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Kinship as a basis for alliance formation between male bottlenose dolphins, *Tursiops truncatus*, in the Bahamas

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Alliances between reproductive males have been described in both cetacean and terrestrial mammal societies, and kin selection theory has often been invoked to explain these stable male coalitions. However, recent studies of bottlenose dolphins, *Tursiops aduncus*, in Australia suggest that mechanisms other than kinship are fundamental in the formation of male alliances. We supplemented skin biopsy sampling with the collection of faecal samples from individually recognized wild bottlenose dolphins, *T. truncatus*, to assess the role of kin selection in alliance membership in the northeast Bahamas. Stable alliances between pairs of males were identified based on association analyses of individual photo-identification data collected over 4 years. Molecular genetic analyses of tissue samples revealed highly significant correlations between patterns of association and both mitochondrial DNA haplotype identity and microsatellite relatedness, indicating that males within long-term alliances are more closely related than expected by chance. These data reinforce the high degree of plasticity previously reported within the genus *Tursiops*, and suggest that social and ecological differences between populations can markedly affect the role of kin selection in determining social alliances between male bottlenose dolphins.

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In the terrestrial environment, male associations and mating strategies have been recognized as an important component of mammalian social structure (Clutton-Brock 1989). Coalitions, or alliances, of males that act jointly to defend territories and secure mating access to females have been described for several mammalian species, namely lions, *Panthera leo* (Packer et al. 1988), cheetahs, *Acinonyx jubatus* (Caro & Collins 1987), mongooses, *Herpestes sanguineus* (reviewed in Waser et al. 1994), kinkajous, *Poto flavus* (Kays & Gittleman 1995), and primates (Pusey & Packer 1987). When members of these cooperating alliances are related, they can benefit both directly, if the alliance leads to enhanced reproductive success, and indirectly, through inclusive fitness resulting from enhanced reproductive success of kin (Hamilton 1963). The role of kinship in establishing and maintaining male alliances has been evaluated for several of these species (e.g. Caro & Durant 1991; Packer et al. 1991; Kays et al. 2000; Mitani et al. 2000). In particular, studies of African lions have shown that kinship is important for the maintenance of larger male coalitions (Packer et al. 1991).

Male alliances have also been reported for cetaceans, most notably bottlenose dolphins, *Tursiops truncatus* (Wells et al. 1987) and *Tursiops aduncus* (Connor et al. 1992; Möller et al. 2001). Within a typically fission–fusion society, membership within these alliances is often stable relative to other more ephemeral associations (e.g. Wells et al. 1987). Such stable alliances have been observed to function cooperatively both in agonistic interactions and in 'herding' to secure breeding access to receptive females (Connor et al. 1992, 2001). However, until relatively recently, it has proved difficult to obtain genetic samples from dolphins in the marine environment, particularly where individual variation in

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behaviour impedes the collection of samples by conventional techniques. Therefore, the lack of empirical data linking association patterns to levels of genetic relatedness in bottlenose dolphins has often hindered direct assessment of the kin selection hypothesis for *Tursiops* species.

In accordance with findings on male chimpanzee, *Pan troglodytes*, social groups (Mitani et al. 2000), recent studies have begun to suggest that, in some bottlenose dolphin populations, factors other than kinship are driving alliance membership. In contrast to hypotheses proposed earlier (Wells 1991), evidence based on direct observations and genetic analyses suggest that, in Sarasota Bay, Florida, U.S.A., members of male alliances tend not to be closely related (Connor et al. 2000). Similarly, recent examination of alliance membership and kinship in Port Stephens, southeastern Australia, supports these findings as evidenced by a lack of preference for kin among allied males (Möller et al. 2001). However, considering the high degree of cultural, taxonomic, morphological and ecological diversity of *Tursiops* species worldwide (Hersh & Duffield 1990; Ross & Cockcroft 1990; Hoelzel et al. 1998; LeDuc et al. 1999; Rendell & Whitehead 2001), it is unclear whether such patterns can be generalized to other populations.

In the present study, we evaluated the strength of associations between male *T. truncatus* in two subpopulations in the northeast Bahamas by examining long-term photographic data on individual patterns of association, and directly assessed their genetic relatedness. We estimated the degree of relatedness between males with both mitochondrial (mtDNA) and nuclear microsatellite DNA markers to assess directly the role of kin selection in the formation of male alliances.

