



Use of molecular genetics for understanding seabird evolution, ecology and conservation

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ABSTRACT: Information on genetic variation within and among populations of highly mobile organisms such as seabirds is necessary for understanding their evolution and ecology, and can be a tool for conservation. Recent developments in molecular genetics, including efficient mutation-detection methods and automated sequencing, are providing detailed genetic information for non-model organisms. Furthermore, theoretical advances such as coalescent theory and molecular assignments are providing powerful tools to determine species' historical and contemporary abundance, distributions and movements. We review advances for studying phylogenetics, population genetics, hybridization, ecology and conservation in seabirds and summarize recent studies in each field. All fields will benefit from larger data sets and more sophisticated analytical methods. Phylogenetic studies will provide a more robust determination of evolutionary history, while studies of population genetics and hybridization will be elevated to genomic-level avenues of inquiry. Ecological studies may benefit from improved molecular assignments, and conservation-focussed studies will benefit from an increased understanding of seabird evolution and ecology. In addition, we highlight that combination of new molecular and analytical tools with data on morphology, behaviour and movements is especially powerful for understanding seabird evolution and ecology, and for aiding conservation.

KEY WORDS: Gene flow · Hybridization · Molecular assignments · Population genetic structure · Phylogeography · Phylogenetics · Population size · Review

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INTRODUCTION

If you are interested in evolution and ecology, why study seabirds? As a group, seabirds possess a number of extreme life history characteristics that make them particularly good model organisms for DNA-based research into evolution and ecology: they can travel great distances, thus violating assumptions of many population divergence and speciation models, yet they generally exhibit natal philopatry (return to the site of birth to reproduce); and, although their breeding sites can be difficult to access, seabirds generally breed in large colonies from which sufficient samples can be collected for robust genetic analyses. Additionally, seabirds tend to be behaviourally complex, and their reliance on the marine environment makes them good candidate species

to study in relation to climate change and other anthropogenic effects. This review explores uses of molecular genetics for understanding seabird evolution and ecology, and highlights the usefulness of this research to understanding broader evolutionary and ecological questions.

Early studies of morphology, behaviour and ecology of seabirds provided much information on their evolution, including estimates of genetic relationships among populations, species and higher taxa (e.g. Storer 1952, van Tets 1965, Nelson 1970, Strauch 1985, see also review by Schreiber & Burger 2002). However, the genetic basis of morphological and behavioural characters is generally unknown, and inferences are limited to patterns, rather than ecological and evolutionary processes. Subsequently, molecular tools such as protein electrophoresis and

DNA–DNA hybridization provided more direct information on population genetic structure, hybridization and phylogenetic relationships (e.g. Sibley 1970, Sibley & Ahlquist 1990). For example, protein electrophoresis revealed the very close genetic related-

ness of some *Larus* spp. (white-headed gull; Snell 1991), and indicated that Atlantic puffins *Fratercula arctica* from different colonies are genetically similar, despite significant differences in body size (Moen 1991).

Table 1. Summary of 2nd and 3rd generation DNA sequencing methods and platforms

Method	Description	Cost
2nd generation (next generation)		
454 pyrosequencing	Parallelized version of pyrosequencing Pyrosequencing uses luciferase to generate light for detection of individual nucleotides added to the growing DNA strand Uses emulsion PCR (DNA amplified inside water droplets; each droplet contains 1 DNA template attached to 1 primer-coated bead, which forms a clonal body) DNA can be extended >1 nucleotide at a time	Cheaper than Sanger sequencing; shorter read length than Sanger, longer than Illumina and SOLiD
Illumina (Solexa)	Sequencing based on reversible dye-termination DNA molecules attach to primers on a slide — causes formation of local clonal bodies (bridge PCR) Four types of reversible terminator bases added to reaction, nucleotides that are not incorporated are washed away Images taken of the fluorescently labeled nucleotides Dye and terminal 3' blocker chemically removed — allows next cycle to begin DNA only extended by 1 nucleotide at a time	Cheaper than 454 pyrosequencing, similar price to SOLiD; shorter read length than both Sanger and 454
SOLiD	Sequencing by ligation DNA amplified by emulsion PCR before sequencing Resulting beads (each contains only copies of the same DNA molecule) deposited on slide Pool of oligonucleotides of fixed length labeled according to sequenced position; annealed and ligated Preferential ligation for matching sequences produces signal informing nucleotide at that position	Cheaper than 454 pyrosequencing, similar price to SOLiD; shorter read length than both Sanger and 454 pyrosequencing, comparable read length to Illumina
3rd generation (next-next generation)		
Ion semiconductor	Sequencing based on detection of hydrogen ions released during DNA polymerization, rather than optical methods System uses a microwell plate (different DNA in each well), wells are flooded with a single type of nucleotide, if nucleotide incorporates a hydrogen ion, it is released and read as the correct base Multiple bases can be added simultaneously if they are the same type	Cheaper and faster than any 2nd generation platform, similar read length to 454 pyrosequencing,
DNA nanoball	Rolling circle replication amplifies small fragments of genomic DNA into DNA nanoballs Sequencing by ligation (unchained) used to determine nucleotide sequence	Low cost compared to other methods, but short read lengths
Helioscope™ single molecular	Extension based sequencing (1 nucleotide at a time as with Sanger sequencing) Uses DNA fragments with added poly-A tail adaptors Cyclic washes with fluorescently labeled nucleotides occur, and reads are performed by Helioscope sequencer	Low cost compared to other methods, but short read lengths
Single molecular real time (SMRT™)	Sequencing by synthesis approach with unmodified polymerase and fluorescently labeled nucleotides	Relatively low cost; 1000 bp read lengths

Table 2 (this and following page). Summary of computer programs for analysis of molecular data, modified from Tables 1 & 2 in Excoffier & Heckel (2006), with the addition of programs for phylogenetic and ecological investigations. POPG, population genetics; POPD, population dynamics; HYB, hybridization; PHY, phylogenetics. MULT, multi-allelic markers (no specific mutation model assumed); DNA, sequence data; STR, short tandem repeat also known as microsatellites (stepwise mutation assumed); hapSTR, linked SNP and STR markers; SNP, single nucleotide polymorphism; FREQ, frequency data; AFLP, amplified fragment length polymorphism; DIST, distance matrix; RAPD, random amplified polymorphic DNA

