy of species identification to assure statistically sound sampling procedures *via* the education of fishermen, scientists and managers (Anon, 1998). In Italy, fraudulent mis-identification of shark seafood products was revealed using DNA barcoding (Barbuto *et al*., 2010). A recent review by Rasmussen *et al*. (2011) describes advances in DNA-based techniques for the detection of seafood species substitution on the commercial market. Application of existing techniques of shark harvest and trade monitoring, and further development of streamlined and cost-effective DNA-based application-specific methods will result in improved baseline information on which conservation agencies and fisheries managers may base future decisions.

PHYLOGEOGRAPHY AND POPULATION STRUCTURE IN ELASMOBRANCHS

Elasmobranch population studies aim to infer historical and contemporary processes responsible for observed patterns of spatial genetic differentiation, and to identify units for fisheries management and conservation (Beheregaray, 2008; Ovenden *et al*., 2011). There have been important technical and analytical advances in these fields in the last three decades such as coalescent theory, which merges phylogenetics and population genetics (Knowles, 2004; Nielsen & Beaumont, 2009). This new method tests the two explanations for the genetic similarity between populations; similarity due to the sharing of a common recent ancestor, or similarity due to extant gene flow (Nielsen *et al*., 2004, 2009*a*; Nielsen & Beaumont, 2009; Garrick *et al*., 2010; Hey, 2010). Earlier applications and a number of pioneering works on elasmobranch phylogeography have been reviewed by others (Heist, 1999, 2004*a*, *b*, 2005; Portnoy, 2010). The emerging syntheses and empirical findings by taxon for five shark orders and the batoids are described below.

STATE OF PHYLOGEOGRAPHIC STUDIES ON ELASMOBRANCHS

Compared with mammals and bony fishes there are few phylogeographic studies on elasmobranchs (Beheregaray, 2008). There is representation, however, from all major categories of species' habits (benthic, benthopelagic and pelagic) and habitats (coastal, oceanic and bathyal) (Musick *et al*., 2004). The majority of the research reviewed here represents the first phylogeographical examination of the species, however, some species have been re-examined by multiple research groups employing different marker systems, sampling intensities or geographical focus, which lends insight into the effect of experimental design. Detailed regional-scale genetic population structure has been studied for some species with important implications for fisheries management.

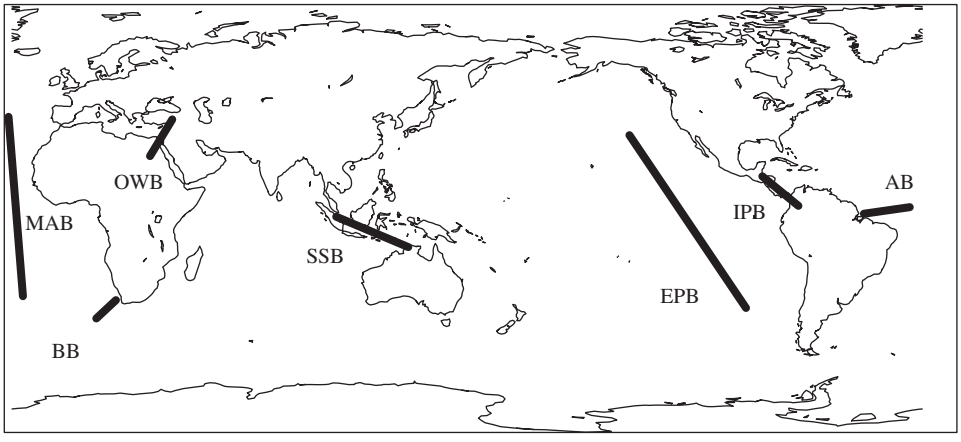

FIG. 1. Traditionally recognized marine biogeographic barriers: MAB, Mid-Atlantic Barrier; BB, Benguela Barrier; OWB, Old World Barrier; SSB, Sunda Shelf Barrier; EPB, Eastern Pacific Barrier; IPB, Isthmus of Panama Barrier; AB, Amazon Barrier. Modified from Rocha *et al*. (2007).

WORLDWIDE PHYLOGEOGRAPHIC BARRIERS AND THEIR INFLUENCE ON ELASMOBRANCHS

There are a number of traditionally recognized biogeographic barriers, including the Old World Barrier (closure of the Tethys Sea), Isthmus of Panama Barrier, Eastern Pacific Barrier, Mid-Atlantic Barrier, Benguela Barrier, Sunda Shelf Barrier and Amazon Barrier (Briggs, 1974, 1995) that are thought to affect, or have previously affected, population connectivity and therefore influence phylogeographic patterns (Fig. 1). The degree to which phylogeographic patterns are concordant across these barriers varies among species, reflecting the species' vagility, habits, preferred environmental conditions and susceptibility to natural and anthropogenic influence on population connectivity.

The closures of the Tethys Sea *c*. 20 million years before present (B.P.) (Old World Barrier, Ricou, 1987) and Panama seaway *c*. 3 MB.P. (=Isthmus of Panama, Cronin & Dowsett, 1996) were important in moulding the present-day distribution of many species and genera, and genealogic lineages within. Even pelagic species with worldwide distributions commonly exhibit genetic differentiation at least across the three major ocean basins based on allele frequency differences in mtDNA (Heist *et al*., 1996*a*); potential exceptions are given by Hoelzel *et al*. (2006) and Castro *et al*. (2007). For large, coastal species, mtDNA typically exhibits genetic partitioning at a genealogical level across ocean basins (Duncan *et al*., 2006; Keeney & Heist, 2006) with the occasional example of genetic leakage across these barriers (Duncan *et al*., 2006; Portnoy *et al*., 2010; Gubili *et al*., 2011).

The Eastern Pacific Barrier is a vast expanse of ocean of *c*. 7000 km. If dispersal occurs across the barrier it is thought to be facilitated by 'stepping stones' of islands (Duncan *et al*., 2006). The barrier has had a spectrum of effects, ranging between complete separation in some coastal species (Schultz *et al*., 2008), the isolation of a monophyletic eastern Pacific Ocean population in others (Jorgensen *et al*., 2009), to no noticeable effect in some oceanic and pelagic species (Duncan *et al*., 2006; Keeney & Heist, 2006).

The Mid-Atlantic Barrier is much shorter in distance than the Eastern Pacific Barrier. Some species share haplotypes across this barrier (Ahonen *et al*., 2009; Portnoy *et al*., 2010; Benavides *et al*., 2011*a*) whereas others do not (Keeney & Heist, 2006; Schultz *et al*., 2008; Benavides *et al*., 2011*b*), indicating contrasting levels of recent connection.

The Benguela Barrier restricts mixing of Atlantic and Indian Ocean populations of tropical species due to a cold current running around the southern tip of Africa (Briggs, 1995). This is evident in globally distributed species where the genealogy is trifurcated among samples from the tropical west Atlantic Ocean, South Africa and Indo-Australian Archipelago (Duncan *et al*., 2006). Some signatures of historical gene flow across the southern tip of Africa were linked with intensified Agulhas leakage during warm interglacial periods (Peeters *et al*., 2004). Conversely, this current does not form a genetic barrier to temperate species, such as the copper shark *Carcharhinus brachyurus* (Günther 1870) (Benavides *et al*., 2011*a*), and tope *Galeorhinus galeus* (L. 1758) (Chabot & Allen, 2009).

The signal from the Sunda Shelf Barrier in the Indo-Pacific Ocean appears complex for elasmobranchs because connectivity between tropical Indian and Pacific Oceans changes with each glacial cycle (Voris, 2000). For relatively less vagile species, the deep waters of the Timor Trench (2000–3000 m) and the strong Indonesian throughflow current along the Makassar and Lombok Straits may facilitate the main genetic subdivisions between Indonesia and Australia rather than historical vicariance by partial land bridge formations (Dudgeon *et al*., 2009; Ovenden *et al*., 2009). Some previously grouped species across this divide have also been taxonomically revised and delimited (White *et al*., 2005).

Studies conducted across the Amazon Barrier using microsatellite and mtDNA markers so far are typified with weak or minimal structure in microsatellite data and strong to moderate subdivision in mtDNA (Schultz *et al*., 2008; Karl *et al*., 2011*a*). These patterns were interpreted as a signature of female philopatry and are supported by tagging studies. Alternatively, the pattern may be explained by mutation-drift equilibrium in one marker (mtDNA) but not the other (microsatellites) marker, across a relatively recent vicariant barrier.

CASE STUDIES FROM MAJOR TAXONOMIC GROUPS

Squaliformes

The Squaliformes are a large group of small to medium-sized, cold-water and mostly bathyal and benthopelagic sharks (Musick *et al.,* 2004). There is generally a high level of unrecognized diversity and lack of knowledge about population structure among sharks within the order Squaliformes (Straube *et al.,* 2011). Recently, phylogeographic studies have started to provide new information to help resolve taxonomic uncertainty and dispersal of these species. Molecular studies found strong genetic divergence within the spiny dogfish *Squalus acanthias* L. 1758 at three different mtDNA gene regions [control region (CR), *coI* and *nd2*] and at eight microsatellite loci (Ward *et al*., 2007; Hauser, 2009; Veríssimo *et al*., 2010) forming two reciprocally monophyletic lineages in the northern and southern Pacific Oceans. Independent management of these two lineages has been recommended, along with a taxonomic revision (Franks, 2006; Hauser, 2009; Ebert *et al*., 2010; Veríssimo *et al*., 2010).

Straube *et al*. (2011) investigated population genetic structure in lantern sharks using AFLP loci and mtDNA *coI* data. This study revealed that southern *E. granulosus* was not endemic to Chile, but was widely distributed in the southern hemisphere and was synonymous with New Zealand *E. baxteri*. Low levels of population differentiation suggested migration of *E. granulosus* between these sites that are separated by *c*.7000 km (Chile and New Zealand). Conversely, specimens previously assigned to *E. baxteri* from South Africa appeared to represent a distinct species, indicating the need for further study.

The Portuguese dogfish *Centroscymnus coelolepis* Barbosa du Bocage & de Brito Capello 1864 showed no evidence of genetic population structure (mtDNA CR and eight microsatellite loci) among collection sites in the eastern Atlantic Ocean. High dispersal potential of this species as well as no major barriers to dispersal within the eastern Atlantic Ocean were thought to explain the lack of differentiation (Veríssimo *et al*., 2011*a*).

Three species of sleeper sharks were studied with mtDNA *cytb* revealing two divergent clades: one for the Greenland shark *Somniosus microcephalus* (Bloch & Schneider 1801) (Cumberland Sound and Iceland) and the other consisting of both the Pacific sleeper shark *Somniosus pacificus* Bigelow & Schroeder 1944 (Taiwan and Alaska) and Southern sleeper shark *Somniosus antarcticus* Whitley 1939 (Tasmania) (Murray *et al*., 2008). These data did not support the separation of *S. pacificus* and *S. antarcticus* and highlighted the taxonomic uncertainty within *Somniosus* (Benz *et al*., 2007). Two hypotheses were generated which need further investigation: (1) *S. pacificus* and *S. antarcticus* are a single species, or (2) the two species are of recent origin and have not yet diverged in *cytb* (Murray *et al*., 2008).

Squatiniformes

Squatiniformes are demersal sharks and appear to demonstrate limited vagility (Musick *et al.,* 2004). The smallest scale over which population genetic structure in any elasmobranch has been demonstrated was found in the benthic California angel shark *Squatina californica* Ayres 1859 (Gaida, 1997). Using allozyme markers, significant differentiation consistent with the low vagility of this species was found between California's Channel Islands, separated by a distance of $<$100 km.

Orectolobiformes

The Orectolobiformes is a diverse order characterized primarily by demersal species although it also includes the pelagic, planktivorous whale shark *Rhincodon typus* Smith 1828. *Rhincodon typus* showed no significant mtDNA CR population structuring within the Gulf of California (Ramirez-Macias *et al*., 2007), but differences were found between the Atlantic and Indo-Pacific Ocean regions (Castro *et al*., 2007). Analyses based on eight microsatellite loci revealed only low levels of worldwide genetic differentiation (Schmidt *et al*., 2009) primarily due to differences between the Atlantic (Caribbean Sea) and Indian Oceans, which may be driven by the cold Benguela Barrier around the southern tip of Africa.