METHODS

Study Site

We conducted boat-based surveys between June and November from 1997 to 2000, in two study sites along the east (26°33′N, 077°04′W) and south (26°00′N, 077°25′W) coasts of Great Abaco Island. The two sites are within the relatively shallow (<7 m) inshore waters of Little Bahama Bank in the northern Bahamas. Both direct observational data and molecular genetic analyses support the existence of two subpopulations (Parsons 2002), with limited movement of individuals between sites and a high degree of residency within the respective areas (Durban et al. 2000; unpublished data).

Field Data and Sample Collection

We documented group composition through photo-identification of the dorsal fin of each animal (Durban et al. 2000). Putative male alliances were identified from repeated dolphin encounters and ad libitum observations (unpublished data). Behaviours that were definitive of alliances included 'herding' or attempted herding of females (with or without dependent calves), 'formation' swimming (Connor et al. 1992), synchronous or apparently coordinated behaviours, and coordinated aggressive behaviours directed at conspecifics without the alliance.

We collected samples for genetic analysis from identified individuals from both sites. Skin samples were obtained opportunistically from free-swimming dolphins, with a remote biopsy technique (Barrett-Lennard et al. 1996). However, when tight and synchronous behaviours or low-profile surfacings prohibited biopsy sampling, or when collecting samples from calves and accompanying females, we used an alternative sampling technique involving the collection of faecal samples from identified individual dolphins (Parsons et al. 1999). When attempting biopsy sampling, we had to ensure we avoided nontarget individuals in the group, but this was not necessary with faecal sampling because simultaneous defecation by multiple, adjacent dolphins was never observed.

The quality of molecular data obtained from faecal samples can be comparable to that of biopsy samples, but it is considerably more costly and time consuming to obtain robust data when working with faecal DNA, and often the quality and quantity of DNA can be limiting when amplifying large or multiplexed fragments (Parsons 2001; Parsons et al. 2003). Furthermore, natural variability in the amount of dolphin DNA present in faecal samples occasionally necessitates collection of multiple samples from the same individual. In addition to skin tissue, biopsy samples also provided blubber cores that have been analysed for contaminants (unpublished data). For these reasons, biopsy sampling was selected as the primary method of sample collection.

We developed sampling protocols to minimize the impact of biopsy sampling on the dolphins (Parsons et al. 2003), and only a single biopsy sample was collected from each individual. Behavioural reactions to the dart impact varied between individuals; however, the intensity and variety of the reaction were comparable in those attempts that did and did not pull a sample. The majority (22 of 25) of the sampled individuals showed no visible reaction or at most a small tail flick and immediate dive. This reaction was similar to, and often less than, the startle reaction of an animal when the dart did not make contact with it, but struck the water nearby (Parsons et al. 2003). Throughout the study, 25 different dolphins were sampled by remote biopsy, and 44 faecal samples were collected representing 23 different dolphins.

The sex of individual bottlenose dolphins was determined either by direct observation of mammaries and the genital region, or by polymerase chain reaction (PCR) amplification of a gender-specific molecular marker (the mammalian SRY gene; see below). All field-based sex determinations were confirmed using the PCR-based method.

Permission for the study was granted by the Bahamas Ministry of Fisheries, and skin tissue samples were transported under CITES export (Bahamas) and import (U.K.) permits to K.M.P.

Table 1. Microsatellite loci used to calculate pairwise relatedness between individual dolphins, and the annealing temperature (°C) and $MgCl_2$ (mM) concentrations optimized for each locus

Locus	Source	Annealing temperature	$MgCl_2$	*k*
EV14a	Valsecchi & Amos 1996	TD65–55	1.5	7
EV37a	Valsecchi & Amos 1996	58	1.5	8
GATA098	Palsboll et al. 1997	TD60–50	1.5	4
D08	Shinohara et al. 1997	56	1.5	2
D14	Shinohara et al. 1997	TD60–50	1.5	5
D22	Shinohara et al. 1997	54	1.5	7
D28	Shinohara et al. 1997	56	1.5	7
TexVet5	Rooney et al. 1999	TD60–50	1.5	5
TexVet7	Rooney et al. 1999	TD60–50	1.5	4
DlrFCB1	Buchanan et al. 1996	TD65–55	1.5	7
DlrFCB2	Buchanan et al. 1996	TD65–55	1.5	5
DlrFCB4	Buchanan et al. 1996	TD65–55	1.5	9
DlrFCB5	Buchanan et al. 1996	TD65–55	1.5	4
KWM1b	Hoelzel et al. 1998	49	1.5	2
KWM2a	Hoelzel et al. 1998	50	0.75	6
KWM9b	Hoelzel et al. 1998	TD65–55	1.5	3
KWM12a	Hoelzel et al. 1998	45	0.75	5

TD65–55 refers to a 'touchdown' PCR programme consisting of 20 annealing cycles starting at 65°C for 30 s and dropping by 0.5°C per cycle, followed by 15 cycles of 30 s denaturation at 92°C and 30 s annealing at 55°C. *k* is the number of alleles resolved in a sample of 58 Little Bahama Bank bottlenose dolphins.