Program	Discipline	Functions	Data type	Platform	Source
Structure	POPG, POPD, HYB	Uses genotypes from multiple markers to determine underlying genetic structure among a set of individuals; can detect immigrants; use in assignment studies	MULT	Java	Pritchard et al. (2000)
NewHybrids	HYB	Designed for the analysis of a single hybrid population; samples can only come from 2 parental populations	MULT	Windows, Linux	Anderson & Thompson (2002)
IM, IMA, IMA2	POPG, HYB	Isolation model testing; multiple versions with migration, 2 population model can estimate divergence time, ancestral population size, divergence time, and migration	DNA, STR, hapSTR	DOS, MacOS	Nielsen & Wakeley (2001), Hey & Nielsen (2004, 2007)
DIYABC	POPG, HYB	Make inferences based on Approximate Bayesian Computation; graphical interface	STR	Windows, Linux	Cornuet et al. (2008)
Migrate	POPG, POPD, HYB	Estimates the effective population sizes and migration rates of 2 constant populations using non-recombining sequences, microsatellite data, or enzyme electrophoretic data	DNA, SNP, STR, MULT	DOS, MacOS, Linux	Beerli & Felsenstein (1999) Beerli & Palczewski (2010)
Bayes Ass+	POPG	Uses multilocus genotypes to estimate contemporary migration between populations	MULT	Windows, MacOS, Linux	Wilson & Rannala (2003)
Cfit	HYB	Fits genotypic and phenotypic data to equilibrium cline models	MULT	Windows	Gay et al. (2008)
ClineFit	HYB	Fits genotypic data to equilibrium cline models	MULT	Windows, MacOS	Porter et al. (1997)
BAPS	POPG	Assigns individuals to genetic clusters; can consider them immigrants or descendants from immigrants	MULT	Windows	Corander et al. (2003, 2004)
GeneClass2	POPG	Uses multilocus genotypes to assign individuals to populations and detect immigrants	MULT	Windows	Piry et al. (2004)
Geneland	POPG	R package that takes into account spatial positions of individuals when detecting population subdivisions	MULT	R	Guillot et al. (2005)
Arlequin	POPG	Multi-use population genetics software environment; computes indices of genetic diversity, F -statistics, Hardy-Weinberg Equilibrium, etc.	DNA, SNP, STR, MULT, FREQ	Windows	Excoffier et al. (2005)
DnaSP	POPG	Estimates several measures of DNA sequence variation within and between populations including linkage disequilibrium, recombination, gene flow and gene conversion	DNA, SNP	Windows	Librado & Rozas (2009)
FSTAT	POPG	Multiple functions – F_{ST} estimates, etc.	STR, MULT	Windows	Goudet (1995)
GDA	POPG	Multiple functions; basic indices of genetic diversity	AFLP, MULT	Windows	Lewis & Zaykin (2002)
Genepop	POPG	Multiple function; basic indices of genetic diversity	STR, MULT	DOS	Raymond & Rousset (1995)
GENETIX	POPG	Multiple function; basic indices of genetic diversity	MULT	Windows	Belkhir et al. (1996–2004)
MEGA	POPG	Multiple function; basic indices of genetic diversity	DNA, DIST	Windows	Tamura et al. (2011)
MSA	POPG	Multiple function; basic indices of genetic diversity	STR, MULT	DOS, MacOS, Linux	Lipman et al. (1989)
SPAGeDi	POPG	Computes genetic distance between populations, inbreeding, kinship, relatedness	STR, MULT	DOS, Windows, MacOS, Linux	Hardy & Vekemans (2002)
BATWING	POPG	Generation Bayesian inference, demographic history, population splits	STR, SNP	DOS, MacOS, Linux	Wilson & Rannala (2003)
COLONISE	POPG	Uses multilocus genotypes to study patterns of colonization events in population histories	MULT	Windows	Foll & Gaggiotti (2005)

(continued on next page)

Table 2 (continued)

Program	Discipline	Functions	Data type	Platform	Source
LAMARC	POPG	For estimating the historical demographics of a number of populations	DNA, SNP, STR	DOS, MacOS, Linux	Kuhner (2006)
MSVAR	POPG	For estimating the historical demographics of a number of populations (expansions and bottlenecks)	STR	DOS, Linux	Beaumont (1999)
Hickory	POPG	Software for analysis of geographic structure in genetic data. Estimates F-statistics from dominant or co-dominant marker data	AFLP, RAPD, MULT	Windows, Linux	Holsinger (1999)
BOTTLENECK	POPG	Detects recent effective population size reductions	MULT	Windows	Cornuet & Luikart (1996)
GENETREE	PHY	Phylogeny reconstruction	DNA	DOS	Griffiths & Tavaré (1994)
PARENTE	POPD	Paternity analysis of genetic data	STR, SNP	Windows	Cercueil et al. (2002)
CERVUS	POPD	Paternity analysis of genetic data	STR, SNP	Windows	Marshall et al. (1998)
PATRI	POPD	Paternity analysis of genetic data	STR, SNP, RFLP	Windows, Linux	Nielsen et al. (2001)
PROBMAX	POPD	Paternity analysis of genetic data	STR, SNP	DOS	Danzmann (1997)
BAlI-Phy	PHY	Phylogeny reconstruction	DNA		Redelings & Suchard (2005)
BayesPhylogenies	PHY	Phylogeny reconstruction	DNA		Page & Meade (2004)
BEAST	PHY	Phylogeny reconstruction	DNA		Drummond & Rambaut (2007)
BUCKy	PHY	Phylogeny reconstruction	DNA		Ané et al. (2007)
Mesquite	PHY, POPG, POPD	Phylogeny reconstruction	DNA		Maddison & Maddison (2011)
MrBayes	PHY, POPG	Phylogeny reconstruction	DNA		Huelsenbeck & Ronquist (2001)
PAML	PHY	Phylogeny reconstruction	DNA	Windows, Unix, Linux, MacOS	Yang (1997)
PAUP*	PHY	Phylogeny reconstruction	DNA	Windows, Unix, MacOS	Swofford (2002)
PHYLIP	PHY	Phylogeny reconstruction	DNA	Windows, DOS, MacOS	Felsenstein (1989)
TOPALi	PHY	Phylogeny reconstruction	DNA	Java	Milne et al. (2004)
Treefinder	PHY	Phylogeny reconstruction	DNA	Java	Jobb et al. (2004)

Increasingly sophisticated DNA-based technologies, especially sequencing methods (Table 1), in combination with new approaches to data analysis (Table 2; Excoffier & Heckel 2006) are now allowing researchers not only to infer genetic relationships among populations and species, but also to address previously intractable questions in both evolution and ecology (Table 3, Fig. 1). Topics now accessible to researchers include (1) rediscovery of species thought to be extinct (e.g. Steeves et al. 2010), (2) identification of speciation events driven by mechanisms previously thought to be improbable (e.g. Friesen et al. 2007a), (3) coevolutionary dynamics of seabirds, their parasites and their immunogenetics

(e.g. McCoy et al. 2005a, Bollmer et al. 2007), (4) understanding why some seabirds exist as highly differentiated populations while others exhibit little genetic differentiation across large distances (e.g. Morris-Pocock et al. 2010, Taylor et al. 2010a; see also review by Friesen et al. 2007b), (5) estimates of historical and contemporary population sizes and movements (e.g. Peery et al. 2008, Boessenkool et al. 2010), and (6) definition of population units for conservation (e.g. Friesen et al. 2005). Additionally, the developing field of ecological genomics (Dupont et al. 2007, Mitchell-Olds et al. 2008, Stapley et al. 2010) is enabling researchers to determine the genetic underpinning of morphological and physiological

Table 3. Potential advances in major sub-disciplines of marine ornithology due to advances in molecular genetics, in particular new sequencing technology and new analytical techniques

Sub-discipline	Advance
Phylogenetics	Genome-level phylogeny construction Larger marker sets Community phylogenetics
Population genetics	Larger marker sets Population genomics Gene expression studies Multispecies approaches Seascape genetic approaches
Hybridization	Larger marker sets Hybridization genomics Candidate genes for reproductive isolation Gene expression studies
Ecology	Demographic estimate accuracy Genetic basis of sexual traits and signaling Movement and local adaptation Genetics of adaptation
Conservation	Higher accuracy molecular assignments Functional differences between populations (gene expression) Disease screening, toxicology

variation in a number of taxa (e.g. McCracken et al. 2009, Sætre & Sæther 2010, Schluter et al. 2010) including seabirds (e.g. Baião et al. 2007).

Advances in other taxa are highlighting key areas of research relevant to the study of seabird evolution and ecology, particularly advances in avian model systems like the *Ficedula* flycatchers (Sætre & Sæther 2010). Thus, research is coming full-circle, with molecular studies providing insight into the genetic basis of morphological and behavioural characters that were once used to infer the genetic relationships among populations and species. As sequencing technology improves and costs decrease, the development of genomic resources for seabirds will also play an increasing role in seabird conservation (Frankham 2010). These advances are particularly timely, as many species are listed as threatened or endangered, global fish stocks continue to decline, and numerous, generally unknown, consequences of climate change are set to impact many seabirds (Grémillet & Boulinier 2009).

