

'O father: where art thou?' – Paternity assessment in an open fission–fusion society of wild bottlenose dolphins (*Tursiops* sp.) in Shark Bay, Western Australia

MICHAEL KRÜTZEN,* LYNNE M. BARRÉ,† RICHARD C. CONNOR,‡ JANET MANN§
and WILLIAM B. SHERWIN*§

*School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney NSW 2052, Australia, †National Marine Fisheries Service, NOAA, Seattle, WA 98115, USA, ‡Biology Department, UMASS-Dartmouth, North Dartmouth, MA 02748, USA, §Department of Psychology and Department of Biology, Georgetown University, Washington, DC 20057, USA

Abstract

Sexually mature male bottlenose dolphins in Shark Bay cooperate by pursuing distinct alliance strategies to monopolize females in reproductive condition. We present the results of a comprehensive study in a wild cetacean population to test whether male alliance membership is a prerequisite for reproductive success. We compared two methods for inferring paternity: both calculate a likelihood ratio, called the paternity index, between two opposing hypotheses, but they differ in the way that significance is applied to the data. The first method, a Bayesian approach commonly used in human paternity testing, appeared to be overly conservative for our data set, but would be less susceptible to assumptions if a larger number of microsatellite loci had been used. Using the second approach, the computer program CERVUS 2.0, we successfully assigned 11 paternities to nine males, and 17 paternities to 14 out of 139 sexually mature males at 95% and 80% confidence levels, respectively. It appears that being a member of a bottlenose dolphin alliance is not a prerequisite for paternity: two paternities were obtained by juvenile males (one at the 95%, the other at the 80% confidence level), suggesting that young males without alliance partners pursue different mating tactics to adults. Likelihood analyses showed that these two juvenile males were significantly more likely to be the true father of the offspring than to be their half-sibling ($P < 0.05$). Using paternity data at an 80% confidence level, we could show that reproductive success was significantly skewed within at least some stable first-order alliances ($P < 0.01$). Interestingly, there is powerful evidence that one mating was incestuous, with one calf apparently fathered by its mother's father ($P < 0.01$). Our study suggests that the reproductive success of both allied males, and of nonallied juveniles, needs to be incorporated into an adaptive framework that seeks to explain alliance formation in male bottlenose dolphins.

Keywords: alliance formation, Bayesian inference of paternity, bottlenose dolphin, paternity index, *Tursiops* sp.

Received 29 November 2003; revision received 11 February 2004; accepted 24 February 2004

Introduction

Evolution by natural and sexual selection predicts that individuals will attempt to maximize their inclusive fitness. This usually leads to diverse forms of competition (Davies 1985), especially in males, and ultimately to the evolution

of different mating systems (e.g. Bradbury & Vehrencamp 1977; Clutton-Brock & Harvey 1978). Differences in mating systems both within and among populations can be attributed mainly either to individual variation and/or the adaptation of male and female behaviour to ecological and social constraints (Rubenstein 1980; Dunbar 1981). Mating systems usually fall under one of the three categories of sexual selection (Smuts & Smuts 1993; Clutton-Brock & Parker 1994). The first category is intrasexual competition,

Correspondence: M. Krützen. Fax: + 61 29385 1558;
E-mail: michael.krutzen@unsw.edu.au

where males either compete directly through physical contests or indirectly through the production of more and higher quality sperm (Harcourt *et al.* 1981). The second category is mate choice, where, for example, females 'choose' a particular male because he can demonstrate his quality either through fighting (Cox & Le Boeuf 1977), energetically costly ornamentation (McGraw *et al.* 2001), or cryptic choice of sperm (Cordero & Eberhard 2003). Finally, there is mate coercion, where males either intimidate, harass a female, or force copulations to increase their mating success (Smuts & Smuts 1993). Whatever mating strategy occurs, selection is expected to maximize inclusive fitness benefits, either directly through reproduction, or indirectly through reproduction of relatives (Hamilton 1964a,b).

In this paper, we focus on the reproductive success of males in a well-documented case of alliance formation and mate coercion in cetaceans: in bottlenose dolphins (*Tursiops* sp.), males form nested levels of alliances to sequester females in reproductive condition (Wells *et al.* 1987; Connor *et al.* 1992a, 1999). Alliances in contests between social groups are commonly found in mammals, but alliances within social groups are comparatively rare (Harcourt 1992). Females form alliances primarily to compete for divisible resources, such as food (Wrangham 1980), while males form alliances in competition for females. Because fertilizations are not divisible, male alliances are probably much less common and thus of considerable interest when found (van Hooff & van Schaik 1994). Males may ally in competition that is indirectly related to female access (e.g. rank competition), but more often the competition is direct, as males attempt to take female consorts from other males (Bercovitch 1988), herd females (Caro 1994), and/or guard females (Packer *et al.* 1991). When males cooperate to guard or sequester a single female, and both have mating access, then the question of who fathers the offspring becomes of great interest. Furthermore, in populations where not all males form alliances, then it becomes important to determine whether alternative (e.g. nonalliance) strategies are ever successful.

Measurement of reproductive skew in social groups is fundamental to the understanding of the evolution and maintenance of sociality, as it determines the immediate fitness benefits to helpers of staying and helping in a particular group. Over the past two decades, various models for an evolutionarily stable strategy (*sensu* Maynard Smith 1982) in which skew varies have been developed (Vehrencamp 1983a,b; Cant 1997; Reeve 1998; Reeve *et al.* 1998; Kokko & Johnstone 1999; Crespi & Ragsdale 2000; Johnstone 2000). Depending on the model, the correlation between relatedness and group size or skew is predicted to be either negative, positive or zero. There is now a growing body of research investigating the relationships between male mating behaviour and variance in male mating success, enabling us to test whether the observed male

competitive tactics are, in fact, successful. In many cases, it has emerged that paternity is significantly skewed in favour of a few dominant males [e.g. savannah baboons *Papio cynocephalus* (Alberts *et al.* 2003); black rhinoceros *Diceros bicornis* (Garnier *et al.* 2001); fallow deer *Dama dama* (Say *et al.* 2003)].

Compared to terrestrial systems, detailed studies of parentage in marine mammals are scarce. Nielsen *et al.* (2001) reported that dominant male humpback whales (*Megaptera novaeangliae*) have a relatively higher reproductive success than subdominant males. Although on a very limited data set, Clapham & Palsbøll (1997) showed multiple paternities for offspring from the same female from different years in the same species. Investigating the breeding behaviour of pilot whales, *Globicephala melas*, Amos *et al.* (1991) showed that for 88% of all sampled foetuses, all sampled males from within the pod could be excluded as fathers. However, several offspring within a pod often had the same father from another pod, creating paternal half-sibling cohorts similar to lion prides.

To date, bottlenose dolphins comprise the only species outside humans where males have been shown to form two levels of nested alliance formation within a social group (Connor *et al.* 1992a,b, 1999). Mating strategies for male bottlenose dolphins in Shark Bay appear to be structured around two strategies of alliance formation, which we will call strategy I and strategy II throughout the paper. Strategy I involves the formation of small and stable first-order alliances, lasting up to 17 years (R. Connor, unpublished data); these alliances are pairs or trios of males who cooperate to sequester and control individual females in reproductive condition. Furthermore, teams of two or more first-order alliances may cooperate to attack other alliances or to defend against such attacks, forming second-order alliances (Connor *et al.* 1992a,b). Strategy II involves the formation of labile first-order alliances within a stable large second-order alliance called a 'superalliance' (Connor *et al.* 1999, 2001). The best documented superalliance consists of 14 males that associate in pairs and trios to control individual females, but is different from stable alliances as individual males frequently switch their alliance partners within the superalliance.

An investigation of the relationship between alliance membership and genetic relatedness showed marked differences in relatedness patterns within the Shark Bay dolphin population (Krützen *et al.* 2003). Animals engaging in strategy I are, on average, more closely related than expected by chance, indicating that males engaging in this alliance strategy could receive inclusive fitness benefits. Thus, it is conceivable that some male bottlenose dolphins in alliances composed of relatives might act as nonreproductive helpers, as suggested for lions *Panthera leo* (Packer *et al.* 1991). In contrast, strategy II males are not closely related (Krützen *et al.* 2003), indicating that this alliance type

provides, at best, less fitness benefits through the reproduction of kin. Any advantage of strategy II must relate to other factors, such as a numerical advantage in competition with strategy I alliances.

Cooperation among male bottlenose dolphins has been reported from at least three other study sites (Wells *et al.* 1987; Möller *et al.* 2001; Parsons *et al.* 2003). In Sarasota Bay, Florida, male bottlenose dolphins (*Tursiops truncatus*) are also found to form stable pairs and consort with females (Wells *et al.* 1987; Wells 1991). In contrast, male bottlenose dolphins (*T. truncatus*) in the Moray Firth, Scotland, do not show the high association coefficients with other males that is typical of alliance formation in other populations (Wilson *et al.* 1992, 1993).

In this paper, we sought to determine the relationship between alliance membership and reproductive success. In particular, we were interested in (1) whether reproduction is limited to males that form alliances, and (2) the distribution of paternities within and among all strategy I alliances. These results will aid in the development of an adaptive framework that seeks to explain alliance formation in male bottlenose dolphins.