The demersal zebra shark *Stegostoma fasciatum* (Hermann 1783) showed genetic subdivision for both mtDNA *nd4* and 13 microsatellite loci between northern Australia and Southeast Asia (Dudgeon *et al*., 2009). The primary regional break was concordant with the Indonesian through-flow current along the Makassar and Lombok

Straits. Mitochondrial DNA, but not microsatellite data, supported further genetic differentiation between North and South Queensland locations (Dudgeon *et al*., 2009).

The demersal nurse shark *Ginglymostoma cirratum* (Bonnaterre 1788) showed surprisingly low haplotypic and nuclear diversity with weak but significant genetic structure in a mtDNA fragment of 1166 bp (*cytb* to CR) along the West Atlantic Ocean coastline (Castro, 2009). Subdivision in microsatellite data (eight loci) was virtually absent. Stronger genetic divisions were found with both CR and microsatellites between Brazilian coastal and offshore locations indicating that deep water is an important dispersal barrier for this species (Karl *et al*., 2011*b*).

Lamniformes

The laminiforms range from highly pelagic to demersal species, with a range of complex movement behaviour and habitat use including cases of routine migratory movements. As such, lamniform species would be expected to display varying levels of population structure and here are examples from three families. The pelagic basking shark *Cetorhinus maximus* (Gunnerus 1765) showed no genetic structuring among ocean basins, with only six global mtDNA haplotypes and low genetic diversity, suggesting either a bottleneck event preceding expansion into the current global distribution, or wide-ranging female-mediated gene flow (Hoelzel *et al*., 2006).

In the pelagic shortfin mako shark *Isurus oxyrinchus* Rafinesque 1810, four microsatellite markers indicated weak evidence of genetic partitioning among ocean basins (Schrey & Heist, 2003). The authors suggest the possibility of sex-biased dispersal in *I. oxyrhinchus* considering earlier mtDNA findings, which suggested population structure between the North Atlantic *v*.South Atlantic and Pacific Ocean samples (Heist *et al*., 1996*a*).

In the great white shark *Carcharodon carcharias* (L. 1758) strong ocean-basin structuring has been detected; for example deep divergence between Indo-Pacific and South Africa–Atlantic mtDNA CR haplotypes (Pardini *et al*., 2001; Gubili *et al*., 2011). Pardini *et al*. (2001) found no evidence for genetic subdivision from five microsatellite loci and proposed natal philopatric behaviour in females to explain the discrepancy between the two marker systems. Recently, significant population structure was detected between juveniles collected from the eastern and southern coasts of Australia from both mtDNA CR and microsatellite loci, lending weight to the possibility that reproductive behaviour restrains gene flow in both sexes in this species (Blower *et al*., 2012).

For the grey nurse shark *Carcharias taurus* Rafinesque 1810 mtDNA CR, six microsatellites and AFLP markers revealed extensive global population differentiation across six widespread geographic regions, with low within-population genetic diversity. All pair-wise comparisons were significant except for Brazil and South Africa, and there was evidence of extensive isolation of populations from the northwestern Atlantic Ocean (Ahonen *et al*., 2009). This deep phylogeographic structuring indicated by multiple marker types may be typical of demersal species that show limited movement or fixed migratory movements.

Carcharhiniformes

Carcharhiniformes, of the family Carcharhinidae in particular, are the most represented in the phylogeographic literature, as many of these species are of economic

importance in commercial and artisanal fisheries. A number of phylogeographic studies on coastally distributed carcharhinids have demonstrated that large oceanic expanses appear to constitute a strong barrier to gene flow (Duncan *et al*., 2006; Keeney & Heist, 2006; Schultz *et al*., 2008; Portnoy *et al*., 2010) and have drawn attention to evidence of female philopatry in large-bodied, coastally oriented species.

Prionace glauca is the most wide-ranging of sharks and mark–recapture studies show cross-ocean basin and cross-hemisphere migrations (Last & Stevens, 2009). Perhaps not surprisingly, no support for genetic partitioning was found between specimens from the east and west Australian coasts, Indonesia and mid-North Pacific Ocean for either the mtDNA CR or five microsatellite loci (Ovenden *et al*., 2009). As yet, there have been no published studies at a global scale.

A global study of *S. lewini* based on mtDNA CR haplotypes showed three lineages: basal haplotypes from southern Africa and the Indo-West Pacific Ocean and two divergent lineages with strong geographical orientation, one occurring from the eastern Pacific to the Indian Ocean and the other in the Atlantic Ocean and Indo-West Pacific Ocean (Duncan *et al*., 2006). There was also evidence of a cryptic lineage (Duncan *et al*., 2006; Quattro *et al*., 2006). A later study employing the same marker increased the sample representation in the western Atlantic Ocean and found a higher degree of genetic partitioning (Chapman *et al*., 2009*a*). No differences were found between populations across the Indo-Australian archipelago using mtDNA CR sequences and three microsatellite loci (Ovenden *et al*., 2009). This regional genetic homogeneity was confirmed along the Australian east coast with mtDNA *nd4* and eight microsatellite loci (Ovenden *et al*., 2011).

Global phylogeographic structure was investigated in the sandbar shark *Carcharhinus plumbeus* (Nardo 1827) employing mtDNA CR and eight microsatellite loci (Portnoy *et al*., 2010). This study revealed complex spatial and temporal relationships between populations that may suggest regional female philopatry, relatively continuous male-mediated gene flow and pulses of female dispersal. No significant differentiation was detected, however, between sites within the Atlantic Ocean, supporting previous assertions of a single genetic stock (Heist *et al*., 1995; Heist & Gold, 1999*b*; Portnoy *et al*., 2010).

The coastal blacktip shark *Carcharhinus limbatus* (Müller & Henle 1839) has been studied by multiple research groups. A global phylogeographic study using mtDNA CR sequences indicated two matrilineal clades with low levels of haplotype sharing amongst localities (Keeney & Heist, 2006). Studies at a smaller spatial scale in the western Atlantic Ocean revealed significant mtDNA haplotype partitioning between nursery sites in the Gulf of Mexico compared with the Atlantic coast of Florida (Keeney *et al*., 2003), and among nine continental nurseries from the south-west U.S.A. coast to the Yucatan Peninsula (Keeney *et al*., 2005). Female natal philopatry was inferred by a contrasting lack of genetic structure in eight microsatellite loci, suggesting higher levels of male-mediated gene flow. *Carcharhinus limbatus* was also studied in the Indo-Australian Archipelago using three mtDNA gene regions and five microsatellite loci, together with its closely related Australian endemic congener the Australian blacktip shark *Carcharhinus tilstoni* (Whitley 1950) (Ovenden *et al*., 2010). Minimal structuring was detected for either species, both among sites that feature dense representation of sampling sites across tropical northern Australia, and also two sites in Indonesia for *C. limbatus*. Evidence of hybridization between

C. limbatus and *C. tilstoni* complicates interpretation of population genetic structure where these species overlap in range (Morgan *et al.*, 2012).

In the spot-tail shark *Carcharhinus sorrah* (Müller & Henle 1839) no significant population subdivision was found across populations within northern Australian waters (Lavery & Shaklee, 1989; Ovenden *et al.*, 2009). Significant division using both five microsatellite loci and mtDNA CR was detected, however, between northern Australia and Indonesia, congruent with the deep waters of the Timor Trench forming a barrier to dispersal for this coastally distributed species (Ovenden *et al.*, 2009).

Comparable genetic subdivision in mtDNA *nd4* markers between Indonesia and Australia, with no significant structure among Australian localities was also found in the milk shark *Rhizoprionodon acutus* (Rüppell 1837) (Ovenden *et al.*, 2011). Similarly, no significant mtDNA RFLP stock structure was detected in its congener, the Atlantic sharpnose shark *Rhizoprionodon terraenovae* (Richardson 1836) in a study incorporating much of its western Atlantic Ocean range along the U.S.A. east coast and the Gulf of Mexico (Heist *et al.*, 1996*b*). Similarly, *R. lalandii* off the south-eastern coast of Brazil showed no population structuring with mtDNA CR data (Mendonça *et al.*, 2011). In contrast, *R. porosus* showed significant genetic structure in mtDNA CR with two distinct management units separated by the Equatorial Current between populations from the Caribbean Sea and several locations along the entire Brazilian coast (Mendonça *et al.,* 2009*b*).

A study on the dusky shark *Carcharhinus obscurus* (LeSueur 1818) revealed significant mtDNA CR differentiation between Australian (east and west coast), Atlantic Ocean (east coast U.S.A. and Gulf of Mexico) and South African regions (Benavides *et al.*, 2011*b*). Significant pair-wise differences between Indonesia and Western Australia were also found, suggesting limited or no dispersal across the Timor Trench. This finding was, however, based on small sample sizes and therefore requires further investigation (Ovenden *et al.*, 2009).

Using fine-scale sampling on the U.S. south-eastern Atlantic Ocean and Gulf of Mexico coasts and north Brazil, a study of bull shark *Carcharhinus leucas* (Müller & Henle 1839) detected significant genetic structure among populations in Brazil compared with the U.S.A. for mtDNA CR markers. This pattern was not observed using five microsatellite loci, and female philopatry was proposed to explain these contrasting patterns (Karl *et al.*, 2011*a*). Female philopatry was also proposed as a driver for population genetic structure in *C. leucas* across northern Australia (Tillett *et al.*, 2012).

In a study of a morphologically sympatric congener to *C. leucas*, the pigeye shark *Carcharhinus amboinensis* (Müller & Henle 1839), sympatric in the Indo-West Pacific and eastern North Atlantic Oceans, this species displayed very different population genetic structure and phylogeny. Populations on the east coast of Australia were genetically distinct from those occupying coastal waters in north-western Australia. Phylogenetic analysis identified three distinct clades suggesting Pleistocene isolation, followed by secondary introgression and restricted contemporary gene flow across northern Australia. Samples from the Arabian Sea and South Africa were found to be genetically similar to one of the Australian clades (Tillett *et al.*, 2012). This appears to be an example of phylogenetic discontinuity in the absence of current spatial separation (Category II or IV, Avise *et al.*, 1987).

A study of the genus *Negaprion* explored population differentiation in the allopatric sister species in the Indo-West Pacific and Atlantic Oceans using both mtDNA CR and microsatellite markers (Schultz *et al*., 2008). Despite the large distances, only weak population structure was detected among populations across the range of lemon shark *Negaprion acutidens* (Rüppell 1837) between Australia and French Polynesia, possibly due to the use of islands as stepping stones that are no greater than 800 km apart. Conversely in the sister species *Negaprion brevirostris* (Poey 1868), there was a significant structure among the populations on either side of the Atlantic Ocean some 2500 km apart. Coastal orientation, tendency to move much shorter distances than many large coastal sharks and female philopatry were proposed as mechanisms driving significant population structure in this species across large oceanic expanses. The eastern Pacific Ocean population was found to be deeply divergent to the Atlantic Ocean population consistent with separation since the rise of the Isthmus of Panama (Schultz *et al*., 2008).

Temperate species are expected to be restricted latitudinally by warmer water. A study employing mtDNA CR in *C. brachyurus* in the southern portion of the species' range (South Africa, Australia, New Zealand and Peru) described two divergent mtDNA lineages, with a major genetic discontinuity across the Indian Ocean and significant structure among three coastal regions separated by wide oceanic expanses (Benavides *et al*., 2011*a*). In a global study of *G. galeus*, a similar genetic subdivision was found across the Indian Ocean suggesting that the warm waters of the Indian Ocean restrict dispersal of temperately distributed species (Chabot & Allen, 2009). Anti-tropical subdivision was also found in *G. galeus*, illustrating the importance of the warm equatorial current as another thermal barrier to dispersal within oceans.

The star-spotted smoothhound *Mustelus manazo* Bleeker 1855 was studied with allozymes and showed significant genetic variation between central Japan and northern Taiwan (Chen *et al*., 2001). Large differences in size and age at maturity were also reported (Yamaguchi *et al*., 2000). These two populations may be assigned to two distinct species with detailed morphometric and meristic data, as already revealed for some species in this systematically troublesome genus (White & Last, 2006). The endangered narrownose smoothhound *Mustelus schmitti* Springer 1939, endemic to the south-western Atlantic Ocean, was studied with mtDNA *cytb* (Pereyra *et al*., 2010). Genetic diversity was low and there was no evidence for genetic structure.

