The use of molecular and observational data to infer the structuring of bottlenose dolphin populations

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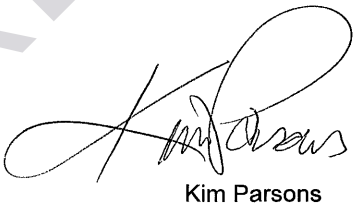
Tursiops truncatus

Abaco, Bahamas

DECLARATION

I declare that I composed this thesis and that it has not been submitted in any previous application for a degree. All quotations have been distinguished by quotation marks and sources of information have been acknowledged. All of the work presented in this thesis is my own, any data collected with the assistance of other workers is specifically acknowledged in the text and in the preface to each chapter.



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ABSTRACT

Knowledge of the structuring of natural populations is important for understanding both evolutionary processes and population ecology, and for supporting management decisions. Conventional methods of direct observation often suffer from a lack of resolution, particularly when studying mobile animals in a marine environment. In this study, I combined direct observation with indirect molecular genetic approaches to infer the social and population structure of coastal (inshore) bottlenose dolphins, *Tursiops truncatus*.

Genetic diversity and structure of bottlenose dolphins around the UK and Ireland was examined using tissue samples from stranded dolphins and incidental fisheries by-catch. Mitochondrial DNA (mtDNA) sequence data indicated significant subdivision among four main sample regions (NE Scotland, Wales, NW Scotland and Ireland). Genetic divergence between NE and NW Scotland populations, and low genetic diversity within the NE Scotland population, provide further support for the precautionary approach currently applied to the management of this population.

Inference from both mtDNA and nuclear microsatellite genetic markers, and direct observational data were used to examine the social and population structure of bottlenose dolphins in the NE Bahamas. Novel strategies for collecting genetic samples (remote biopsy and faecal sampling) from free-ranging dolphins were developed and validated, enabling an individual-based analysis of population subdivision. Patterns of individual associations in two contrasting habitats indicated that environmental pressures affect dolphin grouping patterns; with a genetic basis for social affiliations occurring only where predation pressures are low. Nonetheless, a particularly notable feature of the social structure in both habitats was the persistence of stable alliances among maternally related males. At the population level, the significant degree of genetic structuring revealed among three sampled regions on Little Bahama Bank, supported the high degree of site fidelity suggested by individual-based photo-identification data. Contrary to the patterns of male dispersal and female philopatry common among both mammals and bottlenose dolphins, sex-specific patterns of genetic differentiation inferred from both mtDNA and microsatellite markers were indicative of female-mediated gene flow.

This study provides novel insight into the factors governing the patterns of structuring within populations of highly mobile small cetaceans, and demonstrates the value of integrating both direct (field-based) and indirect (molecular genetic) data in the study of free-ranging animals.

CONTENTS

ABBREVIATIONS

µ	micro
°C	degrees Celsius
A	adenosine
ATP	adenosine 5'-triphosphate
bp	nucleotide base pairs
C	cytosine
CTAB	hexadecyltrimethylammonium
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic acid
EDTA	ethylenediamine tetraacetic acid
G	guanosine
GITC	guanidine thiocyanate
HWI	half weight index
km	kilometre
L	litre
m	milli-
M	molar
PCR	polymerase chain reaction
SE	standard error of mean
T	thymidine
Tris	tris[hydroxymethyl]aminomethane

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PREFACE

The data chapters of this thesis (Chapters Two through Eight) have been presented as a series of full papers and short communications. Therefore, some repetition between chapters, particularly in the methods, is unavoidable. Most of the chapters have multiple authors: Paul Thompson and Les Noble supervised the research which forms the basis of this dissertation; Paul Thompson also provided data on the location of bottlenose dolphin stranding events (Chapter Two, Figure 1b) and essential background information on the field studies, and conservation concerns regarding the NE Scotland bottlenose dolphins; Diane Claridge and Ken Balcomb are the co-founders and directors of Bahamas Marine Mammal Survey, and have been involved in the conception of this project and all aspects of field work; John Dallas provided valuable technical advice and expertise on extracting DNA from mammal faeces; Denise Herzing provided genetic samples from the dolphins at White Sand Ridge; John Durban collaborated on all aspects of the field work, genetic sample collection, and planning of this study. All collaborators contributed valuable comments on earlier drafts of the manuscripts that are the data chapters. I conducted all field and laboratory work, performed all data analyses and wrote all the manuscripts.