Materials and methods

Behavioural sampling

Bottlenose dolphins have been systematically studied in Shark Bay since the mid-1980s (Connor *et al.* 1992a,b; Smolker *et al.* 1992). The core study area covers about 200 km² (Fig. 1). Between 1994 and 1999, tissue samples were opportunistically obtained from 305 free-ranging bottlenose dolphins in the Eastern Gulf, using a biopsy system that was specially designed for use in small cetaceans (Krützen *et al.* 2002).

Age was determined using two approaches. The actual year of birth was used if known. In the second approach, size and ventral speckling information were used. Speckling of the ventral area typically first appears between 7 and 11 years of age and increases through adulthood (Smolker *et al.* 1992). Speckling begins around the genital area, then the belly and eventually spreads to the chest and chin in very old animals. Lateral speckling occurs later than ventral speckling. Grades of speckling range from 1 (no speckling) to 4 (heavy speckling). The grades of speckling were

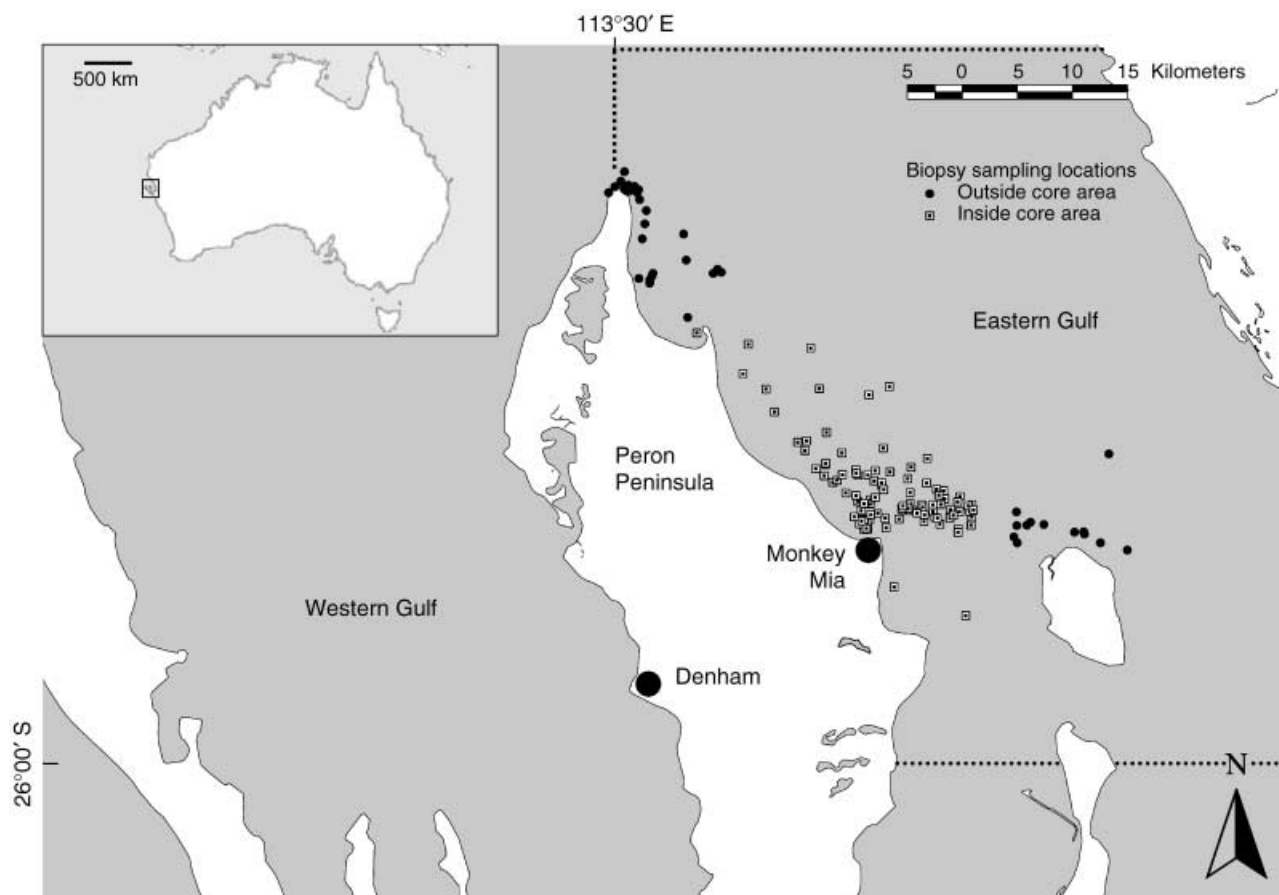


Fig. 1 Geographical location of bottlenose dolphin samples collected for this study. One point may represent more than one individual. The dashed lines indicate the combined boundaries of survey blocks 3 and 5 (see Preen *et al.* 1997). There were no dolphin sightings south of the indicated area in the Eastern Gulf (Preen *et al.* 1997).

calibrated by reference to speckles of animals of known age. Individuals first sighted as juveniles could be more accurately aged compared to those first sighted as adults. For Shark Bay females, 12 years is the earliest known age at first reproduction (Mann *et al.* 2000). Therefore, all females aged 12 years or older at the time of their sampling were considered to be sexually mature. Males were treated differently. Shark Bay males do not appear to form alliances and begin herding females until their mid- to late teens (Connor *et al.* 2000a). However, sexual maturity might begin much earlier, as suggested by bursts in testosterone levels in Sarasota Bay dolphins at 8 years of age (Wells *et al.* 1987). For this reason, all males that could have been least 8 years of age when they were sampled were included as candidate fathers.

For males, alliance membership was known from previously published work (Connor *et al.* 1992a, 1999) and ongoing studies. Males were also sampled whose affiliation to a particular type of alliance could not be entirely resolved using the behavioural data available (see Results). However, preliminary observations suggested that 10 of those males fulfil the alliance membership criteria (Connor *et al.* 1992a): during individual follows they have been observed to engage in aggressive behaviour towards other males and females, and they showed association coefficients in the range of stable alliances (R. Connor, unpublished data). Therefore, those 10 males will be referred to as 'potential strategy I alliance' members. It was not possible to assign alliance partners to the remaining males. However, this may well be the result of limited behavioural data for those males, rather than that these animals genuinely do not have an alliance partner. In this paper, we refer to those males as 'males without assigned alliance partners', bearing in mind that future studies with adequate behavioural sample size may reveal alliance membership for those males.

Tissue sampling and genetic analysis

Tissue sampling and storage was carried out as described in Krützen *et al.* (2002). Total genomic DNA was extracted from skin biopsies using standard methods (Davis *et al.* 1986). The animals were genetically sexed following the method developed by Gilson *et al.* (1998). All samples were genotyped with a panel of eight highly polymorphic dinucleotide microsatellite loci: these were MK3, MK5, MK6, MK8, MK9 (Krützen *et al.* 2001); EV1 (Valsecchi & Amos 1996); KWM12 (Hoelzel *et al.* 1998); and 199/200 (Amos *et al.* 1993). The polymerase chain reaction products were run on an ABI 377 DNA automated sequencer (Applied Biosystems), and the size of the fragments obtained was measured using GENESCAN, version 3.1 and GENOTYPER, version 1.1.1 software (both Applied Biosystems).

To test for duplicate samples, the data were checked for identical genotype entries, using MSTOOLS, version

3.0 (available from <http://oscar.gen.tcd.ie/~sdepark/mstoolkit/>). The level of expected heterozygosity (H_E) and other locus characteristics were estimated. The probability that two unrelated individuals have an identical genotype was calculated using the formula from Paetkau *et al.* (1995):

$$I = \sum_i p_i^4 + \sum_i \sum_{j>i} (2p_i p_j)^2$$

where p_i and p_j are the frequencies of the i th and the j th alleles at each locus in a given population.

Studies of paternity require detailed knowledge about genetic population structure. Previous studies have shown that for nuclear DNA markers, there is weak isolation by distance with male and female gene flow rates (Nm) of 8–130 between adjacent sampling localities within the Eastern Gulf of Shark Bay (Krützen *et al.* 2004). Because of the high gene flow, all individuals in the east Shark Bay study area are considered to be part of the same population and were pooled for subsequent analysis.

Paternity inference

Paternity was inferred using two different approaches. Both methods are identical in that they calculate a likelihood ratio of two competing hypotheses, known as the Paternity Index (PI) in human paternity testing (Pena & Chakraborty 1994). The two competing hypotheses are

$H_{(1)}$: The candidate father is the true father

$H_{(0)}$: The candidate father is an unrelated random male from the same population.

The likelihood ratios $LR_{PO} = PI = H_{(1)}/H_{(0)}$ for each locus are calculated using the formulae developed for cases where the mother's genotype is known (Brenner 1997), and for individuals without maternal data (Brenner 1993) and were generated using the software CERVUS 2.0 (Marshall *et al.* 1998). Both approaches imply that the individuals to be tested are from the same racial background (in human testing) or from the same population (in animal populations).

The difference between the two approaches lies in the way in which significance is applied to the paternity index. The first method (called 'Bayesian inference' throughout this paper) is commonly used in legal paternity disputes in humans. The posterior likelihood (W ; i.e. the probability of paternity, or 'Wahrscheinlichkeit') of $H_{(1)}$ is calculated using Bayes' theorem (Evetts & Weir 1998):

$$W = p_{prior} \Pi PI / [(p_{prior} \Pi PI) + (1 - p_{prior})]$$

where p_{prior} is the prior probability. In human paternity testing, p_{prior} is the probability of $H_{(1)}$ prior to considering the genetic evidence. In cases where there are many candidate

fathers for a child (i.e. orgies in humans, promiscuous mating systems in animals), p_{prior} for $H_{(1)} = 1/n$; n being the number of candidate fathers. Note that for a large PI , p_{prior} has only a very small influence on the probability of paternity W .