Ten populations of the demersal leopard shark *Triakis semifasciata* Girard 1855 on the Californian coast were studied with mtDNA CR and inter-simple sequence repeats (ISSR) (Lewallen *et al*., 2007). Analysis for both marker types including landscape genetic visualization and assignment tests suggested complex genetic structuring and the presence of seven gene pools between Humboldt (northern) and San Diego (southern CA, U.S.A.).

Batoids

With a few exceptions (notably the mobulids), batoids tend to have demersal lifestyles which probably influences observed population genetic structuring at regional scales for many species. Molecular studies have already revealed the need for taxonomic revision for many species (*e.g. A. narinari*; Richards *et al*., 2009; Schluessel *et al*., 2010) and it is likely that future investigations into population subdivision will result in more species being described.

Significant genetic structure in mtDNA CR was detected in three species of sawfishes, *Pristis microdon* Latham 1794, *Pristis clavata* Garman 1906 and *Pristis zijsron* Bleeker 1852 across northern Australia (Phillips *et al*., 2011). *Pristis microdon* showed higher levels of genetic structuring than *P. clavata* and *P. zijsron,* possibly due to localized female philopatry and the species' requirement for fresh water.

The phylogeography of thornback rays *Raja clavata* L. 1758 in European waters was assessed using five microsatellite loci and mtDNA cytb sequences (Chevolot *et al*., 2006). Strong regional differentiation was found between the Mediterranean basin, the Azores and the European continental shelf. This contrasts to the weak population structure found in the thorny skate *Amblyraja radiata* (Donovan 1808) that was sampled with mtDNA *cytb* throughout the North Atlantic Ocean (Chevolot *et al*., 2007). Although both species have similar life-history traits and distributions, the bathymetry of the continental shelf edge appears to limit dispersal for *R. clavata* only. The study by Pasolini *et al*. (2011) revealed that the biscuit skate *Raja straeleni* Poll 1951 in South Africa and European *R. clavata* are recently diverged geographical sibling species, based on the clustering of mtDNA CR haplotypes and significant AFLP F values between the two taxa.

Nine skate species of *Bathyraja* in the Southern Ocean were studied with mtDNA (Smith *et al*., 2008). *Bathyraja* sp. (*cf. eatonii*) from the Antarctic continental shelf and slope is a species distinct from *Bathyraja eatonii* (Günther 1876) from the Kerguelen Plateau (the type locality). Significantly diverged intraspecific clades indicative of geographic differentiation were observed for both *B*. sp. (*cf. eatonii*) and *Bathyraja maccaini* Springer 1971 between the Ross Sea and the South Atlantic Ocean.

PCR-RFLP based mtDNA CR haplotypes identified for *R. productus* (Sandoval-Castillo *et al*., 2004) and PCR-RFLP of mtDNA *nd2* haplotypes in *R. steindachneri* (Sandoval-Castillo & Rocha-Olivares, 2011) showed significant divergence between the Gulf of California and Pacific coast of Baja California, Mexico. Population structuring was also detected between the Gulf of California and the Pacific coast for the Californian butterfly ray *Gymnura marmorata* (Cooper 1864) using mtDNA *cytb* (Smith *et al*., 2009). This species co-occurs in the east Pacific Ocean with the morphologically similar longsnout butterfly ray *Gymnura crebripunctata* (Peters 1869) and large genetic divergence confirmed the valid taxonomic status of these species suggesting distant origins rather than *in situ* evolution (Sandoval-Castillo *et al*., 2004). In contrast, the study of the round stingray *Urobatis halleri* (Cooper 1863) using seven microsatellite loci (Plank *et al*., 2010) did not support an isolation of populations between the Gulf of California and the Pacific coast of Baja California. The population of *U. halleri* sampled at Santa Catalina Island, California, which is separated by a deep-water channel from the coastal sites, however, was significantly divergent from the other populations.

Molecular studies have provided evidence of a species complex for *A. narinari* using mtDNA *cytb* and *nd4* markers (Richards *et al*., 2009; Schluessel *et al*., 2010), and an Indo-Pacific spotted eagle ray species *Aetobatus ocellatus* (Kuhl 1823) was subsequently redescribed (White *et al*., 2010*b*). Within the *A. narinari* complex, central Atlantic, eastern Pacific and western Pacific Ocean populations formed three distinct mtDNA clades (Richards *et al*., 2009) and Schluessel *et al*. (2010) also reported significant population structuring in the Indo-Pacific Ocean. These studies indicate an Indo-West Pacific Ocean origin for the *A. narinari* complex, with

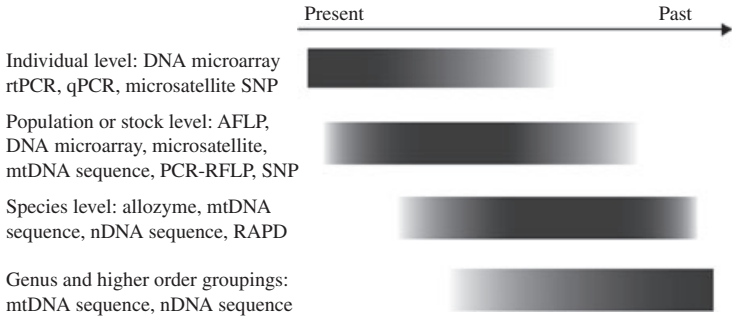

FIG. 2. Molecular techniques and markers and their general application along 'time slices' of population history (see Appendix) (■ = most informative). Modified from Garrick *et al.* (2010). rtPCR, real-time PCR; qPCR, quantitative PCR; SNP, single nucleotide polymorphisms; AFLP, amplified fragment length polymorphism; RFLP, restriction fragment length polymorphism; nDNA, nuclear gene sequence; RAPD, random amplification of polymorphic.

subsequent westerly dispersal around the southern tip of Africa into the Atlantic Ocean followed by possible dispersal into the eastern Pacific Ocean through the Isthmus of Panama prior to closure.

Griffiths *et al.* (2011) analysed longnose skates *Dipturus oxyrinchus* (L. 1758) from the north-eastern Atlantic Ocean and the Mediterranean Sea with mtDNA CR. Bayesian analysis suggested that little gene flow occurred between the regions and that the populations separated 20 kb.p. Analyses with mtDNA CR and microsatellite markers revealed cryptic species in common skate *Dipturus batis* (L. 1758) in northern European waters (Griffiths *et al.*, 2010). Local extinctions and taxonomic confusion among skates are a major issue (Dulvy *et al.*, 2000; Iglésias *et al.*, 2009).

Thus, phylogeographic studies provide the essential baseline information about stock structure that is desirable for appropriate fisheries management and have often provided motivation for taxonomic revision (*e.g.* cryptic species discovery and proposals for synonymy). Increasing numbers of studies are incorporating multiple genetic markers and are using model-driven approaches to infer spatio-temporal patterns. The advantage of studying species with multiple classes of molecular markers is that information on population history can be obtained along a continuum of 'time slices' based on the genetic diversity of each marker and the molecular technique used (Fig. 2), which can provide essential validation of the patterns uncovered.

REPRODUCTIVE PHILOPATRY IN SHARKS, SKATES AND RAYS

Reproductive philopatry challenges traditional concepts of genetic population structure as genetic differences can occur between regions in the absence of physical barriers to movement. The exact definition of this behaviour is a topic of much debate, but here it is referred to as the long-term use of nursery areas by mature females for parturition over multiple breeding cycles. Philopatry has only recently been described in sharks further emphasising the complex patterns of habitat use in elasmobranchs (Feldheim *et al.*, 2001; Pardini *et al.*, 2001; Hueter *et al.*, 2005).

Selective pressures associated with reproductive strategy and body size are factors that influence reproductive philopatry. In many sharks, gestation periods for

females are commonly ≥1 year consuming large energy reserves to produce a few well-developed offspring. As such, the energetic costs of reproduction are large for females compared with male conspecifics. The occurrence of philopatric behaviour is also shaped by selective pressures exerted on species that appear to be associated with reproductive strategy, body size and the overall importance of juvenile survival for species resilience (Portnoy *et al*., 2006). Under this scenario, females are more likely to re-utilize successful nurseries (Wourms & Demski, 1993; Last & Stevens, 2009). The use of nursery areas protects pups from larger conspecifics or other predatory species (Feldheim *et al*., 2004; Knip *et al*., 2010; Bruce & Bradford, In press; Tillet *et al*., 2012). This behaviour has been identified in *C. leucas*, *N. brevirostris*, *C. plumbeus* and *C. tilstoni,* sharks which are coastal, viviparous species (Klimley, 1987), but is also likely to occur in other species such as *C. carcharias* (Blower *et al*., 2012).

Reproductive mode alone does not predict the occurrence of reproductive philopatry in elasmobranchs. Despite being viviparous, this behaviour is not known to occur in *R. taylori*, which also inhabits protected inshore waters (Simpfendorfer & Milward, 1993; Heupel *et al*., 2006). Unlike larger sharks, *R. taylori* inhabit these areas throughout their entire life cycle suggesting reproductive philopatry is more likely in larger species that have size segregation and expend additional energy increasing juvenile survival by using nursery areas. Under this hypothesis, egg-laying species are not predicted to display this type of philopatry. It is also unknown whether batoid species display reproductive philopatry. As for sharks, however, larger viviparous species that utilize nurseries during juvenile life-stages may be philopatric. Further research is required to confirm this.

The designation of a species as philopatric requires an extensive research effort, for example, the prolonged monitoring over multiple years of a large number of individuals. To address this, research must be able to discriminate individuals and trace movement patterns over long time periods, which is often difficult using conventional tagging methods. Genetic tools (genotyping and tracing patterns of genetic variation) are advantageous for identifying philopatry to identify individuals and because they trace movement in generational time providing a wealth of long-term data (Chapman *et al*., 2009*b*).

ASSESSING GENETIC POPULATION STRUCTURE TO INFER PHILOPATRY

One of the first studies to use genetic population structure to discern philopatry was based on significant mtDNA (maternally inherited) and lack of microsatellite (bi-parentally inherited) differentiation in *C. carcharias* between South African and Australian sampling localities (Pardini *et al*., 2001). In this study, philopatry was defined as non-roving (rather than an affinity to nurseries) and the study concluded that limited movement of females had generated population genetic structure between South Africa and Australia. Ideally, genetic marker data would be accompanied by biological data to test for philopatry or sex-specific differences in dispersal potential. If not, genetic data would need stringent interpretation.

mtDNA is solely maternally inherited; it does not undergo recombination as bi-parentally inherited nuclear DNA does (Avise *et al*., 1987). This difference in inheritance will cause genetic fixation, due to reproductive isolation, to occur faster

and remain in mtDNA longer than in nuclear markers (Birky *et al*., 1983). Higher levels of genetic diversity are generally found in microsatellites and can result in lower F_{ST} (measure of population differentiation) values relative to mtDNA markers (Hedrick, 1999; Frankham *et al*., 2002). Furthermore, different microsatellite loci have varying levels of polymorphism within and between species and a sufficient number of loci must be examined to show existing population structure. As such, the mere presence of genetic structure that is present in mtDNA and absent from microsatellite data is not sufficient to conclude female philopatry. Rather, statistical analyses quantifying the power of selected loci to detect population structure if present (Dudgeon *et al*., 2009) or the use of multiple coalescence analyses for estimating gene-flow to explain the cause of observed population structure differences is required (Portnoy *et al*., 2010)

Discerning philopatry using variation in population genetic structure is heavily influenced by sampling strategy. For example, if females remain in an area for a given time and then disperse, the identification of philopatry will depend on whether the philopatric or dispersed phases are sampled. Similarly, philopatric females are not necessarily weaker dispersers. Females may be returning to specific nurseries to pup, but might travel large distances to find a suitable mate or to feed. Distinguishing this behaviour genetically is complex and provides an example of where a holistic approach (including other tagging methods, such as acoustic or satellite tagging) would complement genetic analyses. For example, the philopatric signature identified in female *C. carcharias* (Pardini *et al*., 2001) conflicted with large-scale acoustic and satellite tracking studies that showed female *C. carcharias* were moving between locations (Bonfil *et al*., 2005). Rather than viewing these results as contradictory, the combined results indicate either limited gene-flow between study regions with the number of migrants insufficient to mix population genetic structure, or that individuals are returning to key areas and the identified genetic structure is due to sampling while individuals were breeding rather than dispersing.