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Chapter One

General Introduction

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CHAPTER ONE

General Introduction

The structuring of natural populations

The distribution of genetic variation among subpopulations is governed by a population's mating system, as well as historic and contemporary dispersal patterns (Chesser 1991a, b). Together, these factors create population structuring through the opposing forces of genetic drift and selection within, and restricted gene flow among subpopulations (Rosel *et al.* 1995). Understanding the structuring of natural populations is of interest to evolutionary biologists, ecologists, conservationists and wildlife managers alike.

Because population structure is an expression of the distribution of genetic variation, and it is recognised that the structuring of natural populations plays an active role in both microevolution and speciation (Wright 1932, 1965), resolving these patterns is important for furthering our understanding of both evolutionary processes and population ecology. From an applied perspective, understanding rates of genetic exchange between subpopulations is a key element in identifying biologically meaningful population subdivisions and defining management stocks or conservation units (Baker & Palumbi 1997; Rosel *et al.* 1999). Furthermore, the structuring of populations plays a critical role in determining the degree and direction of gene flow, inbreeding, disease transmission, dispersal and ultimately a population's ability to adapt to environmental disturbance. Hence, population

genetic studies are a priority where the conservation and management of a species is an issue (Templeton 1987; Harrison & Hastings 1996; O'Corry-Crowe *et al.* 1997; Whitehead *et al.* 1998).

Assessing population structure and differentiation has become the focus of numerous studies spanning the continuum of nearly all taxonomic divisions (e.g. Chesser 1983; Bakke *et al.* 1996; Good *et al.* 1997; McConnell *et al.* 1997; Andersen *et al.* 1998; Arens *et al.* 1998; Barratt *et al.* 1999; Burg *et al.* 1999; Segesser *et al.* 1999; Avise *et al.* 2000; Cooper & Lenski 2000; Fernando *et al.* 2000; Gutierrez-Espeleta *et al.* 2000; Piertney *et al.* 2000; Pope *et al.* 2000; Dalebout *et al.* 2001; Girman *et al.* 2001). Natural populations inhabit a variety of ecological and environmental contexts that effectively determine both their distribution in space and time, and the apportioning of individuals into smaller subpopulation units. While the segregation and dispersal of populations of terrestrial vertebrates is often influenced by physical geographic characteristics (e.g. Talbot & Shields 1996; Von Segesser *et al.* 1999; Pope *et al.* 2000), the marine environment lacks obvious barriers to gene flow (Avise 1998; Waples 1998). Many marine vertebrates are highly mobile and undergo extensive migrations as adults (e.g. marine turtles; Nichols *et al.* 2000, and humpback whales; Stone *et al.* 1990). However, this high potential for dispersal does not necessarily translate into high levels of gene flow (Palumbi 1996), and a lack of understanding of these movement patterns constrains attempts to define stocks to be conserved or managed.

Among species of marine mammals, both patterns of speciation and causes of genetic differentiation are poorly understood. Defining structure in

populations of highly vagile marine mammals is further complicated by the knowledge that geographic distance is not necessarily well correlated with genetic distance (Hoelzel 1998). Moreover, marine mammals exhibit a diverse array of patterns of population differentiation with some geographically coincidental populations being genetically divergent (e.g. sympatric killer whale population; Hoelzel & Dover 1991; Hoelzel *et al.* 1998a), and other geographically distant populations being genetically similar (e.g. fin whales; reviewed in Hoelzel 1998).

Although little is known about what limits genetic exchange in cetaceans (whales, dolphins and porpoises), genetic differentiation among populations has been attributed to both resource specialisation and social organisation (Hoelzel and Dover 1991; Palsboll *et al.* 1995; O'Corry-Crowe *et al.* 1997; Hoelzel 1998; Hoelzel *et al.* 1998b; Whitehead *et al.* 1998). Intraspecific resource specialisation, such as foraging or habitat specialisations, can act as an important mechanism in limiting gene flow and causing population differentiation between both sympatric and parapatric populations (Hoelzel 1998). Moreover, the complexity and diversity of social structures that have been described for many cetacean species (Connor 2000) suggests that this may play a significant role in defining subpopulations.

Social structure

Whilst mating systems can be viewed as the culmination of the reproductive strategies of individuals (Clutton-Brock 1989), a population's

social structure comprises all interactions among conspecifics, both reproductive and non-reproductive (Alexander 1974). A population's mating system and social organisation can dramatically affect its demographic parameters (e.g. Whitehead 1987; Lambin & Krebs 1993), patterns of gene flow (Selander 1970; Alexander 1974; Altmann *et al.* 1996), cultural (Connor *et al.* 1998) and disease transmission; (Bell 1983; Loehle 1995), and ultimately population structuring (Chesser *et al.* 1993). Consequently, information on social structuring contributes significantly towards understanding the partitioning of genetic variation within and among populations (Chesser 1991a, b; Surridge *et al.* 1999).