Here we review molecular genetic methods for studying seabird evolution and ecology, and then

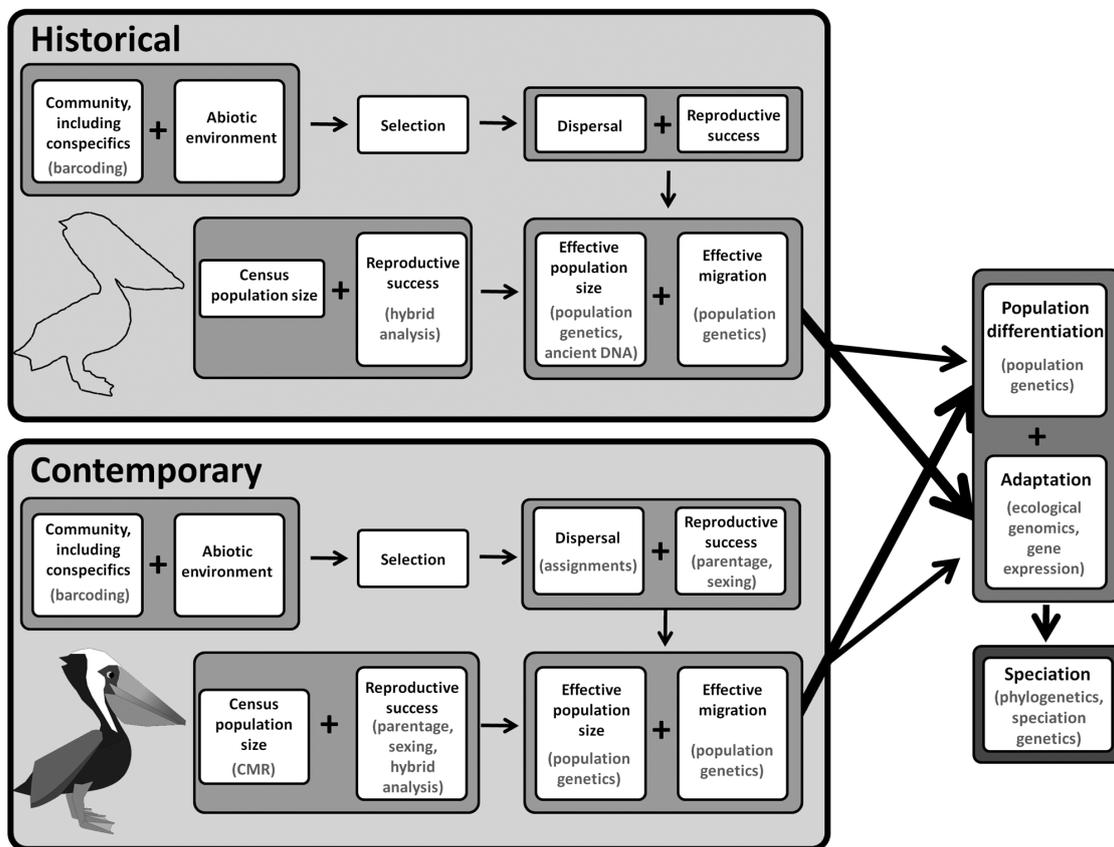


Fig. 1. Applications of molecular tools (in parentheses) for the study of seabird evolution and ecology. Arrow thickness indicates the presumed strength of causal relationships

Table 4. Types of molecular markers used in studies of evolution and ecology, the source of genetic variation, potential uses, and costs of analysis; devel.: development; screen.: screening; mod.: moderate

Marker type	Source of variation	Uses	Cost (devel.)	Cost (screen.)	Notes
mtDNA					
Restriction fragment length polymorphism (RFLP)	Lengths of fragments due to substitutions at restriction sites and in intervening sequences	Population genetics, phylogenetics	Low	Mod.	Superseded by direct sequencing (see following marker type)
Direct sequencing	Any mutation	Population genetics, hybridization, phylogenetics	Low	Low	Inferences restricted by unusual inheritance (matrilineal, no recombination), but high mutation rate makes it useful for recent and/or rapid evolution events
Nuclear DNA					
Minisatellite DNA (classical fingerprinting)	Numbers of repeat units within a fragment	Kinship, individual identification	Low	Mod.	Superseded by use of microsatellite DNA
Randomly amplified polymorphic DNA (RAPD)	Presence/absence of fragments due to substitutions in PCR priming sites and intervening sequences	Kinship, population genetics	Low	Low	Repeatability tends to be poor; some journals will not accept RAPD data; dominant inheritance complicates some inferences
Amplified fragment length polymorphism (AFLP)	Presence/absence of fragments due to substitutions in PCR priming sites, restriction sites, and intervening sequences	Population genetics, hybridization	Low	Low	Dominant inheritance complicates some inferences
Microsatellite DNA; intersimple sequence repeat (ISSR), short tandem repeat (STR)	Numbers of repeat units within a fragment	Kinship, population genetics, hybridization	Mod.	Low	Widely used, but becoming superseded by direct sequencing; vulnerable to homoplasy
Single nucleotide polymorphisms (SNPs)	Any mutation, but usually a substitution	Potential kinship via phylogenetics, but usually population genetics, hybridization	High at present	Low	Highly repeatable; source of variation is usually known
Direct sequencing	Any mutation, but usually a substitution	Population genetics and phylogenetics, but also kinship, hybridization	Low	Mod. at present	Highly repeatable; source of variation is usually known; screening cost is decreasing rapidly

draw examples from studies of phylogenetics, population genetics, hybridization and ecology (Table 3, Fig. 1). For each section, we outline traditional approaches, highlight examples using new molecular tools, and explore how traditional and molecular methods can be combined. We also give an overview of applications of molecular methods to conservation. This paper is not a comprehensive review of all molecular genetic work currently being conducted on seabirds; it is meant to highlight a few areas of research and to expose some ideas about future directions in seabird research.

MOLECULAR GENETIC METHODS

Information about an organism's evolution and ecology is retained in its DNA, and recent methods, especially those based on coalescent theory and Bayesian statistics, let us extract and understand this information. Numerous methods have been developed to assay DNA variation indirectly (several recent reviews, e.g. Baker 2000, Avise 2004; Table 4). One of the earliest methods to be developed was DNA fingerprinting, in which restriction enzymes (bacterial enzymes that recognize and cut short frag-

ments of DNA, typically 4 to 6 base pairs [bp] long) and agarose gel electrophoresis are used to screen individuals for variation in the number of repeats of a 20 to 25 bp unit. Restriction enzymes also can be used to detect mutations resulting in restriction fragment length polymorphisms (RFLPs) in short fragments of purified DNA such as mitochondrial DNA (mtDNA). These methods of mutation detection have been largely superseded by tools based on polymerase chain reaction (PCR), or DNA amplification. Common techniques include analysis of amplified fragment length polymorphisms (AFLPs), which uses a combination of restriction enzymes and PCR to detect sequence variation, and microsatellite analyses, which use specially designed PCR primers to amplify gene regions that tend to vary in the number of short (typically 2 to 6 bp) repeating units (Table 4).