Blower *et al*. (2012) compared population genetic structure in mtDNA and microsatellites mostly in juvenile *C. carcharias*, in Australian populations (east and south-western coasts). This study, unlike Pardini *et al*. (2001), found weak but significant structure in the microsatellites that was also evident in the mtDNA. They concluded that both breeding males and females are philopatric on a continental scale; introducing a new component in the discrimination of philopatry *i.e.* scale. The designation of a species as philopatric requires a knowledge of home-ranges relative to dispersal potential. The challenge is to discriminate between a species displaying philopatry *v*. a limited home range.

Reproductive philopatry examines prolonged affinities to nurseries, as opposed to limited home range, and as stated above is most likely to occur in species that utilize nurseries during juvenile life phases. Conclusions drawn from Blower *et al*. (2012) correlate with recent acoustic and satellite tracking results which strongly suggests long-term juvenile *C. carcharias* affinity (a predicted outcome of reproductive philopatry) was evident at two very specific locations on the Australian east coast (Bruce & Bradford, 2012). Differences in conclusions regarding male movement patterns between Pardini *et al*. (2001) and Blower *et al*. (2012) might reflect differences in their sampling strategies. Tillett *et al*. (2012) conducted a study on juvenile *C. leucas*, to investigate philopatric behaviour by sampling juveniles from nurseries within sections of habitat that could be feasibly connected by dispersal.

This sampling approach excluded locations that might be separated by larger distances or vicariant events. The study identified structure in mtDNA that was absent in microsatellite analyses in juveniles, indicating female reproductive philopatry. This finding was also found in *C. leucas* by Karl *et al.* (2011*a*) based on comparisons of mtDNA and microsatellites, although that study did not directly sample nurseries.

ASSESSING RELATEDNESS BETWEEN INDIVIDUALS

Another method which can be used to identify philopatry is relatedness between individuals using kinship and parentage analysis. While Feldheim *et al.* (2001) were investigating multiple paternity in *N. brevirostris*, they found the same gravid females in the same nursery in consecutive breeding cycles. The power of these kinship and parentage analyses to detect variation strictly depends on the degree of polymorphism of the available markers (Schultz *et al.*, 2008). If the power of markers is sufficient to reveal individual genotypes, these can be used as permanent identification tools (tags) in mark–recapture experiments (Feldheim *et al.*, 2004; Chapman *et al.*, 2009*b*), as well as for inferring spatial and temporal dynamics on individual and population scales.

Ongoing research programmes at Bimini Island in the Bahamas have genotyped a substantial number (*c.* 900) of juvenile *N. brevirostris*. Genetic screening of these juveniles has provided information on the harder to sample, larger adults, by inferring the genotypes of their parents (Feldheim *et al.*, 2001, 2004; Dibattista *et al.*, 2008). Kinship analysis grouped the juveniles into sibling groups and determined maternal and paternal genotypes. Despite only opportunistically genotyping mature *N. brevirostris*, parentage analyses were able to determine which juveniles were sired by the same parents, either sampled or un-sampled. Litter reconstructions combined with individual identification using passive integrated transponder (PIT) tags confirmed that females gave birth to multiple litters biennially at the Biminis. These patterns were not evident in male conspecifics which sired only one litter in 10 breeding cycles indicating sex-specific differences in dispersal or mating success (Feldheim *et al.*, 2004).

Reproductive philopatry identified through kinship and parentage analyses, unlike population structure assessments, has the power to discriminate whether observed behaviours are the exception or the rule. If female philopatry only occurs in a small proportion of the population, it is less likely to be identified through population structure assessment. Whereas, if sample size is sufficiently large, kinship and parentage analysis can identify the proportion of the population displaying this behaviour providing valuable biological information (Veríssimo *et al.*, 2011*b*).

In conclusion, the value of genetic methods is that through genotyping an individual is permanently 'tagged' and therefore research studies become no longer limited by the physical properties of tagging methods (*i.e.* tag attachment or battery life). Furthermore, information encoded in the genetic data (irrespective of method or marker) provides information on long-term movement patterns, which is information beyond that provided by each individual themselves. Caution must be taken when interpreting genetic data as there are many scenarios that may shape movement patterns. Sampling strategies targeting individuals in nurseries (*i.e.* juveniles or females) and grouping analyses by sex or size increase the power for discerning reproductive philopatry.

GENETIC EFFECTIVE POPULATION SIZE

Genetic effective population size (N_e) estimation has featured in terrestrial conservation efforts for several decades (Schwartz *et al*., 2007), but is emerging only now from theoretical realms for application in marine conservation and commercial fisheries. Complementing conventional stock assessment methods, genetic monitoring can estimate a population's effective size (N_e) to evaluate abundance and viability for elasmobranch species. As the size of any isolated population decreases, the rate of genetic drift increases, along with the rate of inbreeding, accumulation of detrimental genes, and loss of adaptive variation (Frankham *et al*., 2010; Hare *et al*., 2011). Assessing the N_e of a species provides an indication of both the breeding population size and of population genetic health (Frankham, 1995; Portnoy *et al*., 2009), making it a potentially invaluable stock evaluation tool for conservation and fisheries management (Luikart *et al*., 1998; Reed & Frankham, 2003).

THE MANY FACES OF N_e

Theoretically, N_e is defined as the size of an ideal population that is experiencing a known amount of genetic drift. Practically, a population's genetic effective size equates to either an estimate of the number of breeders that produced the sampled cohort (N_b) or the harmonic mean size of the breeding population over several generations (N_e). This interpretation depends on the life history of the species and sampling method adopted. Waples (1990*a*, *b*) showed that for semelparous Pacific salmon *Oncorhynchus* spp., the N_e per generation approximates the product of N_b and the generation length when population size is constant. For iteroparous species, however, like elasmobranchs, the relationship between N_b and N_e is still being established and requires further research (Waples, 2010).

The historical or long-term effective population size (denoted here as HN_e) equates to the harmonic mean of the effective population size per generation over *c*. $4N_e$ generations into the past where the population size is scaled by the genetic mutation rate (Hare *et al*., 2011). HN_e estimates have some value for providing rough estimates of virgin biomass prior to anthropogenic exploitation (Crandall *et al*., 1999; Hare *et al*., 2011), and have been estimated for several elasmobranch species including *R. typus* (Castro *et al*., 2007), *S. lewini* (Duncan *et al*., 2006), *N. brevirostris*, *N. acutidens* (Schultz *et al*., 2008), sleeper sharks (Squaliformes: Somniosidae) (Murray *et al*., 2008), *C. maximus* (Hoelzel *et al*., 2006) and *C. leucas* (Karl *et al*., 2011*a*). HN_e estimates, however, lack the rigour required for population management decisions due to: (1) variation associated with the molecular evolutionary rate estimate, (2) unknown population connectivity at ancient timescales, which typically constrain the resolution of HN_e to a global estimate (Frankham *et al*., 2010; Portnoy, 2010) and (3) a bias towards reflecting low breeding population size directly following any population bottlenecks that may have occurred in the species' history (Frankham *et al*., 2010; Portnoy, 2010).

Of much greater interest to fisheries management and conservation is the contemporary effective population size or short-term N_e (denoted here as CN_e). CN_e indicates whether a population's current genetic health is sufficient for sustained adaptation and approximates the recent mean number of breeding individuals (Lande, 1995; Reed & Frankham, 2003; Frankham *et al*., 2010).

CN_e is estimated using two main approaches, requiring either temporally spaced samples from a population or samples from a single point in time. Both methods provide similar information but have different advantages and limitations (Luikart *et al*., 2010), particularly when applied to elasmobranchs.

MULTIPLE COHORT TEMPORAL CN_e ESTIMATION

The temporal method typically requires population samples separated by at least two generations, and preferably more (Williamson & Slatkin, 1999; Waples & Yokota, 2007). The allele frequency variances between these cohorts are used to calculate the CN_e (also termed variance N_e). Generation times can be long for elasmobranchs, however, and sampling successive generations is often impractical. A modified temporal method allows for the sampling of consecutive cohorts as long as their age can be accurately identified, and information regarding reproductive output and survival likelihood can be estimated (Jorde & Ryman, 1995). Elasmobranchs can be aged using characters such as juvenile umbilical scaring, or by correlating age to morphometric properties such as mineralized vertebral bands (Cailliet *et al*., 2006). The temporal method allows selection of either diploid [nuclear (n)DNA: microsatellites, single nucleotide polymorphisms] or haploid (mtDNA) markers (Laikre *et al*., 1998), unlike single time-point methods, for which only nDNA markers can be employed.

The temporal method has been applied to teleosts such as wild populations of *Oncorhynchus* spp. (Waples, 2002, 2006*a*), red drum *Sciaenops ocellatus* (L. 1766) (Gold & Turner, 2002) and Atlantic cod *Gadus morhua* L. 1758 (Therkildsen *et al*., 2010), but estimates for elasmobranch species have appeared only recently. Portnoy *et al*. (2009) used the temporal approach to estimate the CN_e of the commercially exploited western Atlantic *C. plumbeus*. The placental viviparous gestation of *C. plumbeus* leaves an umbilical scar that lasts for several months after birth, allowing researchers to clearly identify the young-of-the-year cohort. Older generations were conservatively placed into age cohorts by their length. Analysis of 902 sharks from five cohorts with eight nuclear microsatellite markers found the number of breeding individuals to be about half of the census estimates (N_C).

The by-catch population of *R. clavata* in the Irish Sea, landed by demersal fishers, was found to have a temporal CN_e estimate of 283 (95% C.I. = 145–857) (Chevolot *et al*., 2008). Although relatively small sample sizes ($n = 363$) and low numbers of microsatellite loci ($n = 5$) were used, the CN_e estimate was bounded by non-zero and non-infinite C.I.s. The small CN_e suggests that the population may be on the cusp of deleterious allele accumulation in the short term and substantial extinction risk in the long term. Such a finding should alert fisheries and conservation managers to implement remedial action, such as species-specific gear modifications, and to increase monitoring activities.

SINGLE TIME-POINT CN_e ESTIMATION

Estimation of CN_e using the single time-point estimate approach requires only a single sampling of the population. The last decade has seen significant development in the variety and utility of single time-point estimation techniques. Some are limited to a narrow range of situations however. For example, co-ancestry analysis (Nomura, 2008) is applicable to small populations where individuals are genetically related and parentage analysis caters for species with overlapping generations, but sex and

age need to be accurately determined (Wang *et al*., 2010). The heterozygote-excess method (Robertson, 1965; Pudovkin *et al*., 1996) is best suited for management of small populations ($N_e \leq 30$) such as endangered or captive species (Nomura, 2008; Pudovkin *et al*., 2010). The CN_e of larger, naturally occurring populations is more commonly estimated from the extent of linkage-disequilibrium (LD) which increases as population size decreases (Hill, 1981; Waples & Do, 2010), although the user needs to be aware of demographic processes, such as immigration and overlapping generations, that can potentially bias an LD N_e estimate (Luikart *et al.,* 2010). The ongoing development of the LD method (Waples, 1991; Bartley *et al*., 1992) including bias correction for low sample sizes (England *et al*., 2006; Waples, 2006*b*) has made it a serious competitor to the temporal method (Waples & Do, 2010; Antao *et al*., 2011), although Russell & Fewster (2009) provide an alternative perspective. Simulations show that the LD method can achieve a similar accuracy and precision with the advantage of a greatly simplified sampling regime (Waples & Do, 2010). Recent modelling to detect population trends using CN_e (requiring at least two temporally spaced sample sets) indicated that the LD method outperformed the temporal approach and that trends of decline can be detected with at least 80% accuracy within one generation (Antao *et al*., 2011). This accuracy, however, requires sufficient power in number of samples and genetic markers, can be vulnerable to false positive results (type I errors), and is dependent on the size of the population in question. The information content of the majority of single time-point estimators is squared by increasing numbers of loci or alleles, whereas for temporal estimates it increases linearly (Waples & Do, 2010). This feature serves to heighten the potential of single time-point methods given the current trend of accelerated marker discovery.