Analysis of Association Patterns

To analyse patterns of association, we used only high-quality photographic identifications, from groups for which all individuals were photoidentified. We calculated levels of association for all pairwise combinations of noncalf males, using the half-weight index (HWI; Cairns & Schwager 1987) which ranges from 0.0 for two animals that were never in the same group together, to 1.0 for constant associates. Association analyses were restricted to individuals that were photodocumented on at least three occasions.

Average linkage clustering was used to examine the patterns and strength of association between individual males. We compared the observed levels of association between all pairs of males to that expected if individuals were associating randomly, without preference or avoidance of potential associates. This expected distribution was based on five independent repetitions of 10 000 permutations of the original pairwise HWI matrix (Bejder et al. 1998), permuting individual dolphins among all groups within annual samples. For all association analyses and permutation tests we used the program SOCPROG (http://www.is.dal.ca/hwhitehe/social.htm) written for the MATLAB computing environment.

Stable male alliances were defined according to the following criteria: (1) groupings (pairs or trios) whose level of association (HWI) over 4 years was greater than the averaged maximum HWI for all males; (2) groupings that associated significantly more often than random; and (3) groupings that were reciprocal top associates, following Möller et al. (2001). These criteria provided a quantitative measure of the strength and stability of alliances, against which the putative alliances identified from behavioural field observations were examined.

Molecular Genetic Analyses

Total genomic DNA was extracted from tissue samples by standard proteinase K phenol/chloroform extraction protocols (Sambrook et al. 1989), and from faecal samples by the guanidine thiocyanate (GITC)/diatomaceous earth method (Parsons et al. 1999). Samples were genotyped at 17 polymorphic published cetacean microsatellite loci (Table 1). Alleles were amplified using the polymerase chain reaction containing γ-^{32}P-labelled forward primers. PCR reactions (10 µl) contained 1 × NH_4 buffer, 0.2 mM of each nucleotide, 0.25 µM of each primer and 0.25 units of *Taq* polymerase (Bioline), and PCR fragments were resolved by electrophoresis on 6% denaturing polyacrylamide gels (Sambrook et al. 1989). A multiple-tubes approach to PCR amplification of microsatellite loci was used for faecal DNA, to ensure that reliable genotypes were obtained (Parsons 2001). Mitochondrial control region haplotypes were determined for each individual by the direct sequencing of a 483-bp fragment, encompassing the hypervariable 5′ section of the mtDNA control region using the PCR primers L15926* (Eggert et al. 1998) and H16498 (Rosel et al. 1995).

Molecular sex of sampled dolphins was determined by the coamplification of a 147-bp fragment of the SRY gene (Richard et al. 1994) and a 211-bp microsatellite locus (EV37; Valsecchi & Amos 1996). Multiplexed PCR reactions (10 µl) contained 1.5 mM of $MgCl_2$, 1 × NH_4 buffer, 0.2 mM of each nucleotide, 0.50 µM of the SRY primers, 0.25 µM of the EV37 microsatellite primers and 0.5 units of *Taq* polymerase (Bioline). Fragments were amplified using a constant 58°C annealing temperature, and PCR products were separated on an ethidium-stained 2% agarose gel. Multiplexing the SRY marker with a microsatellite marker generated an internal positive control

reaction, thereby avoiding erroneous female assignment because of general PCR failure.

Statistical Analyses

To assess the role of kinship in establishing alliances between male bottlenose dolphins, we examined pairwise levels of relatedness between all pairs of sampled males, using both maternally inherited mtDNA haplotype and biparentally inherited microsatellite genotyping data. The significance of shared mitochondrial control region haplotypes (maternal relatedness) within alliance groupings was examined by calculating the probability of a chance occurrence. For this, we used a randomization test, in which 1000 pairs of haplotypes were randomly drawn from the distribution of mtDNA haplotypes resolved from our total dataset of 53 Abaco bottlenose dolphins (Parsons 2002). To account for site-specific differences in mtDNA variability, we treated the two study sites independently (east Abaco, N=32; south Abaco, N=21). These data were used to calculate the probability of two males having the same haplotype by random chance alone.