Indirect methods of mutation detection such as AFLPs and microsatellite screening have the advantage that large numbers of individuals can be assayed quickly and relatively inexpensively. However, these tools are being replaced by direct sequencing of target genes, and analyses of single nucleotide polymorphisms (SNPs—single base pair replacements, insertions or deletions; Vignal et al. 2002, Coates et al. 2009). New sequencing methods are making SNP discovery and direct sequencing much easier and will greatly increase genetic tools for seabird studies (our Table 1; Tautz et al. 2010, Helyar et al. 2011). Financial costs of generating and mapping genomic level SNP datasets, or of identifying variable microsatellites are no longer prohibitive (see reviews by Hudson 2008, Shendure & Ji 2008, Tautz et al. 2010). Numerous studies of seabird evolution and ecology have used DNA sequencing to assess phylogenetic relationships (e.g. Pereira & Baker 2008, Jesus et al. 2009, Patterson et al. 2011), population differentiation (e.g. Morris-Pocock et al. 2008, Gómez-Díaz et al. 2009, Hailer et al. 2011; see review by Friesen et al. 2007b) and hybridization (e.g. Pacheco et al. 2002, Gay et al. 2007, 2008b, Taylor et al. 2010b), and to estimate population parameters such as migration rates or population sizes (e.g. Boessenkool et al. 2010). New sequencing methods now enable whole genomes to be sequenced within months (Hudson 2008, Mitchell-Olds et al. 2008, Shendure & Ji 2008, Thomson et al. 2010, Ekblom & Galindo 2011), although the full genome of a seabird species has yet to be assembled. The potential for genomic level seabird research to contribute to the larger picture in evolutionary biology is huge, particularly given recent findings regarding population divergence, hybridization, and speciation in a num-

ber of seabird species (see 'Population genetics and phylogeography' and 'Hybridization' below).

The drawbacks associated with using molecular genetic tools for studying ecology and evolution are rapidly being overcome (Hudson 2008). Previously, molecular genetic tools could be slow and expensive to adapt to particular research projects, and they often provided little information about functional variation or behaviour (but see e.g. Baião et al. 2007, McCracken et al. 2009). Until recently, assembling enough variable markers was also difficult for numerous seabird species: traditional, first generation sequencing methods suggested that variable markers are less common in birds than in other taxa (Baker 2000). New sequencing methods (summarized in Table 1; see reviews by Schuster 2008, Shendure & Ji 2008) are revolutionizing the study of ecology and evolution and have essentially removed the limitations outlined here (Hudson 2008, Tautz et al. 2010, Gardner et al. 2011); generating large, variable SNP or microsatellite datasets is now a possibility for any species. Additionally, the development of large genomic resources for seabirds will allow more directed studies that can target genes responsible for functional variation and possibly even the genetic underpinnings of behaviour, as has been done in other groups of organisms (Barrett 2010, Hubbard et al. 2010, Stapley et al. 2010, Toth et al. 2010).

Coalescent-based approaches that utilize Markov chain Monte Carlo (MCMC) methods and likelihood-based inference (implemented in Isolation with Migration: IM, Isolation with Migration Analytic: IMA, and Isolation with Migration Analytic 2: IMA2; see Table 2), and, more recently, approaches utilizing Approximate Bayesian Computation (ABC; Cornuet et al. 2008) (Table 2) are rapidly increasing our ability to robustly evaluate hypotheses about population divergence, and to infer population history (Hey 2006, Hey & Nielsen 2007, Csilléry et al. 2010, Lopes & Beaumont 2010, Pinho & Hey 2010). These approaches allow us to robustly estimate divergence times, historical and contemporary population sizes, rates of gene exchange during divergence, and to evaluate competing divergence scenario hypotheses. A number of seabird research groups are now utilizing larger (10+ markers) datasets and have recently developed analytical methods (Peucker et al. 2009, Lopes & Boessenkool 2010, Morris-Pocock et al. 2010, 2011, Taylor et al. 2011a, Welch et al. 2011).

DNA-based investigations of seabird evolution and ecology have the greatest power when combined with traditional methods, for example, by comparing variation in AFLPs with band returns to infer meta-

Table 5. Examples of studies in which molecular data were combined with other types of data to enable new insights into seabird evolution, ecology or conservation. AFLP: amplified fragment length polymorphism

Species	Molecular marker(s)	Additional tool(s)	Inference	Source
Cory's shearwater <i>Calonectris diomedea</i>	Microsatellites	Morphometrics, stable isotopes, trace metals	Individuals could be assigned to breeding colonies with 86% accuracy	Gómez-Díaz & González-Solís (2007)
Procellariiformes	Allozyme, DNA-DNA hybridization, DNA sequence	Morphology, behaviour, life history	More comprehensive inclusion of species than in previous phylogenies	Kennedy & Page (2002)
Phalacrocoracidae	mtDNA	Osteology	Cliques' of comparable characters suggest similar selective pressures	Holland et al. (2010)
Atlantic puffin <i>Fratercula arctica</i>	Allozymes	Morphometrics	Geographic variation in body size is due to environmental effects	Moen (1991)
Band-rumped storm-petrel <i>Oceanodroma castro</i>	mtDNA, microsatellites	Morphometrics, vocalizations	Divergence in morphology and vocalizations of seasonal populations precedes divergence in neutral molecular markers	Smith & Friesen (2007), Bolton et al. (2008), Deane (2011)
Black-legged kittiwake <i>Rissa tridactyla</i>	Microsatellites	Microsatellite variation in ticks	Kittiwakes prospecting among local colonies results in gene flow for kittiwakes but not ticks	McCoy et al. (2005b)
Wandering albatross <i>Diomedea exulans</i>	AFLP	Band returns	Populations are demographically but not genetically isolated	Milot et al. (2008)
Laysan albatross <i>Phoebastria immutabilis</i>	mtDNA, microsatellites	Band returns	Rare migration prevents genetic divergence of populations and enables colonization of new sites despite generally strong philopatry	Young (2010)
Cook's petrel <i>Pterodroma cookii</i>	mtDNA	Light-based data loggers, stable isotopes	Genetic divergence between colonies is ultimately the result of differences in non-breeding distributions and breeding times	Rayner et al. (2011)
Glaucous-winged gull <i>Larus occidentalis</i> and western gull <i>Larus glaucescens</i>	mtDNA, microsatellites	Plumage colouration Bare-part colouration	Restricted introgression of phenotypic traits compared to neutral markers – indicating selection on phenotypic traits and a role in isolation the species	Gay et al. (2008)

population dynamics (e.g. Milot et al. 2008), by combining morphometrics with isotope and genetic data to assign birds killed during winter to breeding populations for impact assessment (e.g. Gómez-Díaz & González-Solís 2007), or by using tracking data in concert with genetic estimates of gene flow in investigations of mechanisms of population differentiation (detailed in 'Ecology' below and in Table 5).