Single time-point CN_e estimation methodology has only recently been applied to elasmobranchs. Similar values were found using LD CN_e methods as for the temporal approach for *C. plumbeus* (Portnoy *et al*., 2009). The critically endangered eastern Australian *C. taurus* population produced approximate Bayesian (Tallmon *et al*., 2008) CN_e estimates of 126·31 (95% C.I. = 67·73–474·11) (Ahonen & Stow, 2009). This population, which receives negligible genetic variation from migration (Ahonen *et al*., 2009), is possibly beyond recovery by natural means, given the magnitude of N_e thresholds thought necessary for retaining evolutionary potential and avoiding the danger of inbreeding depression. A recent investigation of LD CN_e in the critically endangered smalltooth sawfish *Pristis pectinata* Latham 1794 population in south-western Florida found similarly low estimates of 250–350 (95% C.I. = 142–955, eight microsatellite loci, $n = 137$; Chapman *et al*., 2011).

INTERPRETATION AND APPLICATION OF N_e

Genetic effective population size (N_e) can give an indication of the genetic health of a population, which can then be assessed with benchmarks that indicate the urgency and type of management strategy that may be required. Retention of long-term evolutionary potential in an idealized population is thought to require an N_e of at least 500 (Franklin, 1980; Frankham *et al*., 2010) although others estimate that at least 5000 breeding individuals may be required (Lande, 1995). Avoiding deleterious allele accumulation requires an $N_e > 1000$ (Palstra & Ruzzante, 2008; Frankham *et al*., 2010) and inbreeding depression may occur if the N_e falls below 50 (Franklin, 1980). How these thresholds translate to elasmobranch populations is

not clear. Chapman *et al.* (2011) found that despite 95–99% population depletion last century, low CN_e estimates of 250–350 for *P. pectinata* were accompanied by empirical estimates of high genetic diversity and negligible inbreeding. One or a combination of scenarios were hypothesized: (1) the longevity of the species (estimated at 30–60 years) has slowed genetic drift, (2) a critically low N_e was never reached, (3) or general features of elasmobranch life history and reproductive behaviour reduce the effects of genetic bottlenecks and inbreeding. These findings should stimulate further investigation into the diversity of other threatened elasmobranchs and the sustainability of elasmobranch harvest.

Understanding the relationship between N_e and demographic census sizes (N_C) can be informative for management decisions. A review of CN_e:N_C ratios in marine teleosts found that in general CN_e is two to six orders of magnitude lower than N_C (Hauser & Carvalho, 2008), possibly due to high fecundity but low survivorship. For example, the CN_e:N_C ratio for New Zealand snapper *Pagrus auratus* (Forster 1801) was estimated at 10^{-5}. Therefore, in theory, a census population size of 50 $\times$ 10^6 individuals is required to maintain a CN_e of 500 (Hauser *et al.*, 2002). *Raja clavata* also yielded ratios of 10^{-5} and 10^{-6} (Chevolot *et al.*, 2008). In contrast, the available data for sharks suggests that their CN_e:N_C ratios are more similar to those of marine and terrestrial mammals than teleosts. Comparison of CN_e and cohort census size (N_C) for 902 *C. plumbeus* individuals from five cohorts sourced from western Atlantic Ocean nursery grounds showed CN_e:N_C ratios close to 0·5 (range from 95% C.I. = 0·21–1·0; Portnoy *et al.*, 2009). Comparisons of CN_e estimates of *C. taurus* (mean = 126, 95% C.I. = 67–474; Ahonen & Stow, 2009) with mark–recapture estimates of mature adults (>2 m L_T, overall mean number of individuals across different estimation methods = 178, 95% C.I. = 58–321; Otway & Burke, 2004) result in ratios of comparable magnitude. These preliminary results for sharks are strikingly different from those for marine teleosts and indicate that a large part of the population (*i.e* $\geq$50%) is most probably contributing to recruitment in these species, a fact that is not apparent when just census estimates are considered.

Simulation studies show that both temporal and LD CN_e estimators can be used to investigate population trends with careful sampling (Antao *et al.*, 2011). Furthermore, trends in CN_e:N_C ratios over time have been investigated. Ratios were found to be constant when compared between time periods for *P. auratus* (Hauser *et al.,* 2002). Ardren & Kapuscinski (2003) found that CN_e:N_C ratios increased over a period of 17 years for the steelhead trout *Oncorhynchus mykiss* (Walbaum 1792) in response to declining population size, even though CN_e was constant over this time. CN_e:N_C ratios may, however, be more predictable in species with low fecundity and low variance in reproductive success, such as elasmobranchs, than in species with high variance in reproductive success (Luikart *et al.,* 2010). These life-history characteristics, common in elasmobranchs, make this group attractive for further exploration of the relationship between CN_e and N_C, providing fertile grounds for collaboration between resource managers and geneticists.

GENERAL CONSIDERATIONS

Providing meaningful CN_e estimates to conservation or fisheries managers requires careful sampling regimes and marker selection tailored for the species and population under scrutiny. The power of estimates is positively influenced by sample sizes,

numbers of loci and alleles, and the number of generations between cohorts. Factors such as CN_e model assumptions, species life history and population-specific migration must be taken into consideration as these can significantly affect the estimated magnitude and interpretation of CN_e. CN_e data analysis can be computationally challenging, but a variety of software packages have been developed to simplify implementation of the available analyses (reviewed in Luikart *et al*., 2010).

The sampling regime is clearly dictated by the choice of either the cohort-dependent temporal method or the single sample-set approach. The anticipated population size will dictate the number of individuals required for accurate estimation. Small populations ($CN_e < 200$) may require as few as 50 unique samples, although more are always preferable (Waples & Do, 2010). Larger populations ($CN_e \geq 1000$) require far more samples to attain similar levels of accuracy and precision, and undersampling of a large population will tend to result in imprecise upper confidence boundaries (Waples, 1989; Palstra & Ruzzante, 2008; Waples & Do, 2010).

Careful consideration is essential when choosing genetic markers. Selecting appropriate markers *a priori* requires a preliminary sensitivity analysis (*i.e.* from a pilot study) to estimate the numbers of samples and markers required for accurate estimates. An indication of the allelic diversity of the population for each marker is preferable although appropriate sample numbers and loci can be inferred from simulation studies (Waples & Do, 2010), albeit less precisely. In general, $\geq$10 unlinked and highly polymorphic loci are desirable (Palstra & Ruzzante, 2008; Luikart *et al*., 2010; Waples & Do, 2010), although consideration needs to be given to the particular N_e estimation method. For example, having large numbers of loci (*e.g.* >10) when using the LD method may increase the chance of linkage amongst loci and bias results (Waples & Do, 2010). Recent advances in the rapid identification of large numbers of loci and alleles, however, should bring workable CN_e estimates of even large populations within the grasp of elasmobranch resource managers in the near future (Waples & Do, 2010).

Estimates of N_e are sensitive to the assumptions of its underlying construct, the idealized Wright–Fisher population (Wright, 1931). In particular, overlapping generations and migration may result in biased N_e estimates for elasmobranchs. Some formulations of temporal N_e estimation can accommodate overlapping generations (Jorde & Ryman, 1995; Waples & Yokota, 2007) and some levels of migration (Wang & Whitlock, 2003; Waples, 2010; Waples & England, 2011). Analysis of population genetic structure should, however, be quantified to evaluate migration as a potential source of bias. The effect of overlapping generations, as found in elasmobranchs, is largely untested for single time-point estimators (Waples & Do, 2010) with some exceptions (Wang *et al*., 2010). Other processes that contravene the Wright–Fisher population, such as selection, variable or fluctuating population size, unequal sex ratio and non-random mating, have less significant effect on N_e estimation but should be considered for each particular study species and system (Palstra & Ruzzante, 2008; Charlesworth, 2009; Luikart *et al*., 2010; Hare *et al*., 2011).

Comparison of different N_e estimators can be informative and in particular, concordance amongst estimators increases confidence in the results (Miller & Waits, 2003; Nomura, 2008; Waples & Do, 2010). For example, similar magnitude CN_e results in *C. plumbeus* were found with both temporal and LD methods (Portnoy *et al.,* 2009). Conversely, highly discordant CN_e values may indicate bias in a particular approach (*e.g.* LD occurring by means other than population reduction) or unusual

underlying ecological or biological effects (Hauser & Carvalho, 2008; Luikart *et al*., 2010), requiring further investigation and interpretation with caution.

N_e estimators provide a window on life-history characteristics and population dynamics that are often extremely challenging to estimate for elasmobranchs, but essential for their conservation and sustainable harvest. The potential of N_e will hopefully be acknowledged by marine resource managers so that N_e results may be incorporated into their strategic plans and the techniques further advanced.

MOLECULAR EVOLUTIONARY RATE AND ITS CALIBRATION IN SHARKS, SKATES AND RAYS

The rate of DNA change, or the molecular evolutionary rate, is an integral parameter to various analyses in systematics, molecular ecology, evolutionary and conservation genetics (Nei, 1987; Nei & Kumar, 2000; Frankham *et al*., 2002; Avise, 2004). The average molecular evolutionary rate of elasmobranchs appears to be slower than in mammals. This was originally based on the observation that the mtDNA gene regions *cytb* and *coI* in sharks (Lamniformes and Carcharhiniformes) evolve seven to eight-fold slower than in primates and ungulates (Martin *et al*., 1992). This finding was corroborated, more recently, with a phylogenetic analysis using whole mitogenomes where the mean chondrichthyan branch length from the root to the tips was *c*. 43% of that of sarcopterygians (Inoue *et al*., 2010). The molecular evolutionary rate in the shark nuclear DNA genes *dix2*, *rag1* and *hsc70* have also been reported as slower than in mammals (Martin, 1999).

The assumption of slow molecular evolutionary rates across elasmobranchs may not always hold, given the extent of rate heterogeneity among elasmobranch taxa. Large differences in molecular evolutionary rates among 14 elasmobranch generic lineages have been reported. For example, rates among genera have been reported to differ by factors of 6·5 and 65 for mtDNA *cytb* and nuclear rRNA, respectively (Winchell *et al*., 2004). Significant rate heterogeneity was also apparent among batoid lineages in several mtDNA regions (Dunn *et al*., 2003). Further work on this subject is required, as there may be even greater variation in rates across elasmobranch species, as has been reported more recently for mammals and birds (Nabholz *et al*., 2008, 2009; Weir & Schluter, 2008).

Within lineages, the transition from short-term elevated molecular evolutionary rates to a long-term slower rate (so called time dependency of molecular evolutionary rate) has been reported for mammals, birds and fishes (Penny, 2005; Ho & Larson, 2006; Burridge *et al*., 2008). Currently, there is no information about this effect in elasmobranchs, but it has the potential to confound molecular inferences of demographic parameters and dating of many important evolutionary events.