The second method (called CERVUS throughout this paper) uses a simulation approach to define a Δ statistic based on population allele frequencies and resolves paternities between the two males with the highest PI s with a known level of statistical confidence (Marshall *et al.* 1998). CERVUS was developed with studies in mind where not all candidate parents could be excluded using codominant genetic markers; it allows incomplete sampling of the candidate parents as well as potential scoring errors that might occur in large data sets. The input parameters required are: the number of candidate fathers (similar to n in Bayesian inference; this needs to be estimated from ecological data); the proportion of candidate fathers sampled (which needs to be estimated from field data); the proportion of loci typed (which can be calculated from the data set); and the genotyping error rate (which can be estimated from observed mismatches between mothers and their known offspring, assuming that the occurrence of germ-line mutations is negligible and that the mutation rates between males and females are the same). The male with the highest LOD-score, defined as the natural logarithm of the paternity index (Meagher 1986), will be assigned paternity if the difference of his LOD-score to that of the second most likely male is larger than Δ . If a nonzero error rate has been specified, some genotypic mismatches between the candidate fathers and the tested offspring will be allowed. Through its simulation, CERVUS allows the exploration of assumptions and sampling limits on the final result.

For both the Bayesian approach and CERVUS, calculating PI following both methods assumes that there are negligible levels of inbreeding in the population, as indicated by a nonsignificant overall F_{IS} . If the population F_{IS} is found to be significant, the formulae to calculate the likelihood ratios need to be modified (Evetts & Weir 1998). Hence, the global level of inbreeding of the Shark Bay dolphin population was calculated as described in Weir & Cockerham (1984), using the program FSTAT, version 2.93 (Goudet 1995).

Input parameters

The mating system of Shark Bay dolphins is promiscuous (Connor *et al.* 1996). Females have been observed to consort with as many as 13 different males during one breeding season (Connor *et al.* 1996), although this is likely to be a gross underestimate, and females give birth to a single calf usually every 4–5 years (Mann *et al.* 2000). Thus, it is unlikely that there will be a high proportion of full siblings within the population.

The most conservative approach for estimating p_{prior} using Bayesian inference is to estimate the number of males (regardless of their reproductive and social status) that might have physical access to the sampled females. Our estimate of n was based on three previous studies. Aerial surveys undertaken in 1989 and 1991 showed that the average number of all dolphins in the Eastern Gulf was 860 (± 87.5); this area is about 5.5 times larger than our study area (Fig. 1, Preen *et al.* 1997). The sex ratio between adult males and females is not significantly different from 1 : 1 (Krützen, unpublished data). Population genetic studies showed that there is weak isolation by distance for both nuclear and mitochondrial markers throughout Shark Bay (Krützen *et al.* 2004). Both mitochondrial and nuclear markers reveal that animals from the western part of the Western Gulf of Shark Bay are genetically different from animals from the Eastern Gulf (Fig. 1). The animals sampled off the top of the Peron Peninsula are not genetically different to the nearest sampling locations in the Eastern Gulf but are significantly different at both mitochondrial and nuclear loci to animals from the Western Gulf (for sampling locations see Krützen *et al.* 2004); hence, these animals could be included in the analysis. Within our study area in the Eastern Gulf, female dispersal is limited between adjacent sampling localities, and male dispersal appears to be slightly greater than that of females (Krützen *et al.* 2004). Therefore, immigration of candidate fathers into our study area appears to be negligible. Based on these data, the number of candidate fathers was estimated to be 430, which is regarded as a conservative overestimate. In addition, the posterior probabilities were also calculated for two different priors, which are based on the average number of male individuals found in the Eastern Gulf ± 2 SD (Preen *et al.* 1997) to evaluate the effect that different priors have on the probability of paternity.

The software CERVUS 2.0 (Marshall *et al.* 1998) was used and the input parameters were estimated as follows: the number of candidate fathers was derived in the same way as for the Bayesian inference; the proportions of males sampled was calculated from field data the proportion of loci typed was 0.939; and the error rate was set to zero, i.e. a mismatch between candidate fathers and offspring was not allowed. Similar to the Bayesian inference, we explored the assumptions using CERVUS by altering the input parameters for the 10 000 simulation runs. This conservative approach was chosen because, first, only candidate fathers that are genetically compatible with the calf are subjected to the statistical analysis. This eliminates the potential problem of having to interpret parentage assignments where candidate fathers show high LOD-scores due to rare alleles that they share by chance with the offspring, but have one or more genotypic mismatches with the calf, a scenario commonly observed using the CERVUS approach. Second, it allows direct comparison of

both approaches used in this study. An error rate of 1.5% would lead to a nonassignment of an average of 1 in 66.6 paternities using a pure exclusion approach in a mating system with no or low male reproductive skew. Given that there are only 64 offspring in our data set, we regard this potential source of error as negligible.

A recent study showed that males forming strategy I alliances are, on average, more closely related than expected by chance (Krützen *et al.* 2003). To account for potentially elevated levels of relatedness among candidate fathers, we set the relatedness parameter in CERVUS to 0.104, which is the average relatedness of strategy I males (Krützen *et al.* 2003), and included one relative at this relatedness level for each candidate father in the simulation.

Is the assigned father actually a half-sibling?

Without detailed knowledge about genealogical relationships, a potential problem for paternity assignments in wild populations is that the assigned father could be a close relative of the tested offspring. This is especially important in our case because we had to make various assumptions about the reproductive status of males. Given the population and mating structure of Shark Bay dolphins (Connor *et al.* 1992a,b; Krützen *et al.* 2004) and the relatively long birth intervals (Mann *et al.* 2000), one could propose that the assigned candidate father is indeed a half-sibling of the offspring. The two most likely propositions, given the mating systems of the Shark Bay dolphins, are PP_1 : the relationship between the assigned candidate father and the offspring is true; i.e. the DNA profiles are from father and offspring, and PP_2 : the assigned candidate father is indeed a half-sibling of the offspring. Brenner & Weir (2003) recently developed a method that allows the comparison of the likelihoods of PP_1 and PP_2 . In addition to $H_{(0)}$ and $H_{(1)}$, there is one more hypothesis for each observed match to consider:

H_A : The DNA profiles are from two half-siblings

The likelihood ratio for two individuals with alleles ab and cd is

$$LR_{PO/HS} = \frac{PP_1}{PP_2} = \frac{LR\left(\frac{\Pr(ab, cd | H_1)}{\Pr(ab, cd | H_0)}\right)}{LR\left(\frac{\Pr(ab, cd | H_A)}{\Pr(ab, cd | H_0)}\right)} = \frac{P_0 + uP_1 + vP_2}{P_3 + uP_4 + vP_5}$$

(modified from Brenner & Weir 2003), where a, b, c and d represent the four alleles which may or may not be identical in state; P_0, P_1, P_2 and P_3, P_4, P_5 are the probabilities that two individuals that are related as specified in $H_{(1)}$ and H_A , respectively, share zero, one or two pairs of alleles identical by descent; u and v depend on the frequencies of the alleles shared as follows: quantities x_1, x_2, x_3, x_4 are defined for

four possible allelic combinations ac, ad, bc, bd . Each x is set to zero if the two alleles are identical in state, and is the reciprocal of the allele frequencies when they are non-identical in state. Then $u = (x_1 + x_2 + x_3 + x_4)/4$ and $v = (x_1x_4 + x_2x_3)/2$ (Brenner & Weir 2003).

If $LR_{PO/HS} > 1$, then PP_1 is more likely than PP_2 . Conversely, if $LR_{PO/HS} < 1$, then PP_2 is the more plausible explanation for the data. To test the significance of $LR_{PO/HS}$, the likelihood ratio was converted into a χ^2 statistic using the formula $\chi^2 = \log(LR_{PO/HS}) \times (2 \ln 10)$ with one degree of freedom (Lander & Kruglyak 1995). The suitability of this method was examined on two known maternal half-sibling pairs.

Testing for reproductive skew

For further questions such as whether reproduction is limited to allied males, and whether paternities within and among all strategy I alliances are evenly distributed, the CERVUS results at the 80% confidence level were used. Although this increases the chance of a type I error, we believe that this is warranted for three main reasons: first, for each paternity, there were no other matching males in the data set (see Results); second, we did not allow a mismatch between candidate father and offspring; and third, we used a gross overestimate for the number of potential fathers in the CERVUS simulation. The skew of the distribution of paternities within and between strategy I alliances was tested by randomly reassigning all paternities achieved by all strategy I alliance members 1000 times and counting the number of occasions on which the variance of the randomized distribution exceeded the observed.

Results

Sampling success

For this study, a total of 312 dolphins was sampled (Table 1). Seven samples were excluded from further analysis because it was not possible to determine the sex of six adult animals, and one animal was sampled twice. Of the remaining 305 animals, 244 were assigned to the core study area based on their sampling location (Table 1, Fig. 1). In the core study area, 88.1% of the animals had previously been sighted, while for the remainder of East Shark Bay, 72.1% of the sampled animals were known. Thirty-four known mother–calf pairs (verified by behavioural and microsatellite data), 28 sexually mature members of male alliances, and 30 juveniles without paternal data were sampled (Tables 1 and 2). Fifteen of these males were members of seven strategy I alliances, and 13 were members of the superalliance. Ten potential strategy I alliance members were also sampled. It was not possible to assign an alliance partner to the remaining males (101 from East Shark Bay or 76 from the core study area; see Materials and methods).