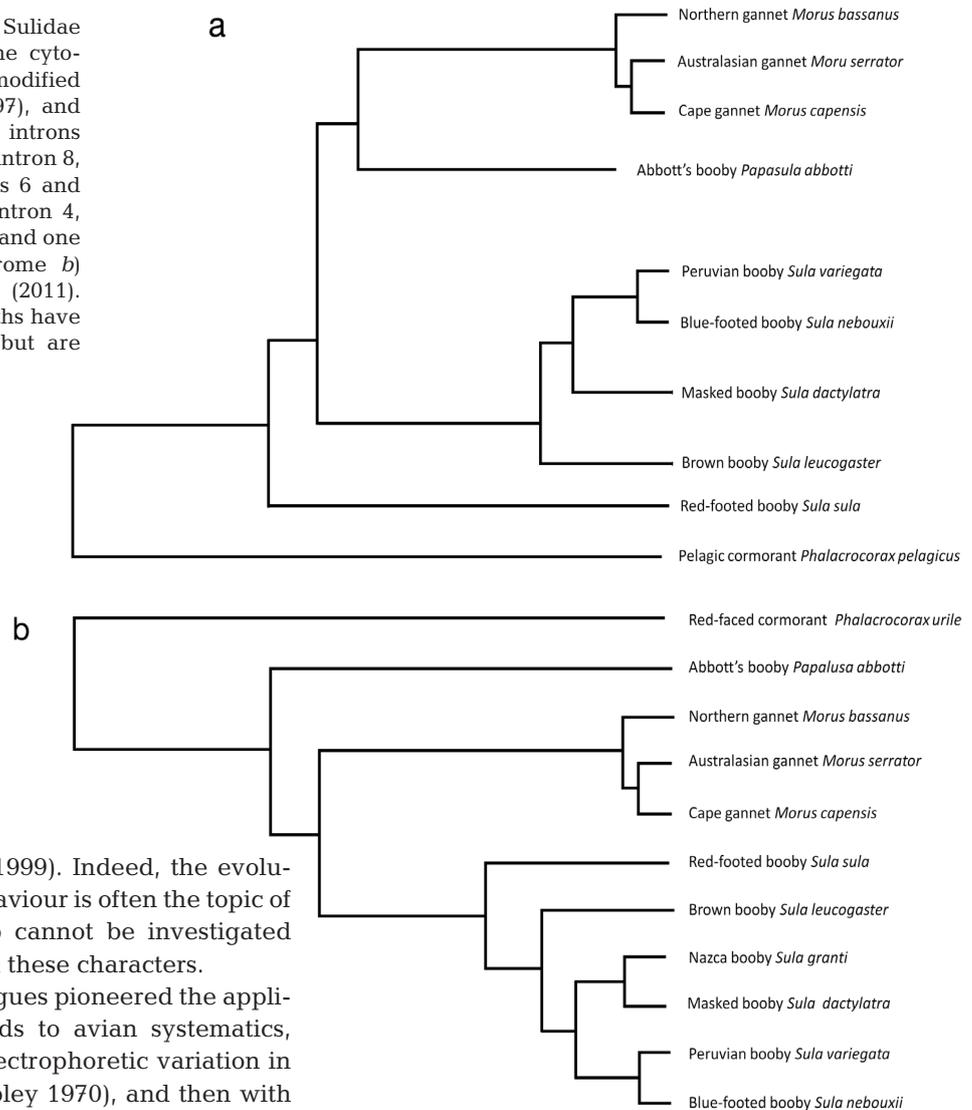
PHYLOGENETICS

Phylogenetics, the study of the evolutionary relationships among organisms, attempts to construct a tree that is representative of the true evolutionary history of a group of organisms. The tree can then aid investigations of taxonomy, character evolution (e.g. mating rituals, or clutch size) and diversification (e.g.

mechanisms of speciation; Avise 2004). However, unless the phylogenetic relationships among species are well resolved, evolutionary hypotheses cannot be reliably tested.

Traditional methods for phylogenetic reconstruction for seabirds rely on external and internal morphology, behaviour, and fossils. Studies based on these methods have provided significant insights into seabird evolution and ecology. For example, behavioural characters, morphology, osteology and allozymes have been used to evaluate evolutionary relationships within the Alcidae, Stercorariidae and Pelecaniformes (van Tets 1965, Strauch 1985, Watada et al. 1987, Kennedy et al. 1996, Chu et al. 2009, Smith 2010). However, the genetic basis for these characters is not usually known, and morphology and behaviour may differ between populations because of selection, phenotypic plasticity, or environmental

Fig. 2. Phylogenetic tree for the Sulidae (a) based on a fragment of the cytochrome *b* gene (gene tree) modified from Friesen & Anderson (1997), and (b) based on multiple nuclear introns (d-crystallin intron 7, a-enolase intron 8, ornithine decarboxylase introns 6 and 7, triosephosphate isomerase intron 4, and lipoprotein lipase intron 2) and one mitochondrial marker (cytochrome *b*) modified from Patterson et al. (2011). Node support and branch lengths have been removed for simplicity, but are high throughout



induction (Barbraud et al. 1999). Indeed, the evolution of morphology and behaviour is often the topic of interest in a study, and so cannot be investigated using phylogenies based on these characters.

Charles Sibley and colleagues pioneered the application of molecular methods to avian systematics, first with their studies of electrophoretic variation in egg white proteins (e.g. Sibley 1970), and then with their very comprehensive DNA–DNA hybridization work (Sibley & Ahlquist 1990). The first phylogenetic studies of seabirds based on DNA sequence data used single markers to infer evolutionary relationships. For example, Nunn et al. (1996) used sequence of the mitochondrial cytochrome *b* gene to generate a phylogeny of 14 albatross species, and Nunn & Stanley (1998) used cytochrome *b* sequences to evaluate evolutionary relationships among 85 species of procellariiform seabirds. Similarly, Friesen & Anderson (1997) used cytochrome *b* sequences to infer a phylogeny for the Sulidae (Fig. 2a), and Kennedy et al. (2005) used mitochondrial sequences to reconstruct evolutionary relationships among darters, cormorants and boobies.

Using a single genetic marker like albumin, or multiple markers from a non-recombining unit like mtDNA, for phylogenetic reconstruction can misrepresent the evolutionary relationships among organ-

isms, because single-gene trees do not necessarily represent the species' true history (Maddison 1997, Degnan & Rosenberg 2006, 2009). Recent advances in sequencing technology have allowed researchers to expand genetic datasets, and developments in phylogenetic analyses now allow researchers to estimate species trees using coalescent theory and Bayesian statistics (Table 2; e.g. Bayesian Estimation of the Species Tree [BEST], Liu 2008). These advances together led to the Tree of Life web project, which started in 1996, but has since expanded with these advances (Maddison & Maddison 1996).

Several recent phylogenetic studies of seabirds illustrate the utility of multilocus datasets. For example, Abbott's booby *Papasula abbotti* is ecologically, morphologically, and behaviourally distinct from other species in the Sulidae (Nelson 1978), and its placement within the family was unclear until recently.

Patterson et al. (2011) used 5 nuclear introns and a mitochondrial marker to estimate a species tree using BEST (Fig. 2b). They found that Abbott's booby was basal to all other members of the Sulidae and likely diverged from the group about 22 million years ago. Interestingly, the results were broadly congruent with van Tets (1965) phylogenetic reconstruction based on behaviour.

In a similar study, Kennedy et al. (2009) used multiple unlinked loci to evaluate the phylogenetic position of the Galapagos cormorant *Phalacrocorax harrisi*, an ecologically and morphologically unique member of the Phalacrocoracidae. Although this species is the only phalacrocoracid that has lost the ability to fly and was previously placed in its own genus, results from Kennedy et al. (2009) indicate that the Galapagos cormorant is most closely related to 2 species that are common and broadly distributed in North America: the double-crested cormorant *P. auritus* and the neotropic cormorant *P. brasilianus*.

Kennedy & Page's (2002) procellariiform phylogeny provides an excellent example of synergy through the combination of different analytical approaches. These authors used 7 incomplete phylogenies based on behaviour, DNA–DNA hybridization, isozymes, life history, morphology, and DNA sequence data to generate a supertree for the Procellariiformes. In another example, Holland et al. (2010) compared strongly supported but incongruent trees based on osteological versus molecular data for the cormorants and shags. Their study found 'cliques' of compatible morphological characters, suggesting groups of taxa under similar selective pressures. Although Cohen et al. (1997) provided a discussion of difficulties in combining morphological, behavioural, and genetic data to construct phylogenies, future researchers should attempt to construct phylogenies based on multiple nuclear and mitochondrial loci and, ideally, combine traditional approaches in phylogeny construction. Studies such as these are needed for other groups of seabirds, and will aid future investigations of both character evolution and mechanisms of diversification in seabirds.

POPULATION GENETICS AND PHYLOGEOGRAPHY

Understanding population genetic structure (the extent to which local populations differ genetically) helps researchers understand many aspects of evolution and ecology (Fig. 1). For example, some seabird species exhibit dramatic geographic variation in morphology and behaviour while others do not (e.g.