To test for the presence of a genetic basis for association patterns, we calculated two measures of genetic relatedness. A pairwise matrix of mtDNA dissimilarity between individual dolphins was generated using a binary scale for control region sequence haplotype identity (0=identical sequence; 1=different sequence). We also estimated microsatellite-based pairwise relatedness between males, with Lynch & Ritland's (1999) regression-based estimator. Relatedness (r) estimates were calculated with the computer program Delrious (http://www.ebc.uu.se/zooeko/JonS/DELRIOUS) and were generated with the allele frequency data obtained for 58 Little Bahama Bank bottlenose dolphins. This estimator is analogous to Queller & Goodnight's (1989) r estimate of genetic relatedness (i.e. $r_{\text{parent–offspring}} \approx 0.5$), but it yields lower sampling variances when applied to hypervariable, multilocus microsatellite data (Lynch & Ritland 1999). We used the Mantel test for matrix correlation to assess the degree of congruence between the calculated levels of association and the two estimates of genetic relatedness between all pairs of males. Statistical significance of the correlation coefficients was determined by comparison with both the standard normal variate and 1000 random permutations of the original matrix.

RESULTS

Over the 4 years, 423 dolphin groups were encountered, with a mean ± SD of 107 ± 36.2 individuals identified from high-quality photographs in any one year (Table 2). Field- and laboratory-based analyses confirmed the presence of 29 noncalf males that were regularly encountered within the study sites ($\bar{X} \pm$ SD=15.03 ± 9.15 encounters) in most years ($\bar{X} \pm$ SD=2.79 ± 0.90 years). Association analyses were based upon 73 groups that contained at least one male.

Table 2. Number of dolphin encounters photodocumented and association indices (HWI) for each alliance grouping

Alliance	Alliance members	1997–2000 HW
I	**Tt56 Tt58**	1.00*
II	**Tt211 Tt212**	0.93*
III	Tt69 Tt70	0.53*
IV	**Tt53 Tt76**	0.84*
V	**Tt42 Tt230**	0.86*§
VI	**Tt64 Tt65**	0.95*
VII	**Tt52 Tt73**	0.73*
VIII	**Tt532 Tt533**	1.00*
IX	**Tt536 Tt537**	0.88*
X	**Tt514 Tt516**	0.82*
XI	**Tt42 Tt72**	0.75*§
Total number of dolphin encounters		423
Number of encounters with noncalf males		173
Number of different dolphins identified		221

Alliances in bold met all three of the association criteria. During the study, Tt72 emigrated from east to south Abaco. Consequently, associations involving Tt72 were examined over two sequential 2-year periods. Alliances affected by Tt72's site transition are indicated by §. Strength of association between all other allied males was calculated over 4 years.

*Indicates statistically significant (α=0.05) associations based on permutation tests.

Strength of association between all pairs of males spanned the entire range of possible values from 0.0 to 1.0 ($\bar{X} \pm$ SD=0.08 ± 0.16, N=406 half-matrix pairwise comparisons), and null associations (HWI=0.0) occurred both within and between sites. Although the mean association did not deviate significantly from random expectation (real $\bar{X}$=0.08, random $\bar{X}$=0.08, P=0.81), the standard deviation of the observed association indices was significantly higher than that calculated from the random data (real SD=0.16, random SD=0.08, P<0.001), suggesting the presence of both preferred and avoided associations between males (Christal & Whitehead 2001). The strength of association between any male and his top associate (maximum HWI) ranged from 0.13 to 1.0 ($\bar{X} \pm$ SD=0.68 ± 0.27, N=29). We used the maximum HWI averaged over all males (HWI=0.68) as a benchmark against which the strength of putative male alliances was compared (Fig. 1, Table 2).

Eleven putative male alliance groupings were identified from behavioural observations (Table 2). All 11 alliances comprised reciprocal top associates, and only one alliance (Alliance III) had an association coefficient that was lower than the averaged maximum HWI (Table 2). The strength of association between top associates was significantly greater for males within alliance groupings ($\bar{X} \pm$ SD= 0.80 ± 0.18) than for nonallied males (0.32 ± 0.15; two-tailed Mann–Whitney U test: U=1.5, N_1=11 alliances, N_2=9 males, P<0.001). Furthermore, males in alliances associated significantly more often (P<0.05 for 10 000 permutations of the original pairwise association matrix) than would be expected if they associated randomly, thereby rejecting the null hypothesis of no preferred companionships between males. Ten of the male alliance dyads met all three of the association criteria outlined above (Table 2).