Table 1 Summary of numbers of bottlenose dolphin samples in respect to the location they were sampled, their age-sex class and their identity status

	East Shark Bay		Core study area	
	All animals	Animals with known ID	All animals	Animals with known ID
Females	105	67	80	63
Males known to be sexually mature	90	84	85	84
Males assumed to be sexually mature	49	24	29	23
Juveniles without parental data	30	16	21	16
Known offspring	34	32	32	32
Total*	305	220	244	215

*Two female and one male offspring are also sexually mature, these were not counted twice.

Table 2 Known alliance membership of sampled sexually mature males

Strategy I first-order alliances							
1	REA	HII	BOT				
2	POI	LUC					
3	WAV	SPU					
4	SHK	CRC	SYL				
5	BIF	BOH					
6	BJA	BUM	BAM				
Potential strategy I first-order alliances							
1	TWI	SMU	DEB				
2	PRI	NAT	WAB				
3	COM	JIM	TYP				
4	FRE	RID	BMB				
Superalliance							
1	LAT	GRI	VAX	KRI	MYR	WOW	HOB
	WBE	HOR	AJA	PIK	ANV	VEE	ROL

Entries in italics indicate that no DNA sample was obtained.

Microsatellite data

All eight microsatellite loci were useful for paternity inference (Table 3). All loci were in Hardy–Weinberg equilibrium, and no linkage disequilibrium could be observed (Krützen *et al.* 2004). The very low probability of identity of 6.7×10^{-10} (Table 3) suggests that finding a genotype match by chance using all eight microsatellite loci is negligible, indicating that the two identical genotypes found in the data set were resampling events. Overall population F_{IS} was estimated to be zero (± 0.017), which was not significant. Hence, no correction factor was used for the calculation of *PI*.

Paternity inference

For 16 offspring with known mothers all but one male were excluded as potential fathers (Table 4). There was no male

Table 3 Levels of variation in East Shark Bay

Locus	No. of alleles	H_O	H_E	PIC	Null allele frequencies	Probability of identity
MK3	10	0.602	0.656	0.623	0.043	0.151
MK5	8	0.803	0.769	0.729	–0.024	0.093
MK6	23	0.878	0.884	0.873	0.004	0.024
MK9	7	0.705	0.753	0.707	0.032	0.106
199/200	8	0.717	0.749	0.715	0.022	0.097
KWM12	17	0.773	0.831	0.808	0.036	0.052
EV1	21	0.864	0.846	0.831	–0.010	0.037
MK8	11	0.802	0.73	0.701	–0.064	0.101
Overall	13.2	0.768	0.777	0.748		6.7×10^{-10}

H_O = observed heterozygosity, H_E = expected heterozygosity, PIC = polymorphic information content (Hearne *et al.* 1992). Null allele frequencies were estimated using the algorithm developed by W. Amos (Summers & Amos 1997) as implemented in CERVUS 2.0 (Marshall *et al.* 1998).

in the data set that matched the remaining 18 offspring. As expected, exclusion power was smaller for juveniles without maternal data: two or more candidate fathers were genetically compatible with 16 juveniles, five juveniles had only one matching candidate father in the data set, while the remaining nine had none (Table 4).

Our success rate in paternity assignments varied depending on the method, the area and the chosen confidence level. In the core area, 16 paternities were successfully assigned to 12 different fathers using CERVUS, while Bayesian inference gave only nine paternities from eight different fathers (Table 4) at the 80% confidence level. At a 95% confidence level, these numbers of paternities were reduced to 11 using CERVUS, and to eight using Bayesian inference. When males from outside the core area were included as candidate fathers, success was only marginally higher, as only one additional paternity was assigned at the 80% confidence level (Table 4). For all assigned paternities, regardless of

Table 4 Paternity results for two methods of paternity inference

		Likelihood ratios (LOD-score)				Bayesian inference Probability of paternity			CERVUS Confidence of paternity								
Estimated proportion of population sampled†									0.541			0.323			0.228		
Number of candidate fathers (± 2 SD)†						605	430	255	605	430	255	605	430	255	605	430	255
Offs.	Moth.	Cand. fath.	$H_{(1)}/H_{(0)}$ (LOD)	Δ	PP_1/PP_2												
Calves with known mother																	
FLI	BLI	LAT	297.35 (5.69)	5.69	4.11	0.3299	0.4094	0.5393	+	+	*	+	+	+	+	+	
RIP	BLI																
BIN	BYT	BOL	940.91 (6.85)	6.85	7.27‡	0.6090	0.6868	0.7874	*	*	*	+	+	*	+	*	
CEB	EED																
MUT	FID	REA	717.73 (6.58)	6.58	3.65	0.5430	0.6259	0.7386	+	*	*	+	+	*	+	+	
GHO	GOB	PIK	926089.04 (13.74)		25.51‡	0.9993	0.9995*	0.9997	*	*	*	*	*	*	*	*	
GOO	GUM																
NIC	HOL																
JOY	HOL	BJA	1297.76 (7.17)	7.17	13.85‡	0.6824	0.7516	0.8363	*	*	*	+	*	*	+	*	
HBT	HOL	TOL	15118.50 (9.62)	9.62	17.64‡	0.9616	0.9724*	0.9835	*	*	*	*	*	*	*	*	
JSE	JFR																
LAU	JOY	BJA	8431.32 (9.04)	9.04	17.05‡	0.9332	0.9516*	0.9708	*	*	*	*	*	*	*	*	
SOG	KWI																
SKI	MIN	BAM	18278.42 (9.81)	9.81	6.53	0.9680	0.9771*	0.9863	*	*	*	*	*	*	*	*	
MOU	MIN	BJA	9015.90 (9.11)	9.11	5.39	0.9372	0.9546*	0.9726	*	*	*	*	*	*	*	*	
NAK	NIC																
HKI	NIC	REA	1235.96 (7.12)	7.12	4.35	0.6717	0.7423	0.8295	*	*	*	+	*	*	+	*	
TUK	NIP																
CRV	NOO	TWI	54582.61 (10.91)	10.91	11.41‡	0.9891	0.9922*	0.9954	*	*	*	*	*	*	*	*	
PIC	PUC	DEB	4062.56 (8.31)	8.31	7.92‡	0.8706	0.9045+	0.9412	*	*	*	*	*	*	*	*	
SQL	SQU																
PEG	SQU																
SHC	SUR																
TF	TAT																
ROO	TRE																
LIT	TRY																
URC	UHF	CRC	477.07 (6.17)	6.17	8.29‡	0.4413	0.5265	0.6526	+	+	*	+	+	*	+	+	
SKF	UHF																
BUR	URP	VAX	71797.60 (11.18)	11.18	10.99‡	0.9917	0.9941*	0.9965	*	*	*	*	*	*	*	*	
FRL	WYL																
IND	MOO																
515	514																
581	580	590	354.44 (5.87)	5.87	4.81	0.3698	0.4524	0.5825	+	+	*	+	+	+	+	+	
POL	BAD	GRI	49039.84 (10.8)	10.8	11.39‡	0.9878	0.9913*	0.9948	*	*	*	*	*	*	*	*	
Juveniles without maternal data																	
542		398	17.89 (2.88)	2.88		0.0288	0.0399	0.0658									
LOS		BOT	143.47 (4.97)	2.53		0.1919	0.2497	0.3610									
LOS		SYL	11.40 (2.43)			0.0185	0.0258	0.0430									
BAY		SPU	21.77 (3.08)	0.82		0.0348	0.0481	0.0789									
BAY		TWS	9.61 (2.26)			0.0157	0.0218	0.0365									
FUF		BJA	445.21 (6.10)	6.10	12.64‡	0.4243	0.5081	0.6367	+	+	+	+	+	+	+	+	
LIC		POI	54.85 (4.00)	0.49		0.0832	0.1129	0.1776									
LIC		EDG	33.72 (3.52)														
HUL						0.1822	0.2379	0.3462									
OLI		369	134.52 (4.90)	1.22		0.0615	0.0841	0.1347									
OLI		SMG	39.55 (3.68)			0.4151	0.4987	0.6279									
DEM		BJA	428.69 (6.06)	2.29		0.0668	0.0912	0.1455									