Storer 1952, Ainley 1980, Power & Ainley 1986, del Hoyo et al. 1992, 1996, Baião et al. 2007). Examining population genetic structure in these species can help determine the adaptive significance (if any) of geographic variation in life history, morphology, physiology and behaviour; how, where and why the variation originated; how populations are connected or what prevents them from being connected; and how species multiply. Furthermore, population differentiation is the first step towards speciation in most speciation models (Coyne & Orr 2004). Consequently, many population genetic studies explore the evolutionary histories of seabirds to understand the speciation history. A subfield of population genetics, phylogeography, studies the correspondence between phylogeny and geography, and can provide insights into evolution and ecology (Avise et al. 1987, Avise 1994).

Studies of morphology, physiology, and behaviour help elucidate population genetic structure. For example, geographic variation in plumage and morphometrics is more extensive in *Cepphus* spp. (guillemots) than in *Uria* spp. (murre). Storer (1952) proposed that this difference is a result of differences in dispersal: guillemots nest in small colonies distributed linearly along coastlines and probably do not disperse far from colonies during the non-breeding season, whereas murre nest in a small number of large colonies, and generally migrate seasonally. However, as in phylogenetic studies, the genetic basis of morphological and behavioural characters is not usually known (but see Baião et al. 2007): even if individuals or populations are morphologically similar, they may differ genetically (e.g. Friesen et al. 1996a, Morris-Pocock et al. 2008, Hailer et al. 2011).

Protein electrophoresis also has provided useful insights into population structure and evolution (Baker 2000, Avise 2004). For example, protein variation suggests that thick-billed murre *Uria lomvia* form kin groups within colonies (Friesen et al. 1996b). In contrast, Moen (1991) found little geographic variation in allozymes among Atlantic puffins from throughout the northeast Atlantic, despite significant variation in body size. Protein electrophoresis yields data rapidly and is inexpensive to conduct; however, birds must be captured and sometimes sacrificed, proteins degrade easily, protein variability is often insufficient to be useful, and some variation is under selection (potentially violating assumptions of neutral mutation theory) and thus may not reflect true evolutionary history (Baker 2000).

Initial uses of DNA to study seabird population differentiation focused on mtDNA (e.g. Pitocchelli et al. 1995), because its unusual mode of inheritance and

relatively high mutation rate make it sensitive to changes in population size and migration rate (Wilson et al. 1985, Baker 2000, Avise 2004, Eda et al. 2008, Rains et al. 2011). Increasingly however, studies of population differentiation, phylogeography, and speciation are using large multilocus datasets of SNPs, microsatellites and sequence data (Table 3) to provide a more complete picture of contemporary versus historical population sizes and dispersal rates (Fig. 1; e.g. Peery et al. 2010, Hailer et al. 2011). For example, Techow et al. (2010) used cytochrome *b* and 6 microsatellite loci to examine the phylogeography and speciation history of giant petrels *Macronectes* spp. Their analyses revealed a complex history that involved population fragmentation, periods of population expansion, and secondary contact.

Population genetic analyses of seabirds have also benefitted from multispecies approaches, at both the seabird (i.e. analyzing multiple species of seabirds) and organismal (i.e. analyzing multiple species of seabird and other non-avian taxa) levels (e.g. parasites). Parasites are useful for studying long-lived species such as seabirds (Nieberding & Olivieri 2007), and multispecies approaches utilizing parasites have increased our understanding of the constraints on the genetic structure and the movements of seabirds (e.g. McCoy et al. 2005b, Gómez-Díaz et al. 2007). McCoy et al. (2005b) compared population genetic structure in black-legged kittiwakes *Rissa tridactyla* with one of their ectoparasites (the tick *Ixodes uriae*). Population genetic structure was much stronger in the ticks than in the kittiwakes, and, given the life history of the ticks, McCoy et al. (2005b) inferred that local movements of kittiwakes during the breeding season result in gene flow for the kittiwakes but not the ticks. Similarly, multispecies approaches at the seabird level have allowed researchers to detect common factors responsible for the presence or absence of population structure (important for making predictions for unstudied species, or informing conservation decisions) (reviewed in Friesen et al. 2007b), and the potential for multispecies approaches is very high given the aforementioned advances in sequencing technology and data analysis techniques.

The strongest population genetic and phylogeographic seabird studies are synergistic and combine molecular data with demographic data, morphology, and/or behaviour to elucidate mechanisms of population differentiation in seabirds. Our goal should be combining this approach with larger, more robust genetic datasets, as some researchers are doing already—though not yet at the scale made possible by

new sequencing methods. For example, by comparing variation in AFLPs with band returns, Milot et al. (2008) showed that populations of wandering albatrosses *Diomedea exulans* are demographically but not genetically isolated. In another synergistic study, Rayner et al. (2011) used geolocator-based tracking, isotopes, and DNA-based methods to examine contemporary and historical populations of Cook's petrel *Pterodroma cookii*, reporting that genetically distinct populations are segregated during the non-breeding season. They suggested that habitat specialization during the non-breeding season may lead to breeding asynchrony, which may restrict gene flow between the populations in conjunction with philopatry (Rayner et al. 2011).

Further studies such as these are needed to understand mechanisms of diversification and adaptation in seabirds, especially in relation to anthropogenic disturbances such as climate change. We see an opportunity for seabird evolutionary biologists and ecologists to move from population genetic to population genomic level studies (Hudson 2008, Tautz et al. 2010, and see Viner & Mitchell 2010 entire issue). Larger, more robust genetic datasets, combined with the ecological knowledge of many seabird researchers, will make population genomic studies more informative as they relate to the mechanisms that generate seabird diversity, than present population genetic studies. The possibility of expanding analyses to examine genes responsible for divergence or to explore differences in gene expression (transcriptomics) between closely related species (e.g. physiological differences, Hedgecock et al. 2007), should be pursued by seabird researchers, especially given the extreme physiology of this taxonomic group and the potential importance of physiological differences in relation to divergence events. These avenues of inquiry will contribute more generally to our understanding of population divergence and speciation and be valuable to the broader evolutionary biology community.

Recently, the term 'seascape genetics' has been used to describe population genetic studies of marine organisms that examine how environmental variables (e.g. ocean currents, ocean productivity) influence differentiation (e.g. Skillings et al. 2011, Amaral et al. 2012, see review by Selkoe et al. 2008; the terrestrial equivalent, 'landscape genetics', is reviewed in Sork & Waits 2010; see also Special issue on landscape genetics Vines & Mitchell 2010). Although no seabird studies to date have explicitly used the term 'seascape genetics', seabird researchers are generally cognizant of the effects of oceanic environmental variables such as fronts, eddies, and climatic phe-

nomena like El Niño on seabird population genetics (Steeves et al. 2003, 2005a,b, Morris-Pocock et al. 2008, Hailer et al. 2011, Rayner et al. 2011, Taylor et al. 2011a). Future population genetic studies of seabirds would benefit from including these variables explicitly, to ensure seabird population genetic studies remain on par with studies of other marine organisms.

HYBRIDIZATION

Hybridization can be either a creative or a destructive force in evolution (Harrison 1993, Allendorf et al. 2001). Global rates of hybridization are increasing and have contributed to species extinctions in a number of taxonomic groups, but have led to the establishment of new species in others (Brumfield 2010). Regardless of the outcome, documenting hybridization and understanding how it affects seabirds is an important aspect of seabird ecology and evolution, but is potentially erroneous without data from multiple, unlinked genetic markers. Hybridization also has conservation implications. For example, as climate change alters marine habitats a number of temperate seabird species may expand their ranges towards the poles. The evolutionary and conservation implications of these range shifts are unknown; however, studies of other species suggest that range shifts may increase hybridization rates (Kelly et al. 2010).