CALIBRATION OF MOLECULAR EVOLUTIONARY RATE FOR ELASMOBRANCHS

Given that molecular evolutionary rate can vary between lineages, whenever possible it should be estimated independently for different lineages using appropriate calibration points. Calibration points so far used are (1) fossil records, (2) paleogeographic events and (3) dated events from other molecular studies (Table I). Fossil

TABLE I. Calibrated mtDNA molecular evolutionary rate in elasmobranchs. Substitution rates are shown in the unit of the number of substitutions per site per lineage per year (S s^{-1} y^{-1})

References	Lineage	Marker	Substitution rate $\times 10^{-8}$ S s^{-1} y^{-1}	Calibration M years
1	All sharks	*cytb* and *coI*	0·07*	20–83 (F)
	Sphyrna tiburo	*cytb*	1·15†	3·5 (IP)
2	*Squalus*	*cytb*	0·099	152 (F)
	Squatina	*cytb*	0·167	157 (F)
	Pristophorus	*cytb*	0·205	157 (F)
	Chlamydoselachus	*cytb*	0·195	200 (F)
	Mitsukurina	*cytb*	0·118	145 (F)
	Alopias	*cytb*	0·096	121 (F)
	Carcharias	*cytb*	0·118	121 (F)
	Galeocerdo	*cytb*	0·168	112 (F)
	Scliorhinus	*cytb*	0·226	112 (F)
	Heterodontus	*cytb*	0·097	194 (F)
	Urobatis	*cytb*	0·634	35 (F)
	Potamotrygon	*cytb*	0·557	35 (F)
	Rhinobatos	*cytb*	0·339	85 (F)
	Raja	*cytb*	0·421	85 (F)
3	*Raja clavata*	*cytb*	0·5–0·8	31 (M)
4	*Sphyrna lewini*	CR	0·4	*c*.3·4 (IP)
5	*Carcharhinus limbatus*	CR	0·215	3·2 (IP)
6	*Negaprion brevirostris*	CR	0·335	3·5 (IP)
7	*Aetobatus*‡	*cytb* + *coI*	0·29–0·45	3·1–2 (IP)
8	*Ginglymostoma cirratum*	*cytb* + tRNAPhe + tRNA thu + CR	0·27	3·5 (IP)
9	*Aetobatus*‡	*cytb*	0·36–0·635	3·5–2 (IP)
10	*Carcharodon carcharias*	CR	0·595	3·5 (IP)
		CR	0·37	5 (SS)

1, Martin *et al*. (1992); 2, Winchell *et al*. (2004); 3, Chevolot *et al*. (2006); 4, Duncan *et al*. (2006); 5, Keeney & Heist (2006); 6, Schultz *et al*. (2008); 7, Richards *et al*. (2009); 8, Castro (2009); 9, Schluessel *et al*. (2010); 10, Gubili *et al*. (2011).

*Rate based on number of transversions at four-fold degenerate sites.

†Rate based on number of transitions at four-fold degenerate sites.

‡See White *et al*. (2010*b*) about taxonomic revision.

F, fossil; IP, rise of the Isthmus of Panama; M, molecular-dated event and SS, the rise of Sunda–Sahul shelves.

calibration points are typically very old (up to 200 MB.P.) reflecting a focus on the elasmobranchs as one of the oldest living groups of jawed vertebrates in deep phylogenetic studies (Winchell *et al*., 2004) on which molecular evolutionary rates are derived. The most common paleogeographic event used for calibration is the rise of the Isthmus of Panama around 2–3·5 MB.P., which physically divided the tropical and warm temperate taxa of the Atlantic and east Pacific Oceans (Collins, 1996; Schultz *et al*., 2008; Richards *et al*., 2009; Schluessel *et al*., 2010). The rise of the Sunda-Sahul shelves *c*. 5 MB.P. was used as an alternative calibration point

in the Southern Hemisphere to study *C. carcharias* (Gubili *et al*., 2011). This land bridge formation, however, is rarely used as the calibration point for marine taxa as it has opened and closed many times with glacial cycles (Voris, 2000). A divergence time of 31 Mb.p. between *Rajini* and *Amblyrajini* was estimated by molecular dating (Valsecchi *et al*., 2005), which was then used for calibration in other studies of skates (Chevolot *et al*., 2006; Griffiths *et al*., 2011).

Major challenges in calibrations are (1) the dating of fossils and geologic events, (2) decisions surrounding assignment of such dates within the tree and (3) the measurement of genetic distances. A vast number of fossil records of elasmobranchs have not been utilized to their potential as calibration points due to the high level of expertize required to identify and age fossils both from extinct and extant lineages (Naylor & Marcus, 1994; López *et al*., 2006). This could be improved through better collaboration with geologists and palaeontologists (Lovette, 2004; Beheregaray & Caccone, 2007).

APPLICATION OF MOLECULAR EVOLUTIONARY RATES FOR ELASMOBRANCHS

Within the elasmobranch literature, just a few calibrated molecular evolutionary rate values are used for a wide-variety of loosely related species. For example, Duncan *et al*. (2006) estimated the rate for *S. lewini* with the rise of the Isthmus of Panama as a calibration point. Their estimate was then used directly for studies of *G. galeus* (Chabot & Allen, 2009), *C. brachyurus* (Benavides *et al*., 2011*a*) and *Orectolobus* spp. as a mean rate (Corrigan *et al*., 2008; Corrigan & Beheregaray, 2009). Other studies have employed similar or averaged rates (Sandoval-Castillo *et al*., 2004; Hoelzel *et al*., 2006; Castro *et al*., 2007; Murray *et al*., 2008; Portnoy *et al*., 2010; Griffiths *et al*., 2011; Karl *et al*., 2011*a*; Phillips *et al*., 2011) or considered the range from calibrated molecular evolutionary rates (Brown *et al*., 1979; Martin *et al*., 1992; Donaldson & Wilson, 1999; Chevolot *et al*., 2006; Duncan *et al*., 2006; Keeney & Heist, 2006; Schultz *et al*., 2008). Currently, calibrated rates for elasmobranchs range between $0{\cdot}215 \times 10^{-8}$ (Keeney & Heist, 2006) and $1{\cdot}2 \times 10^{-8}$ (Hoelzel *et al*., 2006) $S\ s^{-1}\ y^{-1}$ (substitutions per site per year) and are commonly used to make inferences about processes during the Miocene, Pliocene, Pleistocene and Holocene. Any difference in the rate will essentially result in an equivalent difference in divergence time estimates. In most phylogeographic studies, it appears that a small variation in molecular evolutionary rate estimates would not greatly affect the overall interpretation of the results being discussed. Otherwise, the caveat for uncertainty and direction of bias needs to be explicitly stated (Corrigan & Beheregaray, 2009).

MOLECULAR DATING WITH A RELAXED CLOCK

As the molecular evolutionary rate is likely to vary among lineages, the assumption of a strict molecular clock (*i.e.* that rates are consistent across time and across lineages) is likely to be violated. For this reason, Bayesian molecular dating approaches using a relaxed clock are becoming popular (Drummond *et al*., 2006). Bayesian methods have also been employed in the studies of elasmobranchs, including investigations of species radiation in *Orectolobus* spp. (Corrigan & Beheregaray, 2009)

which used a mean rate estimated by Duncan *et al*. (2006); species radiation in *Etmopterus* spp. (Straube *et al*., 2010) which dated 21 nodes with five fossil calibration points encompassing 40–375 Mb.p.; and a Rajidae phylogeny which dated 12 nodes with an age constraint of a node at 31 Mb.p. (Griffiths *et al*., 2010).

MOLECULAR EVOLUTIONARY RATE AND MICROSATELLITES

Microsatellite markers have more recently been investigated with respect to calculating molecular evolutionary rate. Microsatellites evolve primarily through replication slippage. There is, however, a range of other mechanisms that ultimately cause changes in allele length, which has led to the development of a number of statistical models of microsatellite evolution (infinite allele, k allele and stepwise mutation models) (Ellegren, 2004). Molecular evolutionary rates are likely to be locus and taxon specific and even differ among repeat motifs and length. Large alleles have higher mutation rates (Weber, 1990), but there appears to be a limit to allele length (Beckman & Weber, 1992), and interruptions in the repeat motif appear to confer stability (Jin *et al*., 1996). Heterozygote instability is an additional challenge, as it does not fit within the classical population genetic theory (Amos *et al*., 2008). Under this scenario, rates are elevated in expanding populations because of increased levels of heterozygosity.

In general, there is much more uncertainty around microsatellite than around sequence data. The assumption, however, that mutation rates in sharks are on the lower range compared with other vertebrates (Martin *et al*., 1992) appears to hold for at least one microsatellite locus studied, where mutation rates were indirectly inferred using coalescent analysis (*C. plumbeus*, Portnoy *et al.,* 2010). Given the inherent complexity of microsatellite mutations, rates will need to be inferred directly from large-scale pedigree studies, as has been done for humans (Weber & Wong, 1993). Like mutation rates applied to DNA sequences, those applied to microsatellites will need to incorporate a wide range of possible mutation rates and make inferences within the limits of those uncertainties. Coalescent-based methods that require a mutation rate to convert model parameters into demographic parameters may be the most troublesome.

In conclusion, molecular evolutionary rate should, whenever possible, be estimated independently for different lineages using appropriate calibration points. Molecular evolutionary rates will continue to be an important research focus and valuable research tool for micro and macroevolution of elasmobranchs. Numerous papers on molecular evolutionary rate and its applications have been published. For a comprehensive review, see Bromham & Penny (2003), Kumar (2005) and Bromham (2008).

RECENT TECHNOLOGIES, TRENDS AND IMPLICATIONS

The focus of elasmobranch genetics to date has been with established methods. Studies applying state-of-the-art genetic technologies are still rare even though this would undoubtedly provide better genetic resolution for tackling known challenges (*e.g.* for forensic species identification, mixed catch analysis, fine-scale population analysis, and management and conservation prioritization; FAO, 1999, 2000, 2005).

This section of the review will outline the key characteristics, current uses and the future potential of next-generation sequencing (NGS), real-time quantitative (q)PCR and microarray technologies to better understand, manage and protect elasmobranchs.

KEY CHARACTERISTICS AND CURRENT USE OF NGS, qPCR AND MICROARRAYS

Chain termination or Sanger sequencing (Sanger *et al*., 1977*a*, *b*) has been the principal DNA sequencing method for more than two decades of genetic research, delivering a revolutionary opportunity to decipher entire genomes (Fleischmann *et al*., 1995; Venter *et al*., 2001). In 2005, a new generation of sequencing technologies, known as NGS, was introduced (Margulies *et al*., 2005; Shendure *et al*., 2005). NGS technologies allow millions of volumetrically reduced sequencing reactions to be performed in parallel and at constantly decreasing costs, thereby enabling a shift in focus from gene to genome-scale research (genomics) even for small laboratories and individual investigators (Schuster, 2008). Several platforms using different types of NGS technologies are now commercially established, and several reviews provide details about their technical functioning and capabilities (Mardis, 2008, 2011; Shendure & Ji, 2008; Metzker, 2010). The comparative performance, benefits and pitfalls of different commercial NGS platforms for ecological and population-oriented applications have also been reviewed (Hudson, 2008; Harismendy *et al*., 2009; Ekblom & Galindo, 2010). Some general concerns associated with NGS that have been expressed are high sequencing error rates, short read lengths and considerable effort with the analysis and management of mega or giga-bases of genetic code. While data analysis and management are likely to pose longer-term challenges, none of these concerns will hinder widespread integration of NGS technologies across various scientific disciplines in the very near future (Shendure & Ji, 2008; Ekblom & Galindo, 2010; Hawkins *et al*., 2010). First studies employing NGS for investigating elasmobranchs are emerging, *e.g.* to identify and characterize expressed sequence tags and microsatellites (Chabot & Nigenda, 2011; Takechi *et al*., 2011; Chabot, 2012).

Real-time qPCR and microarray technologies (Schena *et al*., 1995; Heid *et al*., 1996) are two other highly influential molecular technologies that were released over a decade ago, which however have not been well utilized for elasmobranchs. A qPCR assay can measure the number of amplified copies based on fluorescence levels during each cycle of a PCR without the requirement for any post-PCR measurements (Arya *et al*., 2005). Combined with reverse transcription, this represents a revolutionary technique for gene expression profiling (VanGuilder *et al*., 2008). It has been applied in several studies of elasmobranchs, primarily because this group features model species for biomedical research (Luer, 1989). In some of the primary model species, such as the clearnose skate *Raja eglanteria* Bosc 1800, qPCR-based gene expression profiling dates back more than a decade (Miracle *et al*., 2001). A more recent example is a study by Rytkonen *et al*. (2010), which used qPCR in the epaulette shark *Hemiscyllium ocellatum* (Bonnaterre 1788) to examine the suitability of nine reference candidate genes for studying hypoxic stress. The recent combination of qPCR with amplicon melting analysis (Gundry *et al*., 2003) has expanded the utility of this technique to include rapid diagnostics based on high-resolution melt (HRM) analysis which is capable of detecting SNPs and other genetic mutations (Reed *et al*., 2007). Morgan *et al*. (2011) were among the first to apply this method

to elasmobranchs in order to distinguish among three morphologically cryptic carcharhinid species that are common targets in fisheries along the northern Australian coast.