Reviews of analyses of social behaviour have demonstrated that the patterns of social organisation determining population dispersion are intricately linked to a species' ecology (Crook & Gartlan 1966; Crook 1970; Eisenberg *et al.* 1972; Clutton-Brock & Harvey 1977; Emlen & Oring 1977). In particular, both group size and social behaviour are strongly influenced by the pressures exerted by the distribution of resources and the risk of predation (Clutton-Brock and Harvey 1977). Intraspecific variation in social structure in response to these variables has been reported in many species (Lott 1984; Clapham 1996; Connor *et al.* 2000), and as such, species-wide generalisations cannot be made (Clutton-Brock 1989). Consequently, studies of multiple populations of a species occupying different ecological environments can provide valuable insight into the ecological determinants of social organisation and its evolution (Fernando & Lande 2000; Baird & Whitehead 2000).

Examining the nature and stability of associations among individuals can prove useful in determining the value of sociality for a species or population (Myers 1983; Whitehead *et al.* 1991; Christal *et al.* 1998). The social structure of a population can be characterised by examining the size, stability and composition of social groups, which can be broadly defined as interacting individuals that are organised in a co-operative manner (Chesser *et al.* 1993). Where members of social groups are either close relatives, and/or long-term associates, there is the potential for both kin selection (Hamilton 1963, 1964) and reciprocal altruism (Trivers 1971) to operate. Although social behaviour is defined by interactions among individuals, and not by genetic relatedness, gene correlations can underpin the maintenance and evolution of social behaviours (Hamilton 1964; Chesser *et al.* 1993).

Kinship often plays an integral role in determining the level of association between conspecific individuals across taxa, and, as such, is regularly cited as a central tenet in the evolution of sociality (Hamilton 1964; Pamilo *et al.* 1997; Ross 2001). The benefits accrued through kin selection have been proposed to explain social groupings in a variety of species. Associations among related individuals form the framework of the social organisation of some cetaceans (Bigg 1990; Baird 2000), social insects (Wilson 1975; Ross 2001), bats (Kerth *et al.* 2000), carnivores (Girman *et al.* 1997), elephants (Fernando and Lande 2000), birds (Parker *et al.* 1995; Piertney *et al.* 1999; Shorey *et al.* 2000), and some primate species (Clutton-Brock and Harvey 1977). It has been theorised that these types of associations encourage co-operation between related individuals, thereby enhancing their inclusive fitness (Hamilton 1963, 1964).

Assessing population structure: the use of molecular genetics

Examining the structuring of wildlife populations, not only at the level of subpopulations but also at the level of social groups and individuals, has yielded a wealth of information on population structuring and dispersal patterns in terrestrial animals. However, delimiting stocks or populations for species of whales and dolphins has been confounded by their relative inaccessibility, longevity, and mobility. Direct field-based observations have proved valuable for describing social affiliations based on recognisable individuals, but can require several years of study to achieve substantial sample sizes. Furthermore, behavioural observations are limited in their power to assess gene flow and determine genetic relatedness among associates. Direct assessment of cetacean population structuring is restricted not only by the vastness of their three-dimensional environment, but also by a lack of understanding of the barriers to movement and gene flow encountered by highly mobile animals within a seemingly continuous marine habitat (Rosel *et al.* 1995; Avise 1998). Consequently, it has proved difficult to study these species at the appropriate spatial and temporal resolution.

Molecular genetic data can be used to estimate genetic relatedness of social affiliates, and indirectly infer patterns of dispersal and gene flow. Therefore, genetic techniques can provide additional and/or alternative information on the degree of effective genetic exchange between population subdivisions. A notable breakthrough in the field of molecular genetics was the advent of the polymerase chain reaction (PCR; Saiki *et al.* 1988). This technique, which greatly reduced the quantity of tissue required for genetic surveys, and led to the successful application of highly variably markers such

as microsatellites to the study of natural populations (Schlotterer & Pemberton 1994), sparked a revolution in the study of free-ranging cetaceans.