Table 4 Continued

		Likelihood ratios (LOD-score)				Bayesian inference Probability of paternity			CERVUS Confidence of paternity					
Estimated proportion of population sampled†									0.541		0.323		0.228	
Number of candidate fathers (± 2 SD)‡						605	430	255	605	430	255	605	430	255
Offs.	Moth.	Cand. fath.	$H_{(1)}/H_{(0)}$ (LOD)	Δ	PP_1/PP_2									
DEM		589	43.26 (3.77)			0.0667	0.0910	0.1453						
APH		SPU	43.17 (3.77)	2.27		0.0074	0.0103	0.0173						
NUM		579	31.11 (3.44)	0.33		0.0490	0.0673	0.1091						
NUM		BOL	22.34 (3.11)			0.0357	0.0493	0.0808						
SUM		SPN	23.76 (3.17)	0.54		0.0378	0.0522	0.0855						
SUM		501	13.78 (2.62)			0.0223	0.0310	0.0515						
524		520	608.77 (6.41)	1.69		0.5020	0.5855	0.7056						
524		531	111.78 (4.72)			0.1562	0.2059	0.3056						
529														
545		ELR	32.60 (3.48)	1.64		0.0512	0.0703	0.1138						
545		REA	6.33 (1.85)			0.0104	0.0145	0.0243						
546		GNA	165.91 (5.11)	3.54		0.2155	0.2779	0.3951						
546		POO	4.84 (1.58)			0.0079	0.0111	0.0187						
548		CAP	23.58 (3.16)	0.34		0.0376	0.0519	0.0850						
548		FAR	16.73 (2.82)			0.0270	0.0374	0.0618						
549		DNO	968.57 (6.88)	3.50		0.6159	0.6920	0.7922						
549		DEB	29.32 (3.38)			0.0463	0.0637	0.1035						
578														
008														
SLO		RSP	76.19 (4.33)	4.33		0.1120	0.1502	0.2308	+	+				
LAN														
SMO		VIP	404.25 (6.00)	3.11		0.4009	0.4840	0.6141						
SMO		DEB	18.04 (2.89)			0.0290	0.0402	0.0663						
120		543	23.86 (3.17)	2.75		0.0380	0.0525	0.0859						
120		NIX	1.52 (1.52)			0.0025	0.0035	0.0060						
MAG		398	177.28 (5.18)	1.81		0.2269	0.2914	0.4111						
MAG		BOH	28.96 (3.37)			0.0457	0.0630	0.1023						
HHD														
HED														
387		BAM	21.48 (3.07)	3.07		0.0343	0.0475	0.0780						
551														
506		WOW	44.21 (3.79)	1.07		0.0682	0.0930	0.1483						
506		NON	15.19 (2.72)			0.0245	0.0340	0.0564						

The columns shaded in grey indicate the paternity assignments using input parameters derived from ecological data and represent the most likely scenario. Only the two most likely fathers are shown (if applicable). The likelihood ratio for $H_{(1)}/H_{(0)}$ equals the paternity index PI . Animals that were sampled outside the core area are given in italics.

Offs., offspring; Moth., mother; Cand. Fath., candidate father; Δ , Difference in LOD-scores between most likely and second most likely candidate father.

†Based on the estimates of Preen *et al.* (1997) ± 2 SD; ‡ $P < 0.05$.

*Strict confidence of paternity (95% level); +relaxed confidence of paternity (80% level).

the level of confidence, $LR_{PO/HS}$ was larger than one, indicating that the father–offspring relationship was 3.6–25.5 times more likely than a possible half-sibling relationship. All offspring share at least one allele with the assigned father at each locus, which would not necessarily be the

case if both individuals were half-siblings. However, it was possible to show this at the 95% confidence level for only 11 out of 17 assigned paternities (Table 4). As predicted, $LR_{PO/HS}$ was smaller than one for both known maternal half-siblings in the data set (data not shown).

Table 5 Number of assigned paternities for males with different alliance strategies, using relaxed and strict confidence levels in cervus

Strategy I alliances		Superalliance		Potential strategy I alliances		Males without assigned alliance partners	
80%	95%	80%	95%	80%	95%	80%	95%
2 REA	1 REA	1 PIK	1 PIK	1 TWI	1 TWI	1 TOL	1 TOL
4 BJA	3 BJA	1 VAX	1 VAX	1 DEB	1 DEB	1 590	
1 BAM	1 BAM	1 GRI	1 GRI			1 BOL	
1 CRC		1 LAT					

Table 6 Estimated ages (in years) at conception for fathers assigned at a 80% confidence level

Assigned father at relaxed confidence level					Calf			Mother			
Code	YOB	Range	Most likely age at conception	Range	Code	YOB	Range	Code	YOB	Range	Age at conception
BJA	1966	1960–71	12	7–18	JOY	1979		HOL	1960	1958–62	28
BJA	1966	1960–71	23	16–27	FUF	1987	1986–87			unknown	
BJA	1966	1960–71	24	20–31	MOU	1991		MIN	1964	1959–69	26
BJA	1966	1960–71	28	23–34	LAU	1994		JOY	1979		14
BAM	1971	1960–71	11	11–22	SKI	1983	1980–84	MIN	1964	1959–69	18
REA	1972	1966–72	20	16–20	MUT	1993		FID	1981	11	
REA	1972	1966–72	22	18–22	HKI	1995		NIC	1975	19	
BOL	1984	1979–89	10	5–15	BIN	1995		BYT	1973	22	
CRC	1971		18		URC	1990		UHF	1972	1970–74	17
PIK	1974	1971–77	16	13–19	GHO	1991		GOB	1979	11	
DEB	1971		20		PIC	1992		PUC	1976	16	
TOL	1986	1984–88	6	4–8	HBT	1993		HOL	1960	1958–62	32
TWI	1980	1977–80	13	13–16	CRV	1994		NOO	1974	19	
GRI	1982		11		POL	1994		BAD	1981	1980–82	12
VAX	1977		17		BUR	1995		URP	1976	1973–79	18
LAT	considered adult at time of sampling				FLI	1984	1983–84	BLI	1970	1965–70	12
590	considered adult at time of sampling				581			580	unknown		

YOB = year of birth.

When our assumptions were explored by changing the simulation parameter in the CERVUS simulation, it became evident that both estimated input parameters (proportion of population sampled, number of candidate fathers in the population) have a large influence on the statistical significance. Not surprisingly, there seems to be a positive correlation between the number of assigned paternities and the proportion of population sampled, and a negative correlation between assignment success and number of potential fathers (Table 4). In contrast, Bayesian inference does not seem to be susceptible to violation of assumptions: the significance of only one paternity assignment changed from the strict to the relaxed level when the number of potential fathers was increased. Overall, however, Bayesian inference led to a much lower number of successfully assigned paternities given our data set. This is regarded as overly conservative as it based on the assumption that every male

in the Eastern Gulf potentially copulated with the mother, which is an unlikely scenario even in promiscuous dolphins.

At the 80% confidence level, two males from two different strategy I alliances (BJA — four paternities, REA — two paternities) accounted for six out of eight paternities achieved by all males in the data set engaging in this alliance strategy (Table 5). The other two paternities were assigned to two dolphins from one potential alliance, four different superalliance members, and four males without assigned alliance partners. Interestingly, it appears that BJA fathered JOY in 1978 as well as JOY's daughter LAU in 1993 (Table 6). Therefore, we calculated the probability that LAU is the product of an incestuous mating using the likelihood ratio formula developed by Edwards (1988). The likelihood ratio for the two hypotheses incestuous vs. nonincestuous mating is 109.6, a highly significant result ($P < 0.01$), strongly suggesting that BJA is not only LAU's

father, but also her grandfather. The paternities achieved by BJA were separated by 16 years (Table 6).

Skewed distribution of paternities among strategy I first-order alliance members and alliances

Most of the paternities were obtained by a minority of individuals and alliances: at the 80% confidence level, two out of 15 first-order alliance members achieved 75.0% (mean number of paternities per male = 0.53, variance = 1.27), and two out of six alliances achieved 62.5% of all paternities (mean = 1.33, variance = 3.87). When all eight paternities were randomly distributed among all sampled strategy I first-order alliance members and strategy I first-order alliances 1000 times, the observed variance was exceeded only seven and five times ($P = 0.007$ and $P = 0.005$, respectively).

Three out of 17 paternities were achieved by males without obvious stable alliance partners at the time of conception: TOL, BOL and the unknown individual from outside the core area (590). TOL and BOL achieved their paternities when they were 6 years (range 4–8 years) and 10 years of age (range 5–15 years), respectively (Table 6). TOL's offspring was conceived in 1992, but there are no sighting records for TOL before 1998. BOL achieved a paternity in 1994, 1 year after he was first seen. BOL was seen in 1993 (four sightings with 20 different males) and 1994 (three sightings with 17 different males). While strong associations are evident between some immature males, adult alliance behaviour, including the regular formation of consortships with parous females, is not in evidence for males under the age of 14 (Connor *et al.* 2000a), rendering it unlikely that TOL and BOL had a stable alliance partner at the time they achieved a paternity. Hence, albeit with caution because of the limited behavioural data and the fact that BOL's paternity was assigned at an 80% confidence level, the paternities of BOL and TOL are regarded as having been achieved when they were juvenile animals without alliance partners. This is further supported by the finding that $LR_{HS/PO}$ for each paternity was 7.27 and 17.64, both significant results (Table 4). It is not known whether individual 590 had an alliance partner when he successfully reproduced because he was the only male from outside the core study area with no behavioural data. Hence, this individual was omitted entirely from all analyses of reproductive success for different alliance types.

For two assigned paternities survey or focal data were available indicating that the respective mothers were seen together with the assigned fathers 1 year prior to parturition, around the time of conception. BJA was seen with MIN during three surveys in September and October 1990, although no data on possible herding events were available. Focal data showed that NIC was actively herded by REA, HII and BOT for a month in August 1994, 1 year prior to HKI's birth.

Discussion

The number of assigned paternities is small, but there are five findings that require further discussion. (i) Adult males known to be in alliances enjoy the lion's share of reproduction in Shark Bay. (ii) Reproductive success appears to be significantly skewed within at least some strategy I first-order alliances, using paternity data at the 80% confidence level. (iii) A small number of paternities were obtained by juvenile males without any alliance partner at the time. (iv) One mating appears to be incestuous. (v) At the 80% confidence level, we could assign paternities to 16 out of 34 (47.1%) of the calves with maternal data, and were successful in only one out of 30 cases (3.3%) when there were no maternal genotypes available.