Microarray analysis is a term used to describe multiple systems and devices for highly parallel hybridization of DNA or RNA whose primary use is similar to qPCR, *i.e.* to monitor gene expression or to detect SNPs. The potential of microarray technologies is, however, far greater (Heller, 2002; Hoheisel, 2006). Even if the efficiency of microarrays for RNA quantification may be limited compared with qPCR (Morey *et al*., 2006), they have greatly increased multiplexing capabilities, covering tens to millions of test sites in a platform measuring as little as 1 cm^2 (hence their synonym: DNA chips). Yoon *et al*. (2009) provide an example of an oligonucleotide microarray designed for the rapid identification of six species of skate, five species of ray and one shark species in the waters of Korea based on differences in their mtDNA *coI* sequence.

TECHNOLOGY-MEDIATED TRANSITION INTO GENOMICS OF ELASMOBRANCHS

Implications of recent technologies for molecular evolutionary and ecological studies are numerous and beyond the scope of this review (Hauser & Seeb, 2008; Tautz *et al*., 2010). The overarching effect across scientific fields is a transition from genetics into genomics. As yet, genomic information about elasmobranchs is limited. The first complete mitochondrial genomes of elasmobranchs were published more than a decade ago, for the small-spotted catshark *Scyliorhinus canicula* (L. 1758) (Delarbre *et al*., 1998), and *M. manazo* (Cao *et al*., 1998) (see Table II for more), but publication of the first complete nuclear genome is outstanding. Venkatesh *et al*. (2007) analysed a significant part (28%) of the genome of a holocephalian, the elephant shark *Callorhinchus milii* Bory de Saint-Vincent 1823, and found it to be a critical reference for comparative analysis. Unlike the genomes of teleosts, the *C. milii* genome is not duplicated, making it more similar to the 3200 Mb human genome (Venkatesh *et al*., 2005). It was initially proposed as a first cartilaginous fish model genome primarily because of its small size (1200 Mb) compared with the genomes of some popular elasmobranch model species, such as *G. cirratum* and *S. acanthias*, which range between *c*. 3800 and 7200 Mb (Venkatesh *et al*., 2007). Genomes of other elasmobranchs vary in size, but generally exceed those of other vertebrates such as teleosts (Stingo & Rocco, 2001; Gregory, 2011). Within the elasmobranchs, genome size does not appear to be linked to phylogenetic position, while numerous small chromosomes (the so-called micro-chromosomes) and high AT contents may be indicative of a more primitive condition (Heist, 2004*a*). Sequencing of the first complete nuclear genomes will provide further insights and increased availability of NGS will rapidly speed up this process. At least three elasmobranch whole genome sequencing projects have already been initiated, for the little skate *Raja erinacea* (Mitchill 1825), *S. acanthias* and *R. typus*. In the following, four potential methodical priorities in future studies that will be part of a gradual transition into elasmobranch genomics research with a focus on applied management and conservation are outlined. Several reviews about genomics provide a more complete perspective on these topics (Luikart *et al*., 2003; Nielsen *et al*., 2009*b*; Allendorf *et al*., 2010; Avise, 2010; Ouborg *et al*., 2010; Stapley *et al*., 2010).

TABLE II. Complete mitochondrial genomes of elasmobranchs

Species	Common name	Genome size (bases)	Genbank reference	Reference
Scyliorhinus canicula	Lesser-spotted dogfish	16 697	Y16067	1
Mustelus monazo	Starspotted smooth-hound	16 707	AB015962	2
Squalus acanthia	Spiny dogfish	16 738	Y18134	3
Amblyraja radiata	Thorny skate	16 783	AF106038	4
Heterodontus franscisci	Horn shark	16 708	AJ310141	5
Plesiobatis daviesi	Deepwater stingray	17 514	AY597334	6
Okamejei kenojei	Ocellate spot skate	16 972	AY525783	7
Mitsukurina owstoni	Goblin shark	17 743	EU528659	8
Chiloscyllium plagiosum	Whitespotted bamboo shark	16 726	FJ853422	9

1, Delarbre *et al*. (1998); 2, Cao *et al*. (1998); 3, Rasmussen & Arnason (1999*a*); 4, Rasmussen & Arnason (1999*b*); 5, Arnason *et al*. (2001); 6, Chen & Hsiao, direct genbank submission (2004); 7, Kim *et al*. (2005); 8, Ferrara *et al*., direct genbank submission (2008); 9, Xie *et al*., direct genbank submission (2009).

More effective development of an established marker

DNA sequences and microsatellites are currently the most widely applied markers in the studies of elasmobranchs. Microsatellites are the primary workhorse for high-resolution analysis of neutral genetic variation in various fish species (Chistiakov *et al*., 2006), although in many elasmobranch species their identification and development remains difficult (Heist, 2004*a*). NGS technologies can help tackle this challenge by allowing for the identification of hundreds to thousands of microsatellite loci throughout the genomes of non-model taxa. Less than a full picotitre plate using the currently dominant Roche 454 (www.454.com) platform may be sufficient to achieve this (Castoe *et al*., 2010), particularly so if microsatellite-enriched libraries are prepared for optimal results (Santana *et al*., 2009; Lepais & Bacles, 2011). As such, NGS approaches will not only be important to reduce both the costs and effort to acquire extensive libraries, but they will also provide many more loci and thus bring about a substantial increase in the power and precision of current and upcoming microsatellite-based studies (see Table III). With respect to the identification of units for applied management and conservation, increased numbers of variable microsatellite markers will provide greater confidence in differentiating between none, or weak but consistent, genetic divergence. In high gene-flow situations, however, or where the migration–drift equilibrium is not yet established, it is possible that even large numbers of highly polymorphic microsatellite loci will not identify neutral genetic differentiation, while adaptive genetic differentiation may nevertheless be present (Hemmer-Hansen *et al*., 2007; Nielsen *et al*., 2009*a*, *c*).

Integrating adaptive genetic variation

One important aspect of the transition from genetics into genomics is a paradigm shift towards studying adaptive genetic variation (Stapley *et al*., 2010). Including markers under selection in future studies of elasmobranchs will have several important advantages, *e.g.* for identifying and prioritising locally adapted units of species for management and conservation purposes, and for studying micro-evolutionary

TABLE III. Current use and future directions of genetics for applied elasmobranch management and conservation. See Appendix for definition of terms. For more information on technologies and costs regarding specific genetic applications, see Allendorf *et al.* (2010)

Elasmobranch genetic research				Management and conservation implications	Benefits through recent technologies	
Applications		Markers	Scientic information		Functional	Applied
Prevailing	Phylogeny	nDNA; mtDNA	Evolutionary biology and taxonomic status	General basis	Accumulating reference data	Greater resolution to infer phylogenetic relationships
	Population differentiation (F_{ST}) and gene flow (m)	mtDNA; msats, *SNPs; candidate genes (nDNA)*	Population dynamics	Identification of intraspecific MU–CUs	Identification and development of numerous powerful msats and SNPs; genome screening and identification of variable nDNA–candidate genes; high-throughput genotyping (particularly SNPs)	Cost reduction and increased power and precision for 1. Trade and fisheries monitoring 2. Mixed catch analysis 3. Cryptic species detection 4. Spatial management 5. Stock assessment 6. Recruitment studies 7. Threatened species conservation
	Species identification	mtDNA; nDNA, msats; *SNPs*	Validation of taxonomic status	Validation of principal MU–CUs; monitoring		

TABLE III. Continued

Elasmobranch genetic research				Management and conservation implications	Benefits through recent technologies	
Applications		Markers	Scientic information		Functional	Applied
Upcoming	Effective population size (N_e)	mtDNA; msats, *SNPs*	Number of breeding individuals; genetic health of populations	Prioritization of MU–CUs	As above	As above
	Kinship–parentage analysis	msats; *SNPs*	Fine-scale population dynamics; reproductive behaviour; dispersal	Targeted management and conservation	As above	As above
	Individual identification	msats; *SNPs*	Individual behaviour	Targeted management and conservation	As above	As above
Future	Adaptation	*Candidate genes (nDNA); SNPs*	Fine-scale population dynamics; phenotype–genotype–environment links	Fine-scale validation of MU–CUs; assisted management	Accumulating reference data; genome screening for selection signals–identification of candidate genes; gene expression profiling	Emerging field for assisted management of local populations (*e.g.* assisted climate change adaption)

nDNA, nuclear gene sequence; mtDNA, mitochondrial gene sequence; msat, microsatellite; SNPs, single-nucleotide polymorphisms, MU–CUs, management and conservation units; Genetic markers given in italics are not currently used for the type of application.

implications of natural and anthropogenic pressures, such as environmental change and fisheries exploitation (Allendorf *et al*., 2008, 2010; Nielsen *et al*., 2009*b*; Stapley *et al*., 2010). Nielsen *et al*. (2009*b*) provide references and detail several options to address genetic adaptation in marine fishes. The two general approaches are to (1) use existing reference data for the identification of candidate genes, or (2) scan previously unexplored genomes for selection signals using NGS (Allendorf *et al*., 2010). For the many data deficient species of elasmobranchs, NGS approaches will be integral. Studying adaptive variation will allow microgeographical population differences to be inferred on an ecological timescale. One likely drawback of studying adaptive radiation in elasmobranchs is that the ultimate demonstration of adaptive evolution generally involves common garden experiments to provide evidence of the link between genetic variation and fitness of resident *v*. non-resident individuals (Nielsen *et al*., 2009*b*). This will typically be difficult for large and mobile species of elasmobranchs, but may be possible using young or relatively small demersal species with the collaboration of public aquariums. The emerging fields of landscape or seascape genetics or genomics (Manel *et al*., 2003; Hansen & Hemmer-Hansen, 2007; Selkoe *et al*., 2008; Schwartz *et al*., 2009; Segelbacher *et al*., 2010) enable valuable insights into local adaptation by studying population genetics in relation to natural environmental gradients (Hemmer-Hansen *et al*., 2007; Nielsen *et al*., 2009*a*, *c*).

Integrating SNPs

Single nucleotide polymorphisms (SNP) are becoming a routine marker across various fields of animal genetics (Vignal *et al*., 2002; Morin *et al*., 2004). There are convincing arguments for SNPs to be used for future population studies of elasmobranchs, but to date there are no published examples. First, SNPs are the most common form of genetic variation in most genomes, including both coding and non-coding regions, which enable both neutral and adaptive genetic variation to be examined in one marker class. Secondly, most SNPs are bi-allelic and thus suitable for high-throughput genotyping. Third, their simple mutation model makes SNPs easy to score, interpret and compare across species and laboratories. Mutationally complex microsatellites, in contrast, are much more difficult to score and interpret (Li *et al*., 2002; Morin *et al*., 2004), but they do have high numbers of alleles per locus and are therefore individually more powerful to examine neutral genetic variation. Utilizing many or a targeted selection of SNPs (Liu *et al*., 2005), however, offers an attractive alternative for management and conservation oriented research of natural populations (Morin *et al*., 2009), which will also be applicable to non-invasive sample collection and historical DNA of threatened or extinct elasmobranchs (Morin & McCarthy, 2007). SNP discovery and selection schemes need to be well prepared and resulting data corrected in order to avoid ascertainment bias (Rosenblum & Novembre, 2007). Garvin *et al*. (2010) provide a technical review and Seeb *et al*. (2011) devote a special issue to the development and application of SNPs in non-model organisms such as elasmobranchs.

Integrating high-throughput genotyping

Genotyping is an important aspect of various types of molecular applications, including but not limited to the assignment of samples at the level of species, populations or individuals. Microarray and qPCR technologies are highly efficient tools

for genotyping although there are several factors that influence their relative effectiveness. Hybridization-based genotyping is generally more effective for SNPs than for microsatellites, even if electronic chips may offer an alternative (Radtkey *et al*., 2000). If SNPs are preferred partly because of the range of genotyping opportunities, the choice of an appropriate technique, device or platform will depend on the scale and design of the study, *i.e.* the relative numbers of SNPs, species and samples (Ragoussis, 2009; Allendorf *et al*., 2010; Garvin *et al*., 2010). For example, Morgan *et al*. (2011) developed a qPCR assay based on mtDNA SNPs among three morphologically cryptic carcharhinid species, which was at least twice as fast and roughly 75% cheaper than DNA sequencing. Yoon *et al*. (2009) chose a microarray oligonucleotide chip for the same purpose, demonstrating the increased capacity of this technique (>10 species). High-throughput genotyping technologies are likely to become a critically important tool for elasmobranch management and conservation. Existing diagnostic assays using species-specific primers and multiplexed PCR reactions for elasmobranchs (Pank *et al*., 2001; Chapman *et al*., 2003; Abercrombie *et al*., 2005; Magnussen *et al*., 2007) may readily be expanded to cover ≥ 30 species-specific probes on a single oligonucleotide chip (Kochzius *et al*., 2010), not only for trade monitoring but also for fisheries monitoring, mixed catch and population analysis. The FishPopTrace project (http://fishpoptrace.jrc.ec.europa.eu/) is an outstanding example for finfish (Stokstad, 2010). Expansion of this technology into elasmobranchs would be timely.