Mitochondrial DNA (mtDNA) is often selected as a genetic marker to infer population structure due to its maternal mode of inheritance (Gyllensten *et al.* 1985) and very low probability of recombination (Brown 1985; Gyllensten *et al.* 1985). The inheritance of the mitochondrial genome as a single organelle from the maternal cytoplasm means that nearly all changes in the molecule are due to mutation, and these differences can be used to trace matrilines. Furthermore, within the mitochondrial genome is the non-coding control region containing the D-loop, the most variable part of the mtDNA genome (see Aquadro & Greenburg 1983; Hoelzel *et al.* 1991). These characteristics render the mtDNA control region ideal for inferring phylogeography at or below the level of species (Avise *et al.* 1987; Harrison 1989; Avise 1994). However, there are limitations when drawing inference from a single class of genetic marker. MtDNA markers lack the ability to resolve male-mediated gene flow, due to maternal transmission, and may not be sufficiently variable to examine relationships among individuals within a single population. Hence, a lack of differentiation in mtDNA may not be sufficient evidence to conclude that structuring is absent (Dowling *et al.* 1992; Ramey 1995). Consequently, the use of multiple genetic markers can help in identifying the processes that shape patterns of intraspecific variation (Avise 1994; Piertney *et al.* 2000). Specifically, the use of markers with contrasting modes of inheritance can prove useful in resolving sex-biased patterns of dispersal and gene flow (e.g. Burg *et al.* 1999)

Microsatellites, biparentally-inherited short (2-6 basepair [bp]) tandem repeat sequences, are another commonly used class of genetic marker for population studies (Bruford & Wayne 1993; Queller *et al.* 1993). Microsatellite arrays consisting of longer units (i.e. 3 or 4bp, tri- or tetranucleotide repeats) evolve faster than those containing shorter (2 bp, dinucleotide repeats) (see Chambers & MacAvoy 2000). However, selection of microsatellite loci involves balancing the relative rates of evolution and the frequency of occurrence throughout the genome (dinucleotide repeat arrays occur more frequently than tri- or tetranucleotide arrays), with practical considerations (dinucleotide arrays often show 'stutter' bands (Hauge & Litt 1993) making the scoring of alleles difficult) (Chambers and MacAvoy 2000). These highly polymorphic (Tautz 1989), neutral nuclear markers have proved useful in addressing a range of both behavioural and ecological questions including population demographics, social structure, reproductive success, dispersal and population structure (e.g. Queller *et al.* 1993; Morin *et al.* 1994; Allen *et al.* 1995; Petri *et al.* 1997; Coulson *et al.* 1998; Goodman 1998; Pemberton *et al.* 1999; Piertney *et al.* 1999; Wilmer *et al.* 1999). Moreover, the ability to estimate levels of genetic relatedness among pairs of individuals, and infer relationships, enables studies of natural populations to examine the pattern, context and function of conspecific interactions (e.g. Packer *et al.* 1991; Morin *et al.* 1994; Kays & Gittleman 1995; Girman *et al.* 1997; Kerth & Konig 1999; Piertney *et al.* 1999; Mitani *et al.* 2000; Burland *et al.* 2001). The number of different studies employing mtDNA and microsatellite markers to address key questions in the fields of ecology, evolution, behaviour and conservation is rapidly increasing. However, while genetic data can provide

information on the degree of genetic exchange between population subdivisions, direct observational data from behavioural studies can strengthen inferences about population structure.

Recently, molecular techniques have permitted detailed investigations of social structure and population structure in marine systems (eg. Amos *et al.* 1993a; Amos *et al.* 1993b; Hoelzel 1994; McMillan & Bermingham 1996). However, while studies of the molecular ecology of whale and dolphin species are increasing in number, they are still restricted by the challenges of obtaining samples for genetic analysis, especially from the smaller delphinid species. During the last decade, non-destructive tissue sampling has been increasingly used in such studies. Tissue collection, using a biopsy punch deployed from a pneumatic rifle or crossbow, has proved an effective method for obtaining small skin samples from several species of both large (Brown *et al.* 1991; Palsboll *et al.* 1991; Barrett-Lennard *et al.* 1996) and small (Weller *et al.* 1997; Fossi *et al.* 2000) cetaceans (see Bearzi 2001 for review). However, there are often situations in which biopsy sampling is not feasible, on the grounds of public relations or practical limitations, and recent concern expressed by some about the possible disturbance and physical impact of biopsy sampling has necessitated the development of alternative, less-invasive methods of tissue sampling (Amos *et al.* 1992; Harlin *et al.* 1999; Parsons *et al.* 1999). Therefore, although the aforementioned advances in laboratory techniques allow previously intractable questions to be addressed, their full potential has not yet been realised, and the number of studies linking empirical data on individual animals' movements and associations to indirect genetic estimates of gene flow and relatedness are still few.