(i) Alliance formation among male dolphins in Shark Bay is clearly a strategy to obtain access to females (Connor *et al.* 1992a,b, 1999, 2001). Alliance formation in males has been hypothesized to increase male fitness either through mate guarding (Connor *et al.* 1992a,b; Kempenaers *et al.* 1995; Watts 1998) or female choice of males that synchronize their behaviour (Connor *et al.* 1992b; Trainer & McDonald 1995). Many mating systems represent variations of mate-guarding adapted to the spatial and temporal distribution of oestrous females (Clutton-Brock 1989). There is a positive correlation between alliance formation and reproductive success in horse stallions (*Equus caballus*), where low ranking males form alliances with unrelated males and father about 25% of all offspring, which is significantly more than other nonallied low-ranking males adopting a 'sneaking' strategy (Feh 1999). Our data show that the vast majority of offspring in the core study area are sired by males engaging in some kind of alliance behaviour in that same area. Therefore, it appears that our identification of an alliance member also predisposes us towards assigning him a paternity of a calf in the same area. There are two possible reasons for this finding: first, allied males actually achieve more paternities; or second, this pattern is an artefact of the logistical constraints on our study. This question will be resolved by further studies outside the core area.

(ii) Various models of group reproduction make predictions about the presence or absence of reproductive skew between group members (Vehrencamp 1983a,b; Cant 1997; Reeve 1998; Reeve *et al.* 1998; Kokko & Johnstone 1999; Crespi & Ragsdale 2000; Johnstone 2000). Which of these models are consistent with our data? There appears to be a skew in reproductive success at least within stable strategy I alliances: two males from two different alliances achieved all five paternities obtained by this alliance type, suggesting dominance in obtaining fertilizations by relatively few alliance members. Thus, our results are consistent with models that predict some skew: concession, manipulation and, to a lesser extent, the restraint model. However, the lack of precise predictions for the level of skew, and our incom-

plete data set, make firm tests of these models impossible, and further work will be required to test these models.

(iii) The fact that two juvenile males without alliance partners at the time obtained one paternity each (which were assigned at the 80% and 95% confidence levels, respectively) gives reason to assume that there might be an alternative mating strategy which does not involve allying with other males. It is not unusual to find males from the same population engaging in different mating strategies (Caro & Bateson 1986; Gross 1996). The maintenance of more than one strategy within a population is typically explained by different tactics having asymmetrical pay-offs and depending on environmental or endogenous cues, which indicate their probability of success (Gross 1996). Different male mating strategies within a population have been reported from only one other bottlenose dolphin study site: Sarasota Bay (Wells *et al.* 1987). However, it was shown recently that in the Sarasota Bay population, pairing is the norm and that solitary males are not single roving males, as previously described (Wells *et al.* 1987), but are actually young males in a transitional stage (Owen *et al.* 2002).

Until recently it was thought that male dolphins attain their social maturity gradually over several years, with a burst in testosterone levels in males about 8 years of age (Wells *et al.* 1987), leading to a distinction between physical maturity (ability to produce sperm) and social maturity (ability to successfully compete for females). Our results of juvenile males achieving paternity provide support for the statement that it is not useful to discriminate between physical and social maturity in male bottlenose dolphins (Connor *et al.* 2000a). Although immature male dolphins are not observed in alliances consorting adult females in the manner of adult males (Connor *et al.* 1992b), intromission and sexual play have been frequently observed in both Shark Bay and Sarasota (Connor *et al.* 2000b), which may reflect an opportunistic mating tactic as much as 'practice'. Similar mechanisms have been observed in Soay rams (*Ovis aries*), where a significant number of juvenile sires were assigned a paternity (Coltman *et al.* 1999).

(iv) Incest avoidance may counter other influences on reproductive skew (Haydock *et al.* 2001) and is of particular importance in species with limited dispersal of either sex, particularly in small populations (Smith *et al.* 1997; Griffin *et al.* 2003). Both genetic and behavioural data suggest that both sexes in Shark Bay dolphins are philopatric (Connor *et al.* 2000b; Krützen *et al.* 2004), which raises the possibility of incest and/or incest avoidance mechanisms. However, our data suggest that incest avoidance does not play a large role in the mating behaviour of Shark Bay dolphins. This finding is also supported by observations of sexually mature males herding their own mother (R. Connor, unpublished data). Although incestuous matings apparently do occur, their apparent low frequency and the fission–fusion structure and the large population size

should minimize the inbreeding coefficient. This suggestion seems to be supported by the nonsignificant population F_{IS} .

(v) Where are all the other fathers? Given the large number of potential males in the data set, the overall number of paternities assigned was low (47.1% for mother–offspring pairs, 3.3% for juveniles without maternal data). Determining parentage in marine mammals is quite demanding because of the lack of geographical boundaries, and the relative inaccessibility of most marine mammals makes their social structure difficult to elucidate. In general, success rates of paternity assignments in wild populations are usually below expectations. For instance, in polygynous pinnipeds, paternity was assigned to 46% and 29% of grey seal (*Halichoerus grypus*) pups on two different islands (Worthington Wilmer *et al.* 1999). The majority of the pups born at a particular colony were not sired by males found in that particular colony. Similarly, only 28% of Antarctic fur seals (*Arctocephalus gazelle*) pups could be assigned a father, although 90% of all candidate fathers were thought to have been sampled (Gemmell *et al.* 2001).

One obvious problem does not appear to afflict our study: inadequate numbers of genetic markers. The number of microsatellite loci used in this study appears to be high enough, because by using only eight hypervariable loci, there were no cases in which we had two candidate fathers for a calf with maternal data. However, to increase statistical significance, it would be desirable to use more microsatellite loci. In human paternity testing, it is not uncommon to obtain an average paternity index of 3.3 per locus (Okamoto *et al.* 2003). For instance, a PI of 10^7 , which should be possible to obtain using 12–14 loci with fairly equal allele frequencies, would be significant at the 95% level even if one had to assume that there were 500 000 candidate fathers! Given that microsatellite loci are already available for many taxa, or can be readily cloned (Zane *et al.* 2002), we recommend that future studies should use large numbers of loci, and use Bayesian inference as this approach will reduce the number of assumptions that need to be made, because large PI s swamp any prior assumptions. Even the possibility of mutations and/or erroneous genotypes as a result of mis-scoring can be implemented in such a framework, as there are likelihood ratio formulae available that take into account mutation rates in the germ line (C. Brenner, personal communication; <http://www.dna-view.com>).

Another explanation for the low success rate in paternity assignments is the combination of the population structure and the timing of our sampling. In a previous study, we showed that there is isolation by distance (Krützen *et al.* 2004). Within the core study area, however, microsatellite data suggested that gene flow was high enough to prevent population subdivision by drift. Thus, it is likely that there were unsampled candidate fathers in the eastern part of the bay. Additionally, four major first-order alliances from the core study area disappeared between 1989 and 1992,

about 3–5 years before biopsy sampling was initiated in Shark Bay (R. Connor and J. Mann, unpublished data). These males probably herded many of the females that were sampled during the course of this study so that the actual fathers of some calves in our data set may not have been available for sampling.

The relationship between paternity, alliance membership and genetic relatedness is crucial in seeking adaptive explanations for the formation of male alliances in dolphins. The data from this paternity study and a previous relatedness study of Shark Bay dolphins (Krützen *et al.* 2003) show that it will be possible to evaluate some of the predictions of current models of group formation given a larger data set. However, as yet, there are insufficient data to evaluate all of the predictions. Our study suggests that not only the reproductive success of allied males, but also that of nonallied juveniles, need to be incorporated into an adaptive framework that seeks to explain alliance formation in male bottlenose dolphins.

Acknowledgements

Special thanks to Hugh Finn, Michael Heithaus, Doro Heimeier, Per Berggren, Kerstin Bilgmann, Nick Gales, Helen McLachlan-Berggren, Leah Page, Colleen Sims and Rachel Smolker who helped by collecting samples and information in the field. Charles Brenner gave helpful information for the use of the Bayesian approach to assign paternities and the likelihood approach to discriminate between different genealogical relationships. Bill Amos gave valuable advice for the use of NEWPATXL. Anna Lindholm's comments improved earlier drafts of this manuscript. The Monkey Mia Dolphin Resort and Monkey Mia Wildlife Sailing supported us during our field studies. The field work was carried out under the permit #SF002958 issued by Conservation and Land Management. Ethics approval was given from the University of New South Wales (# 99/52). The study was partly funded by the Australian Research Council (ARC), the W. V. Scott Foundation (Australia), the Linnean Society of New South Wales (Australia), and the Dolphins of Monkey Mia Research Foundation (Australia and USA).

References

Alberts SC, Watts HE, Altmann J (2003) Queuing and queue-jumping: long-term patterns of reproductive skew in male savannah baboons, *Papio cynocephalus*. *Animal Behaviour*, **65**, 821–840.

Amos B, Barrett J, Dover GA (1991) Breeding system and social structure in the Faroese pilot whale as revealed by DNA fingerprinting. In: *Genetic Ecology of Whales and Dolphins* (eds Hoelzel AR, Donovan GP), pp. 255–268. International Whaling Commission, Cambridge.