GENERAL CONCLUSION

Molecular tools have, and continue to be, successfully used to address many aspects of ecological and evolutionary science. They are directly applicable to issues that complicate fisheries management and conservation, including species identification, stock structure and stock size assessment. With the move into the age of genomics, many of the limitations of the genetic tools currently used will be overcome using advanced technologies. Integrating recent genomic methods for marker development and genotyping, for example, will facilitate effective taxonomic identification and trade monitoring, as well as the analysis and monitoring of fisheries catch compositions, which are often unknown. High-resolution population analysis based on the assessment of both neutral and adaptive genetic variation will enable the identification and prioritization of local management units and facilitate targeted management and conservation plans at the species and population level with respect to changing environmental conditions. Intensified elasmobranch genetic research will also help in obtaining and using information on migratory and remote, high-seas stocks of elasmobranchs, which may need to be co-managed by different countries. Genetic applications are already, and will continue to be, an integral component of elasmobranch research. The past 25 years have seen major advances in elasmobranch taxonomy, systematics, conservation and management as a direct result of genetic characterization and comparison. The next decade promises breakthroughs in genetic technologies that will rapidly advance elasmobranch research capabilities in population, ecological and evolutionary genetics.

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APPENDIX

GLOSSARY OF TERMS

Allozyme electrophoresis: Separates proteins on a gel depending on size, shape and charge. Co-dominant marker assumed to be neutral. Samples must be fresh, so enzymes do not denature.

Amazon Barrier (AB): The biogeographic barrier caused by the immense freshwater and sediment outflow of the Amazon River separating Brazil and the Caribbean.

Amplified fragment length polymorphism (AFLP): DNA is digested with restriction enzymes then adapter sequences are ligated to the fragments. Selective PCR amplification using adapter primers is followed by product separation by length using gel electrophoresis. A dominant marker assumed to be neutral. Fragment-size homoplasy can cause incorrect scoring of bands across gels.

Ascertainment bias: Systematic error in parameter estimates or observed genetic patterns due to the selection of inappropriate or unrepresentative genetic markers.
Benguela Barrier (BB): The biogeographic barrier caused by the cold water upwelling around southern Africa separating the Indian Ocean from the tropical Atlantic Ocean.
Candidate gene: Gene that is suspected to have a large impact on a given trait.
Catch-per-unit-effort (CPUE): Standardization of the effort (*e.g.* area sampled or total time) required per catch.
Coalescent theory: A collection of stochastic models used to generate predictions about patterns of genetic variation and make inferences from samples of genetic data.
Cohort: Animals grouped by age, generally of the same generation.
Common garden experiment: Experimental procedure to test the genetic basis of phenotypic variation by studying individuals from different environments under the same controlled conditions (in a 'common garden' setup).
Eastern Pacific Barrier (EPB): The biogeographic barrier caused by the vast open water distances between the islands of the central Pacific Ocean and the tropical eastern Pacific Ocean.
F_{ST} (F-statistic): Measure of population differentiation. Generally $F_{ST} < 0{\cdot}05$ indicate little genetic differentiation whereas $F_{ST} > 0{\cdot}15$ indicate large genetic differentiation. Many analogues (*e.g.* Φ_{ST}, R_{ST} and others) have been developed.
Genetic drift: Random sampling of gametes in small populations resulting in stochastic fixation of genes and ultimately population-wide chance allele frequency changes.
Genetic effective number of breeders (N_b): The number of breeders that produce offspring in a single year within the constraints of an idealized Wright–Fisher population.
Genetic effective population size (N_e): The size of a theoretical 'Wright–Fisher' population that reflects the observed rate of genetic drift in a real population.
Genomics: Broadly defined here as the study of genetic variation throughout the genomes of species and/or populations.
Genotyping: Non- or post-sequencing based method to determine genetic polymorphisms within and among species, populations or individuals.
Harmonic mean: The reciprocal of the arithmetic mean of reciprocals, which is always smaller than or equal to the arithmetic mean as low values are disproportionately influential.
High-resolution melt analysis (HRM): Following rtPCR the double-stranded PCR product is slowly heated until the two strands of DNA separate. Mutations and polymorphisms between sequences are detected by differences in melt temperatures.
Inbreeding depression: Loss of fitness in reproductive capacity or survival as a result of inbreeding.
ISSR: Inter-simple sequence repeat PCR is a fast, inexpensive genotyping technique based on the variation in the regions between microsatellites.
Isthmus of Panama Barrier (IPB): The biogeographic barrier and disjunction separating the Caribbean Sea and the tropical eastern Pacific Ocean formed after the closure of the Isthmus of Panama.
Iteroparous: Reproductive strategy of multiple reproductive events prior to death producing over-lapping generations.
Kinship analysis: Two individuals are classified as half siblings, full siblings, parent–offspring or unrelated

Landscape and seascape genetics: Approaches using environmental variables to explain genetic structuring observed over space and time.
Linkage disequilibrium: Genetic artefact observed in small populations where alleles of different loci are non-randomly associated.
Local clock: A rate assigned to a set of lineages (often a clade) within a given phylogeny. Several rate categories may be assigned.
Macroevolution: Evolution at, or above, the level of species; the patterns and processes of diversification and extinction of species over evolutionary time.
Microarray: Fluorescence-based multiplexing device containing tens to thousands of probes (oligonucleotides) that hybridize to specified targets (DNA or RNA samples).
Microevolution: The process of evolution in populations that changes allele frequencies over generations, due to mechanisms including selection, mutation, gene flow and drift.
Microsatellites: Simple repeat unit of two to six nucleotides tandemly repeated with unique flanking sequences. Alleles at a locus differ in repeat number and products are separated by size using electrophoresis. Co-dominant marker assumed to be neutral. High cost and labour intensive to develop loci and mode of evolution is poorly understood.
Mid-Atlantic Barrier (MAB): The biogeographic barrier caused by the open ocean distances between the Americas and Africa
Mitochondrial DNA (mtDNA): Circular multi-copy genome external to the cell nucleus and maternally inherited so does not undergo recombination. No dominance (behaves as a haploid marker) and assumed to be neutral. Provides maternal history only so unable to detect hybrids, nuclear integration of mtDNA sequences can complicate and confound analyses.
Molecular clock: A concept assuming constant and uniform rate of substitutions in molecular evolution in all lineages under consideration. Also see 'universal clock', 'strict clock', 'local clock' and 'relaxed clock'.
Molecular evolutionary rate: Molecular clock rate. Number of substitutions per site and year and usually given as a fraction of one or a per cent. Rates are either based on substitution or divergence rates (divergence rate = substitution rate $\times$ 2).
Multiplex assay: Simultaneous analysis of multiple genetic polymorphisms using a single analytical procedure.
Mutation rate: The instantaneous rate at which non-lethal nucleotide changes occur in the genome. When measured over known number of generations, some authors call it 'pedigree rate'.
Next generation sequencing: Multiple novel and non-Sanger based technologies for sequencing in a volumetrically reduced and massively parallel mode.
Non-model taxa: Taxonomic unit for which genomic reference data is non-existent or very limited.
Nuclear DNA (nDNA): Bi-parentally inherited linear genome distributed over numerous chromosomes that undergoes recombination. Co-dominant marker selection pressure depends on target. Interpretation can be complicated if genes have an unknown function. Currently cost and labour intensive to find variable markers.
Old World Barrier (OWB): The biogeographic barrier and disjunction separating the Mediterranean Sea and Indian Ocean, formed after the closure of the Tethys Sea.

Parentage analysis: Siblings of shared parentage are grouped by estimating how many specific individuals parented a group of juveniles, as well as the most likely parent of a target offspring given the selection of parents.

Philopatry: Tendency of an individual to stay at or return to particular sites, such as females returning to locations to give birth.

Phylogenetics (molecular phylogenetics): Estimation of evolutionary relationships, and hence history, among species and higher taxa. DNA sequence data is commonly used.

Phylogeography: The field of study concerned with principles and processes governing the geographic distributions of genealogical lineages, especially those within and among closely related species.

Picotiter plate: Analytical device geared towards massively parallel sequencing analysis (>1 million reads) using the Roche 454 platform. Sequencing effort and costs may be reduced by physically subdividing picotiter plates (with a potential reduction in read numbers), or by joining different sequencing projects using aliquots of tagged DNA fragments.

Polymerase chain reaction (PCR): Technique to amplify millions of copies of a particular DNA sequence from one or a few starting copies.

Population genetics: The study of naturally occurring genetic differences among organisms, but more commonly the study of polymorphism and divergence within and among populations.

Random amplified polymorphic DNA (RAPD): Short primers of arbitrary sequence are used to amplify anonymous regions of genomic DNA and products are separated on a gel. Dominant marker assumed to be neutral. Fast and cheap but poor reproducibility, interpretation of results (presence or absence of bands) can be ambiguous and DNA degradation can alter results.

Real-time quantitative PCR (qPCR): PCR technique with fluorescent detection used to amplify and simultaneously quantify the amount of a targeted DNA molecule. Dominance and selection pressure depends on DNA target. Quantification can be complicated and reproducibility across platforms may vary.

Real-time PCR (rtPCR): PCR technique with fluorescent detection of amplified DNA sequence giving results in real time compared with regular PCR which requires gel electrophoresis to visualize end products. Dominance and selection pressure depends on DNA target. Smaller DNA targets increase sensitivity but assays are more susceptible to contamination than regular PCR.

Relaxed clock: The assumption of a single substitution rate within a phylogeny is relaxed and allowed to vary across phylogeny according to a predefined model of rate change.

Restriction fragment length polymorphism (RFLP): DNA (mostly mtDNA) is digested with restriction enzymes and fragments are separated by length using gel electrophoresis. Interpretation of results can be ambiguous if DNA degradation or partial digestion occurs.

Roche 454: One of the first and dominant systems for next-generation sequencing developed by 454 Life Sciences and run on the Roche GS FLX Titanium platform (www.454.com).

Saturation: The loss of evolutionary information in a sequence through multiple changes that erase the signal of descent.

Semelparous: Reproductive strategy of a single reproductive event prior to death producing discrete generations.

Single-nucleotide polymorphism (SNP): Variations in DNA sequence occurring at individual nucleotides.

Strict clock: A single substitution rate along all branches of a given phylogeny.

Substitution rate: The rate at which mutations are fixed in the population (slower than the mutation rate). Substitution rate is here used in terms of number of fixed mutations (substitutions) per site and time unit and used synonymously with the term 'molecular clock rate' and 'molecular evolutionary rate' and given in a unit of substitution (S) per site per lineage per year ($S\ s^{-1}\ y^{-1}$).

Substitution model: A wide variety of models developed to describe DNA nucleotide substitutions. These models use different number and types of parameters that are free to vary.

Sunda Shelf Barrier (SSB): The biogeographic barrier or disjunction caused by the land bridge during low sea-levels which restricted exchange between the western Pacific and tropical Indian Oceans.

Transition: A change in a DNA sequence from a pyrimidine to a pyrimidine (*e.g.* C to T), or from a purine to a purine (*e.g.* G to A).

Transversion: A change in a DNA sequence from a pyrimidine to a purine (*e.g.* G to T), or from a purine to a pyrimidine (*e.g.* A to C).

Universal clock: A single substitution rate of a gene for all taxa of a broader group.

Wright–Fisher population: An idealized representation of a population of diploid individuals bound by assumptions of constant size, random mating, discrete generations, no migration and no selection.

Young-of-the-year: Animals < 1 year of age.