In this thesis, I focus on the value of molecular genetics for inferring the population structure of a species of small cetacean, the bottlenose dolphin (*Tursiops truncatus*). In particular, I develop novel methods for obtaining genetic samples, and apply these to the study of the social and population structure of free-ranging bottlenose dolphins.

The bottlenose dolphin

Odontocete cetaceans are distributed widely about the world's oceans and coastal waters. It has been hypothesised that environmental conditions are a major influence on the social structure, and ultimately population subdivision, of these species, particularly through the impact which habitat complexity can have on prey distribution and exposure to predators (Wells *et al.* 1980). Bottlenose dolphins are one of the most widespread odontocetes, occurring throughout tropical and temperate waters and occupying a wide range of habitats (Wells & Scott 1994). This species, therefore, offers an excellent opportunity to examine how environmental conditions influence cetacean social organisation and population structuring. Furthermore, as a coastal cetacean species, *Tursiops truncatus* populations are often directly or indirectly impacted by human activities (e.g. Acevedo 1991; Simoes-Lopes 1991; Law *et al.* 1995), and the bottlenose dolphin is the most commonly live-caught species for display in oceanaria. As such, regional examination of population structuring and the combined inference of genetic and ecological data are prerequisites for defining biologically significant subdivisions.

Previous behavioural studies have highlighted variability in the social organisation of this species, characterised by patterns of association, in several different regions. Group size, structure and the persistence of social affiliations appear to vary among study sites, with a general trend towards larger, more transitory groups occurring in more exposed habitats (Norris & Dohl 1980; Shane *et al.* 1986). Despite the long history of research on bottlenose dolphins, assessments of the role of the environment in shaping social structure and consequent gene flow remain constrained by dissimilar sampling methodologies and group definitions, short temporal scales (eg. Wells *et al.* 1987; Smolker *et al.* 1992; Wilson 1995), and the absence of parallel genetic studies.

Thesis synopsis

This thesis is organised into three primary sections, demonstrating the progression from studying a cetacean population using samples collected from dead stranded or by-caught dolphins (Section I), through developing methodologies for obtaining samples from individually-recognised live, free-ranging dolphins (Section II), and culminating in the use of these approaches in the analysis of bottlenose dolphin social and population structure, integrating both direct and indirect data sources (Section III).

In the first data chapter (Chapter Two), I examine the genetic diversity of bottlenose dolphins around the UK and Ireland using mtDNA control region sequences. Samples were obtained from by-caught and beach-cast dolphins from five geographic regions representing the core areas indicated by the

contemporary distribution of this species. MtDNA sequence data are used to compare genetic diversity within each region and infer current population structuring. The results of these analyses are discussed in light of the limitations to inference from stranded samples, and with reference to current concerns for the conservation and management of bottlenose dolphins throughout British and Irish waters.

Chapters Three, Four and Five (Section II) are focussed on the development and validation of methods for obtaining tissue samples for genetic analysis from free-swimming bottlenose dolphins. In Chapter Three, I introduce a novel method for collecting and extracting dolphin mtDNA from faecal samples. In Chapter Four I present a validation of the use of faecal-derived dolphin DNA in microsatellite genotyping studies. For this validation analysis, I compare the multilocus genotypes obtained from matched tissue and faecal samples, using a multiple tubes approach, and empirically determine the number of repeat amplifications required to obtain the correct genotype with a high degree of certainty. The final chapter in Section II, Chapter Five, is a direct comparison of the two methods employed throughout this study for obtaining DNA from wild, free-swimming bottlenose dolphins; skin biopsy sampling and faecal sampling.

In Section III, I present an analysis of the population structure and social organisation of bottlenose dolphins in the NE Bahamas. Chapter Six is an assessment of the social organisation. Here, I examine the patterns and persistence of social affiliations, as well as the genetic relatedness among individual dolphins, in two study sites. The resolved social structure is compared and contrasted between sites in light of their contrasting

environmental characteristics, and inferences are made regarding the environmental influence on the role of kinship in a social cetacean population. In Chapter Seven, using both nuclear and mtDNA markers, I characterise the strength of associations among male dolphins in the two study sites and assess whether or not kinship functions to maintain male alliances.

Finally, in Chapter Eight, I examine the population structuring of bottlenose dolphins on Little Bahama Bank using samples from identifiable individuals from three study sites representing the three geographic poles of a shallow sandbank. Structure is assessed using both mtDNA control region sequences and microsatellite genotyping data. A Bayesian framework is used to examine population structuring based on the microsatellite data, enabling the integration of direct field-based estimates of between-site movements. The results of this molecular genetic survey are presented within the context of the behavioural data collected throughout this study.