Amos B, Schlötterer C, Tautz D (1993) Social structure of pilot whales revealed by analytical DNA profiling. *Science*, **260**, 670–672.

Bercovitch FB (1988) Coalitions, cooperation and reproductive tactics among adult male baboons. *Animal Behaviour*, **36**, 1198–1209.

Bradbury JW, Vehrencamp SL (1977) Social organization and foraging in emballonurid bats III: mating systems. *Behavioural Ecology and Sociobiology*, **2**, 1–17.

Brenner CH (1993) A note on paternity computation in cases lacking a mother. *Transfusion*, **33**, 51–54.

Brenner CH (1997) Symbolic kinship program. *Genetics*, **145**, 535–542.

Brenner CH, Weir BS (2003) Issues and strategies in the DNA identification of World Trade Center victims. *Theoretical Population Biology*, **63**, 173–178.

Cant MA (1997) A model for the evolution of reproductive skew without reproductive suppression. *Animal Behaviour*, **55**, 163–169.

Caro TM (1994) *Cheetahs of the Serengeti Plains*. University of Chicago Press, Chicago.

Caro TM, Bateson P (1986) Organization and ontogeny of alternative tactics. *Animal Behaviour*, **34**, 1483–1499.

Clapham PJ, Palsbøll PJ (1997) Molecular analysis of paternity shows promiscuous mating in female humpback whales (*Megaptera novaeangliae*, Borowski). *Proceedings of the Royal Society of London — Series B: Biological Sciences*, **264**, 95–98.

Clutton-Brock TH (1989) Mammalian mating systems. *Proceedings of the Royal Society of London — Series B: Biological Sciences*, **236**, 339–372.

Clutton-Brock TH, Harvey PH (1978) Mammals, resources and reproductive strategies. *Nature*, **273**, 191–195.

Clutton-Brock TH, Parker GA (1994) Sexual coercion in animal societies. *Animal Behaviour*, **5**, 1345–1365.

Coltman DW, Bancroft DR, Robertson A *et al.* (1999) Male reproductive success in a promiscuous mammal: behavioural estimates compared with genetic paternity. *Molecular Ecology*, **8**, 1199–1209.

Connor RC, Smolker RA, Richards AF (1992a) Two levels of alliance formation among male bottlenose dolphins (*Tursiops* sp.). *Proceedings of the National Academy of Sciences, USA*, **89**, 987–990.

Connor RC, Smolker RA, Richards AF (1992b) Dolphin alliances and coalitions. In: *Coalitions and Alliances in Humans and Other Animals* (eds Harcourt AH, de Waal FBM), pp. 415–443. Oxford University Press, Oxford.

Connor RC, Richards AF, Smolker RA, Mann J (1996) Patterns of female attractiveness in Indian Ocean bottlenose dolphins. *Behaviour*, **133**, 37–69.

Connor RC, Heithaus MR, Barre LM (1999) Superalliance of bottlenose dolphins. *Nature*, **397**, 571–572.

Connor RC, Read AJ, Wrangham R (2000a) Male reproductive strategies and social bonds. In: *Cetacean Societies* (eds Mann J, Connor RC, Tyack PL, Whitehead H), pp. 247–269. Chicago University Press, Chicago, London.

Connor RC, Wells RS, Mann J, Read AJ (2000b) The bottlenose dolphin — social relationships in a fission–fusion society. In: *Cetacean Societies* (eds Mann J, Connor RC, Tyack PL, Whitehead H), pp. 91–126. Chicago University Press, Chicago, London.

Connor RC, Heithaus MR, Barre LM (2001) Complex social structure, alliance stability and mating success in a bottlenose dolphin 'super-alliance'. *Proceedings of the Royal Society of London — Series B: Biology Sciences*, **268**, 263–267.

Cordero C, Eberhard WG (2003) Female choice of sexually antagonistic male adaptations: a critical review of some current research. *Journal of Evolutionary Biology*, **16**, 1–6.

Cox CR, Le Boeuf BJ (1977) Female incitation of male competition: a mechanism of mate selection. *American Naturalist*, **111**, 317–335.

- Crespi BJ, Ragsdale JE (2000) A skew model for the evolution of sociality via manipulation: why it is better to be feared than loved. *Proceedings of the Royal Society of London — Series B: Biological Sciences*, **267**, 821–828.
- Davies NB (1985) Cooperation and conflict among dunnocks, *Prunella modularis* in a variable mating system. *Animal Behaviour*, **33**, 628–648.
- Davis LG, Dibner MD, Batty JF (1986) *Basic Methods in Molecular Biology*, 1st edn. Elsevier Science Publishing, New York.
- Dunbar RIM (1981) Intraspecific variations in mating strategy. In: *Perspectives in Ethology* (eds Klopfer P, Bateson P), pp. 385–431. Plenum Press, New York.
- Edwards JH (1988) The use of multiple hypervariable probes for the probable detection or exclusion of incest. *Annual Review of Human Genetics*, **52**, 351–353.
- Evetts IW, Weir BS (1998) *Interpreting DNA Evidence*. Sinauer Associates, Sunderland, MA.
- Feh C (1999) Alliances and reproductive success in Camargue stallions. *Animal Behaviour*, **57**, 705–713.
- Garnier JN, Bruford MW, Goossens B (2001) Mating system and reproductive skew in the black rhinoceros. *Molecular Ecology*, **10**, 2031–2041.
- Gemmell NJ, Burg TM, Boyd IL, Amos W (2001) Low reproductive success in territorial male Antarctic fur seals (*Arctocephalus gazella*) suggests the existence of alternative mating strategies. *Molecular Ecology*, **10**, 451–460.
- Gilson A, Syvanen M, Levine K, Banks J (1998) Deer gender determination by polymerase chain reaction: validation study and application to tissues, bloodstains, and hair forensic samples from California. *California Fish and Game*, **84**, 159–169.
- Goudet J (1995) Fstat Version 1.2.: A computer program to calculate *F*-statistics. *Journal of Heredity*, **86**, 485–486.
- Griffin AS, Pemberton JM, Brotherton PNM *et al.* (2003) A genetic analysis of breeding success in the cooperative meerkat (*Suricata suricatta*). *Behavioral Ecology*, **14**, 472–480.
- Gross MR (1996) Alternative reproductive strategies and tactics: diversity within sexes. *Trends in Ecology and Evolution*, **11**, 92–98.
- Hamilton WD (1964a) The genetical evolution of social behaviour, I. *Journal of Theoretical Biology*, **7**, 1–16.
- Hamilton WD (1964b) The genetical evolution of social behaviour, II. *Journal of Theoretical Biology*, **7**, 17–52.
- Harcourt AH (1992) Coalitions and alliances: are primates more complex than non-primates?. In: *Coalitions and Alliances in Humans and Other Animals* (eds Harcourt AH, de Waal FBM), pp. 445–471. Oxford University Press, Oxford.
- Harcourt AH, Harvey PH, Larson SG, Short RV (1981) Testis weight, body weight and breeding system in primates. *Nature*, **293**, 55–57.
- Haydock J, Koenig WD, Stanback MT (2001) Shared parentage and incest avoidance in the cooperatively breeding acorn woodpecker. *Molecular Ecology*, **10**, 1515–1525.
- Hearne CM, Ghosh S, Todd JA (1992) Microsatellites for linkage analysis of genetic traits. *Trends in Genetics*, **8**, 288–294.
- Hoelzel AR, Dahlheim M, Stern SJ (1998) Low genetic variation among killer whales (*Orcinus orca*) in the eastern north pacific and genetic differentiation between foraging specialists. *Journal of Heredity*, **89**, 121–128.
- van Hooft JARAM, van Schaik CP (1994) Male bonds: affiliative relationships among nonhuman primate males. *Behaviour*, **130**, 309–337.
- Johnstone RA (2000) Models of reproductive skew: a review and synthesis. *Ethology*, **106**, 5–26.
- Kempenaers B, Verheyen GR, Dhondt AA (1995) Mate guarding and copulation behaviour in monogamous and polygynous blue tits – do males follow a best-of-a-bad-job strategy? *Behavioral Ecology and Sociobiology*, **36**, 33–42.
- Kokko H, Johnstone RA (1999) Social queuing in animal societies: a dynamic model of reproductive skew. *Proceedings of the Royal Society of London — Series B: Biological Sciences*, **266**, 571–578.
- Krützen M, Valsecchi E, Connor RC, Sherwin WB (2001) Characterisation of microsatellites in *Tursiops aduncus*. *Molecular Ecology Notes*, **1**, 170–172.
- Krützen M, Barre LM, Möller LM *et al.* (2002) A biopsy system for small cetaceans: darting success and wound healing in *Tursiops* spp. *Marine Mammal Science*, **18**, 863–878.
- Krützen M, Sherwin WB, Connor RC *et al.* (2003) Contrasting relatedness patterns in bottlenose dolphins (*Tursiops* sp.) with different alliance strategies. *Proceedings of the Royal Society London — Series B: Biological Science*, **270**, 497–502.
- Krützen M, Sherwin WB, Berggren P, Gales NJ (2004) Population structure in an inshore cetacean revealed by microsatellite and mtDNA analysis: Bottlenose dolphins (*Tursiops* sp.) in Shark Bay, Western Australia. *Marine Mammal Science*, **20**, 28–47.
- Lander E, Kruglyak L (1995) Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nature Genetics*, **11**, 241–247.
- Mann J, Connor RC, Barre LM, Heithaus MR (2000) Female reproductive success in bottlenose dolphins (*Tursiops* sp.): life history, habitat, provisioning, and group-size effects. *Behavioral Ecology*, **11**, 210–219.
- Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.
- Maynard Smith J (1982) *Evolution and the Theory of Games*. Cambridge University Press, Cambridge.
- McGraw KJ, Stoehr AM, Nolan PM, Hill GE (2001) Plumage redness predicts breeding onset and reproductive success in the house finch: a validation of Darwin's theory. *Journal of Avian Biology*, **32**, 90–94.
- Meagher TR (1986) Analysis of paternity within a natural population of *Chamaelirium luteum*. I. Identification of most-likely male parents. *American Naturalist*, **128**, 199–215.
- Möller LM, Beheregaray LB, Harcourt R, Krützen M (2001) Alliance membership and kinship in wild male bottlenose dolphins (*Tursiops aduncus*) of southeastern Australia. *Proceedings of the Royal Society of London — Series B: Biological Sciences*, **268**, 1941–1947.
- Nielsen R, Mattila DK, Clapham PJ, Palsbøll PJ (2001) Statistical approaches to paternity analysis in natural populations and applications to the north Atlantic humpback whale. *Genetics*, **157**, 1673–1682.
- Okamoto O, Yamamoto Y, Inagaki S *et al.* (2003) Analysis of short tandem repeat (STR) polymorphisms by the PowerPlex 16 system and capillary electrophoresis: application to forensic practice. *Acta Medica Okayama*, **57**, 59–71.
- Owen ECG, Wells RS, Hofmann S (2002) Ranging and association pattern of paired and unpaired adult male Atlantic bottlenose dolphins, *Tursiops truncatus*, in Sarasota, Florida, provide no evidence for alternative male strategies. *Canadian Journal of Zoology*, **80**, 2072–2089.
- Packer C, Gilbert DA, Pusey AE, O'Brien SJ (1991) A molecular genetic analysis of kinship and cooperation in African lions. *Nature*, **351**, 562–565.

- Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.
- Parsons KM, Durban JW, Claridge DE *et al.* (2003) Kinship as a basis for alliance formation between male bottlenose dolphins, *Tursiops truncatus*, in the Bahamas. *Animal Behaviour*, **66**, 185–194.
- Pena SDJ, Chakraborty R (1994) Paternity testing in the DNA era. *Trends in Genetics*, **10**, 204–209.
- Preen AR, Marsh H, Lawler IR, Prince RIT, Shepherd R (1997) Distribution and abundance of dugongs, turtles, dolphins and other megafauna in Shark Bay, Ningaloo Reef and Exmouth Gulf, Western Australia. *Wildlife Research*, **24**, 185–208.
- Reeve HK (1998) Game theory, reproductive skew, and nepotism. In: *Game Theory and Animal Behaviour* (eds Dugatkin LA, Reeve HK), pp. 118–145. Oxford University Press, Oxford.
- Reeve HK, Emlen ST, Keller L (1998) Reproductive sharing in animal societies: reproductive incentives or incomplete control by dominant breeders. *Behavioral Ecology*, **9**, 267–278.
- Rubenstein DI (1980) On the evolution of alternative mating strategies. In: *Limits to Action* (ed. Staddon JER), pp. 65–100. Academic Press, London.
- Say L, Naulty F, Hayden TJ (2003) Genetic and behavioural estimates of reproductive skew in male fallow deer. *Molecular Ecology*, **12**, 2793–2800.
- Smith D, Meier T, Geffen E *et al.* (1997) Is incest common in grey wolf packs? *Behavioral Ecology*, **8**, 384–391.
- Smolker RA, Richards AF, Connor RC, Pepper JW (1992) Sex-differences in patterns of association among Indian Ocean bottle-nosed dolphins. *Behaviour*, **123**, 38–69.
- Smuts BB, Smuts RW (1993) Male aggression and sexual coercion of females in nonhuman primates and other mammals: evidence and theoretical implications. *Advances in the Study of Behaviour*, **22**, 1–63.
- Summers K, Amos W (1997) Behavioural, ecological and molecular genetic analyses of reproductive strategies in the Amazonian dart-poison frog, *Dendrobates ventrimaculatus*. *Behavioral Ecology*, **8**, 260–267.
- Trainer JM, McDonald DB (1995) Singing performance, frequency matching and courtship success of long-tailed manikins (*Chiroxiphia linearis*). *Behavioural Ecology and Sociobiology*, **37**, 249–254.
- Valsecchi E, Amos W (1996) Microsatellite markers for the study of cetacean populations. *Molecular Ecology*, **5**, 151–156.
- Vehrencamp SL (1983a) A model for the evolution of despotic versus egalitarian societies. *Animal Behaviour*, **31**, 667–682.
- Vehrencamp SL (1983b) Optimal degree of skew in cooperative societies. *American Zoologist*, **23**, 327–335.
- Watts DP (1998) Coalitionary mate guarding by male chimpanzees at Ngogo, Kibale national park, Uganda. *Behavioural Ecology and Sociobiology*, **44**, 43–55.
- Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.
- Wells RS (1991) The role of long-term study in understanding the social structure of a bottlenose dolphin community. In: *Dolphin Societies: Discoveries and Puzzles* (eds Pryor K, Norris KS), pp. 198–225. University of California Press, Berkeley.
- Wells RS, Scott MD, Irvine AB (1987) The social structure of free-ranging bottlenose dolphins. In: *Current Mammalogy* (ed. Genoways H), pp. 247–305. Plenum Press, New York.
- Wilson B, Thompson P, Hammond P (1993) The ecology of bottle-nosed dolphins, *Tursiops truncatus*, in the Moray Firth. In: *European Research on Cetaceans 6* (ed. Evans PGH), pp. 114–115. Cambridge, European Cetacean Society.
- Wilson B, Thompson P, Hammond P (1993) An examination of the social structure of a resident group of bottlenose dolphins (*Tursiops truncatus*) in the Moray Firth, N. E. Scotland. In: *European Research on Cetaceans 7* (ed. Evans PGH), pp. 56–57. Cambridge, European Cetacean Society.
- Worthington Wilmer J, Allen PJ, Pomeroy PP, Twiss SD, Amos W (1999) Where have all the fathers gone? An extensive microsatellite analysis of paternity in the grey seal (*Halichoerus grypus*). *Molecular Ecology*, **8**, 1417–1429.
- Wrangham R (1980) Female choice of least costly males: a possible factor in the evolution of leks. *Zeitschrift für Tierpsychologie*, **54**, 357–336.
- Zane L, Bargelloni L, Patarnello T (2002) Strategies for microsatellite isolation: a review. *Molecular Ecology*, **11**, 1–16.

This work is the result of a collaborative effort of various researchers from different disciplines who are involved in the Dolphins of Shark Bay Research Project. Michael Krützen is a postdoctoral research associate at UNSW and is interested in cetacean social structure, molecular ecology and population genetics. This paper formed an integral part of his PhD thesis about paternity and relatedness among male bottlenose dolphins in Shark Bay, Western Australia. Lynne Barré is a marine mammal researcher with NOAA and was involved in most aspects relating to the field work of this study. Richard Connor is interested in the evolution of cooperation among male bottlenose dolphins. Janet Mann's research focuses on the behavioural ecology of female dolphins and the development of calves. Bill Sherwin's interests are in molecular ecology, conservation genetics and demographic modelling.

Appendix I

Allele frequencies for eight microsatellite loci used in this study.

	MK3	MK5	MK6	MK9	199/200	KW12	EV1	MK8							
147	0.0046	201	0.0022	145	0.0022	168	0.0024	114	0.0045	155	0.0024	141	0.0327	87	0.0023
151	0.0023	205	0.1550	147	0.0022	170	0.3071	116	0.0762	157	0.0942	143	0.0101	89	0.0721
157	0.0718	209	0.0087	149	0.1485	172	0.2714	118	0.4103	159	0.0024	145	0.3266	105	0.0766
159	0.0069	211	0.2795	151	0.0437	174	0.1738	120	0.1659	163	0.0048	147	0.0276	107	0.0901
161	0.0463	213	0.2467	153	0.2402	176	0.2238	122	0.2018	165	0.0048	149	0.0151	109	0.1847
163	0.5417	215	0.2598	155	0.0022	178	0.0190	124	0.0561	167	0.2488	151	0.0779	111	0.0495
165	0.1806	217	0.0087	157	0.0852	180	0.0024	126	0.0830	169	0.0072	153	0.0402	113	0.4617
167	0.0417	219	0.0393	161	0.0022			134	0.0022	171	0.2415	155	0.0779	115	0.0541
169	0.0995			163	0.0175					173	0.0072	157	0.1583	117	0.0068
171	0.0046			165	0.0109					175	0.1643	163	0.0101	119	0.0023
				167	0.0066					177	0.0072	169	0.0050		
				169	0.0808					179	0.0217	170	0.0327		
				171	0.0568					180	0.0024	172	0.0176		
				173	0.1092					182	0.0290	174	0.0678		
				175	0.0109					184	0.1063	176	0.0151		
				177	0.0175					186	0.0483	178	0.0302		
				179	0.0590					188	0.0072	180	0.0075		
				181	0.0131							182	0.0352		
				183	0.0371							184	0.0025		
				185	0.0240							186	0.0075		
				187	0.0197							190	0.0025		
				189	0.0066										