Traditional studies of hybridization in seabirds involved reports of birds with unusual plumage patterns (e.g. Cairns & DeYoung 1981), or geographic variation in morphology and/or allozyme allele frequencies (e.g. Bell 1996). These studies are complicated by the fact that back-cross hybrids, and even F1 hybrids, can be difficult to distinguish from parental species based on morphology (e.g. Friesen et al. 1993). Researchers now have the ability to generate sufficiently large genetic datasets (see above; Table 1) and possess appropriate computer programs to characterize hybridization between species thoroughly (see 'Molecular genetic methods' above and the following paragraph; Table 2; Gay et al. 2007, 2008, 2009, Taylor et al. 2010a, 2011b).

Although hybridization is generally considered rare in seabirds, an increasing number of studies are documenting hybridization and interspecific gene flow using multilocus cline and coalescent-based analyses (e.g. Reinhardt et al. 1997, Gay et al. 2007, 2008, Brown et al. 2010, Carneiro et al. 2010, Taylor et al. 2010b, 2011b, Taylor 2011). Molecular markers can also aid in determining whether an individual has

hybrid ancestry, how many generations ago hybridization occurred, and which species was maternal vs. paternal. Several recent studies of seabird hybridization illustrate the utility of using molecular markers.

An example comes from Taylor's (2011) analysis of hybridization between blue-footed boobies *Sula nebouxii* and Peruvian boobies *Sula variegata*. Taylor (2011) used a multilocus data set and cline analysis to examine introgression and to characterize the hybrid zone. He found that blue-footed and Peruvian boobies hybridize, but that pre- and postzygotic barriers are well established, particularly in light of the recent divergence of this species pair (Patterson et al. 2010). Additionally, Taylor et al. (2010b) and Taylor (2011) used a multilocus dataset to determine that morphologically aberrant individuals within the hybrid zone are most likely first generation hybrids and that the majority of hybridization between blue-footed and Peruvian boobies takes place between female Peruvian boobies and male blue-footed boobies, with subsequent backcrossing primarily between female hybrids and male blue-footed boobies. Finally, Taylor (2011) evaluated the likelihood that this species pair diverged from their common ancestor with gene flow, and found that a divergence with gene flow scenario was more likely than divergence without gene flow, using the isolation with migration model implemented in IMA. A dataset generated using new sequencing methods would significantly improve the ability of any study similar to the one completed by Taylor (2011) to explore the evolutionary history of a species pair, particularly given the low level of genomic divergence detected in this other studies using the current datasets.

A synergistic investigation of seabird hybridization was conducted successfully by Gay et al. (2007, 2008, 2009). They used multiple unlinked genetic markers, morphological characters, and cline analyses to determine the nature of the hybrid zone for each species pair. White-headed gulls appear more prone to hybridization than most other seabirds, possibly because of recent divergence and subsequent secondary contact between morphologically and behaviourally similar species (discussed in Gay et al. 2007, 2008, 2009). Gay and colleagues were able to document introgression at neutral loci and subsequently compared the rate of introgression of neutral loci with phenotypic introgression. They inferred that selection against phenotypic introgression is strong in both hybrid zones, and that sexual selection may be important for maintaining the species barriers.

Seabird researchers interested in hybridization should take a synergistic approach whenever possi-

ble. Future investigations of hybridization in seabirds will benefit from larger, genome-spanning datasets, and approaches that target functional differences between hybridizing species. As with hybrid-zone research on other non-model organisms, seabird researchers should seek to determine the genetic regions, and ultimately genes, responsible for reproductive isolation.

ECOLOGY

Tracking population fluctuations and dispersal patterns is important for understanding how species respond to natural and anthropogenic stressors. Such information is critical both for understanding ecology and evolution, and for conservation (see next section). Capture-mark-recapture (CMR) studies (banding or ringing) help to clarify population relationships and dynamics, and the technology required for banding studies is relatively cheap (e.g. Nisbet 1989, Wooller et al. 1992, Wanless et al. 2007). For example, a long-term banding study of blue-footed boobies *Sula nebouxii* on Isla Isabel, Mexico, revealed that individuals are more faithful to their first nest site than their natal site and may have a 'dispersal phenotype' (Kim et al. 2007). This behaviour is not shared by other members of the Sulidae and would not have been predicted based on long-term banding of the Nazca booby *S. granti*, a close relative of the blue-footed booby, in the Galapagos (Huyvaert & Anderson 2004). Banding has also revealed long distance movement between seabird colonies and a general trend of natal philopatry in many seabirds (Wooller et al. 1992). More recently, GPS tracking has provided previously unattainable high-resolution data on seasonal and daily movements (Grémillet & Boulinier 2009, Wilson & Vandenabeele 2012, this Theme Section). Unlike banding, tracking studies require expensive equipment and have only recently become common in the literature. Both banding and tracking require extensive effort and are virtually impossible for secretive species.

DNA-based methods allow researchers to assign individuals to genetic populations and to estimate population parameters like dispersal and population size in non-invasive ways (e.g. using moulted feathers, fecal samples, scavenged carcasses). Most notably, molecular assignments can provide insights into movements with minimal disturbance to individuals. Molecular assignments involve using an individual's multilocus genotype to infer its origin (Manel et al. 2005). If an individual's genotype clusters with high

probability with individuals from a different sampling site, that individual is likely a migrant. Molecular assignments are most successful when populations are significantly genetically differentiated (i.e. $F_{ST} > 0.1$) but can be used even when populations are only weakly differentiated (i.e. $F_{ST} < 0.05$) (e.g. Peery et al. 2008, Hall et al. 2009).

Although using molecular assignments to identify dispersal events can require large numbers of samples from many populations and so can be logistically challenging, this method is increasingly common in the seabird literature (e.g. Boessenkool et al. 2009, Schlosser et al. 2009) and often provides insights unavailable from banding data. Rare dispersal events, which are difficult to detect if band returns are low, may be captured in the multilocus genotype of an individual. For example, Boessenkool et al. (2009) used multilocus assignments in their study of yellow-eyed penguins *Megadyptes antipodes* and found evidence of rare migration events that would not likely have been detected otherwise. Similarly, Hall et al. (2009) used molecular assignments to characterize dispersal patterns in marbled murrelets *Brachyramphus marmoratus* in central California, and Faria et al. (2010) used both molecular assignments and methods based on coalescent theory to infer source-sink dynamics in South American terns *Sterna hirundinacea*. In an innovative modification of molecular assignments known as parent-offspring dyad analysis, Peery et al. (2008) determined that the central California population of marbled murrelets is a demographic sink.

Application of molecular assignments to species for which extensive CMR and/or tracking data exist is an especially promising but largely unused method to elucidate the connection between evolution and ecology, e.g. by disentangling the relative effects of historical versus contemporary seabird movements on local adaptation, or inferring the effect of non-breeding distributions on population differentiation and speciation (Friesen et al. 2007b). For example, band returns indicate that Laysan albatrosses *Phoebastria immutabilis* are strongly philopatric to natal breeding colonies, making them highly vulnerable to anthropogenic disturbance. However, geographic variation in mtDNA and microsatellites suggests that Laysan albatrosses have sufficient dispersal over longer timeframes to maintain population genetic variation and enable rapid colonization (Milot et al. 2008, Young 2010, Rayner et al. 2011). We could not find any studies that combine data from molecular markers and satellite tracking, highlighting the need for research using this combination of methods.

DNA-based methods allow seabird researchers to census populations through non-invasive molecular 'mark-and-recapture'. Rudnick et al. (2008) presented the method in their study of non-breeding imperial eagles *Aquila heliaca* in Kazakhstan. Direct counts of eagles in the Naurzum Zapovednik suggested that fewer than 32 individuals used the area; however, analysis of moulted feathers using the program MARK indicated a roosting population of 308 individuals (Rudnick et al. 2008). Although this approach has yet to be conducted on seabirds, the utility of the method and the nature of seabird roosting areas (numerous feathers available for sampling) suggest that it may be useful. Population sizes and changes in size can also be estimated indirectly by applying genetic theory to molecular variation. For example, deviations in heterozygosities from values predicted at mutation-drift equilibrium can be used to infer recent population increases or declines using programs such as BOTTLENECK (Cornuet & Luikart 1996) and FLUCTUATE (Kuhner et al. 1998). Using this approach, Birt et al. (2012) found support for census results suggesting that Xantus's murrelets *Synthliboramphus hypoleucus hypoleucus* on Guadalupe Island, Mexico, have declined over the last century, and by comparing variation in contemporary and historical samples of yellow-eyed penguins, Boessenkool et al. (2010) estimated an effective size an order of magnitude lower than the census size for the southern population than for the northern population.

Additionally, DNA-based methods allow the evaluation of sex-specific questions, since the sex of a bird can now be determined with relative ease, using only a blood or feather sample. Given that many seabirds are sexually monomorphic, and that morphological details are not always recorded during sample collection, molecular sexing of seabirds is invaluable. Indeed, a search of the literature highlights the prevalence of this method in recent years: over 400 papers on seabird ecology and evolution have used molecular sexing since 2002. The questions addressed by these studies are highly diverse and include investigations of physiological ecology (e.g. Giudici et al. 2010), breeding investment and foraging (e.g. Torres et al. 2011), sociochemical compounds (e.g. Leclaire et al. 2012), sex-specific foraging behaviour (e.g. Weimerskirch et al. 2006), disease transmission (e.g. Wojczulanis-Jakubas et al. 2011), and the utility of morphology or behaviour in sex determination of wild birds (Zavalaga et al. 2009).

Finally, DNA-based methods are useful for investigations of parentage and kinship in seabirds.

Microsatellites and SNPs can be used to generate genealogies, to detect extra pair paternity, and to assign offspring to adults when sampling is sufficient. The literature on this topic is vast and new sequencing methods will aid the development of the variable markers required for paternity assignment (e.g. Baião & Parker 2009, Wojczulanis-Jakubas et al. 2009, Huyvaert & Parker 2010).

CONSERVATION

In addition to providing managers with better understanding of seabird evolution and ecology, molecular genetic methods can aid conservation (recent reviews: Allendorf & Luikart 2007, Frankham 2010, Haig et al. 2011). Probably the most important direct contribution at present involves defining units of conservation. To be most effective, species-level conservation must be based on sound taxonomy: failure to recognize reproductively isolated species can result in loss of diversity. Taxonomy was traditionally based on morphology and ecology, but recent studies have unveiled several examples of mistaken taxonomy such as 'cryptic' species that are similar in appearance and ecology but that represent reproductively isolated, sometimes ancient lineages (e.g. Friesen et al. 1996a, Bolton et al. 2008, Rayner et al. 2011). The New Zealand storm petrel *Oceanites maorianus* provides an example. Presumed extinct for 150 years, birds similar to the New Zealand storm petrel were documented off New Zealand in 2003. Analyses of variation in both mtDNA and a nuclear intron confirmed that these birds belonged to the same taxon as the type specimen and that represents a distinct species (Robertson et al. 2011).

In addition, most conservation legislation recognizes phenotypically, ecologically and/or genetically distinct populations of vertebrate species as worthy of protection (e.g. 'distinct population segments' under the US Endangered Species Act; 'diagnosable units' under the Canadian Species at Risk Act). The rationale is that loss of these populations would result in loss of some of the species' genetic resources, including potential local adaptations, and that such populations may be genetically and demographically isolated from each other. Distinct populations are often termed evolutionarily significant units (ESUs), and may be identified from unique molecular, morphological, behavioural or ecological characteristics (e.g. Moritz 1994, Fay & Nammack 1996). Marbled murrelets illustrate the importance of defining distinct population segments. Murrelets breeding in

Washington, Oregon and California were originally treated as a threatened population segment, distinct from British Columbia and Alaska birds (USFWS 1992), but when molecular studies failed to find genetic differences between these regions (Friesen et al. 2005), distinct population status was removed; the viability of the species is now being evaluated as a single unit.

Additionally, local populations may be demographically but not genetically isolated from each other. Such populations may have sufficient dispersal to prevent genetic differentiation and local adaptation, but not enough to function as a single demographic unit. Thus, birth rates, death rates, and other demographic parameters may differ. Such populations are often referred to as management units (MUs) and may be recognized from differences in allele frequencies at molecular markers (e.g. Moritz 1994). Results from molecular studies often provide insights not expected from ecological data. For example, the high dispersal ability of the yellow-eyed penguin *Megadyptes antipodes* suggests that the species represents a single demographic unit, but differences in frequencies of mitochondrial haplotypes and microsatellite alleles, and results of assignment tests indicate that birds breeding on New Zealand's South Island are demographically isolated from those elsewhere and should be treated as a separate MU (Boessenkool et al. 2009).

Molecular markers can also aid conservation. For example, estimates of effective population size are needed both to assign species to conservation categories and to develop recovery plans; these estimates are often one or more orders of magnitude lower than census sizes (e.g. yellow-eyed penguins; Boessenkool et al. 2010). Molecular markers can identify hybrid individuals and their descendants (see 'Hybridization' above), and so aid recovery plans by allowing managers to exclude hybrid individuals from captive breeding programs, or from the population if maintenance of genetically pure populations is a conservation goal (e.g. Haig et al. 2004). Population markers can also help to assess the population-specific effects of anthropogenic disturbances (e.g. oil spills, Riffaut et al. 2005; fisheries by-catch, Walsh & Edwards 2005). Gómez-Díaz & González-Solís (2007) provide an example of the synergistic application of molecular genetics with other tools for impact assessment. They were able to assign individual *Calonectris* spp. shearwaters that had been caught in long-line fisheries in the Mediterranean to their population of origin with 86 to 100% accuracy using morphometrics, stable isotopes, trace metals, and

multilocus genotypes. As DNA technology becomes more accessible, many new applications to conservation are emerging (e.g. disease screening, Ishak et al. 2008; toxicology, Haig et al. 2011).

CONCLUSIONS

Studies of phylogenetics, population genetics, hybridization, speciation and ecology in seabirds have improved our understanding of evolution, ecology and conservation, and this can only grow as more seabird research groups incorporate DNA-based analyses including new sequencing technologies into their investigations. Seabird research in evolutionary biology has the potential to contribute in a significant way to evolutionary biology, given the ecological knowledge of seabird researchers, advances in sequencing technology and data analysis, and the fact that collection of a sufficient number of samples for robust genetic analyses is usually a possibility for colonially nesting organisms.

DNA-based methods are providing seabird researchers with tools to address previously intractable questions about seabird evolution and ecology. Although the majority of new genetic resources were not developed specifically for seabirds, advances in sequencing technology and analytical methods are resulting in improved genetic and genomic resources for seabirds within the capabilities of many seabird research groups. Many seabird researchers do not include genetic data in their studies; however genetics can provide versatile tools to aid in studying evolution, ecology and conservation of seabirds (Table 3, Fig. 1). Some molecular methods are still being optimized, but the potential applications of molecular markers will likely increase as DNA-marker generation becomes cheaper and faster, and methods of data interpretation become increasingly sophisticated. Results of studies that combine molecular markers with other tools will be especially useful to understanding the evolution, ecology and conservation not only of seabirds but also of other highly mobile organisms in a rapidly changing world.

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