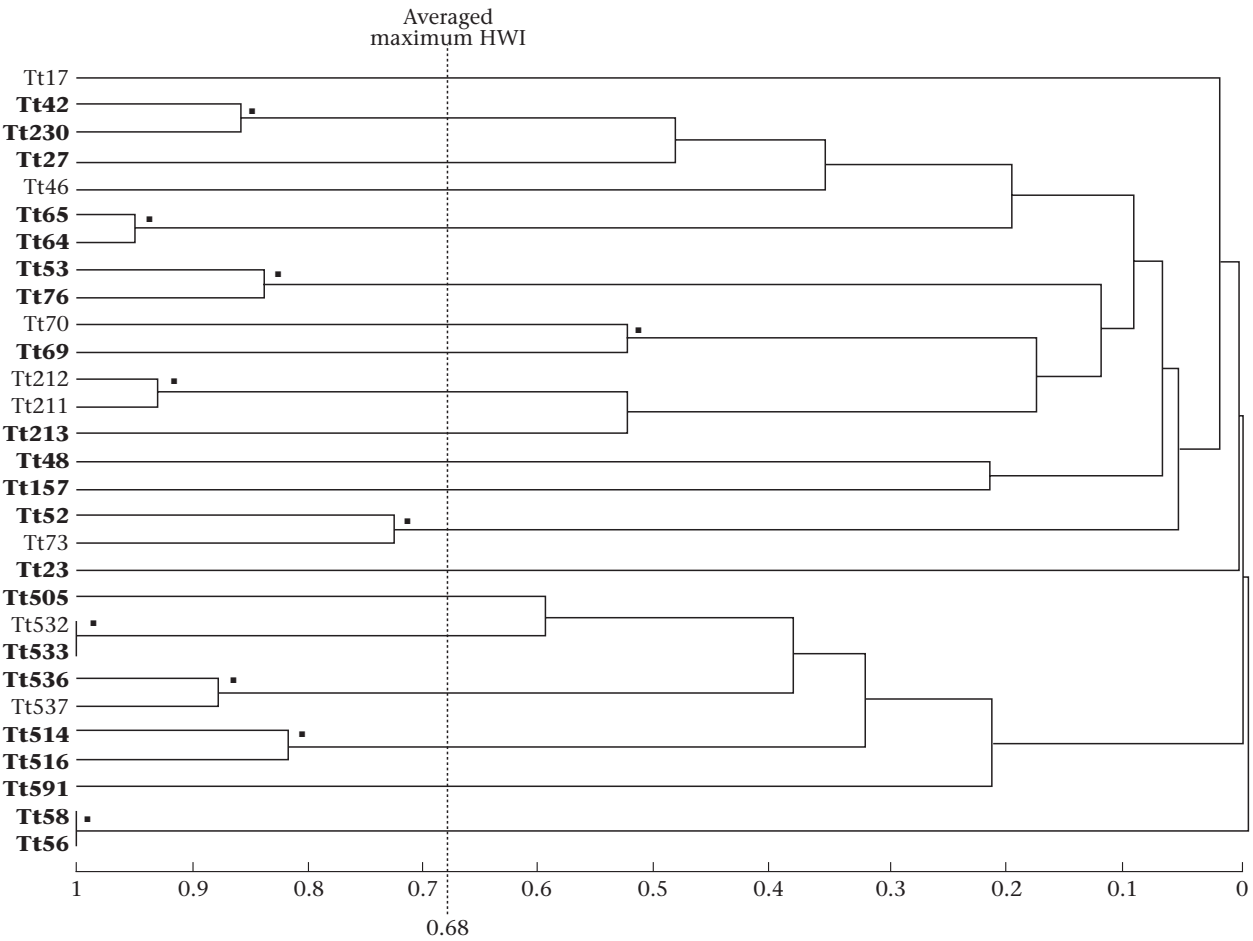

Figure 1. Dendrogram of half-weight index (HWI) measures of association between 29 noncalf males. Identification numbers in bold indicate individuals represented in the microsatellite and mtDNA relatedness analysis. An asterisk indicates the 10 male alliances identified from behavioural observations (Alliance XI is not indicated because it is possible to indicate only one of Tt42's two sequential alliances on the dendrogram).

Skin biopsy or faecal samples were collected for molecular genetic analyses from 21 of 29 identified males. Unambiguous genotypes were obtained for all 21 individuals at between 12 and 17 dinucleotide and tetranucleotide loci ($\bar{X}$=16). The mean number of alleles resolved per locus ± SD was 4.6 ± 1.9, and no locus was found to deviate from Hardy–Weinberg expectations. Analysis of 483 bp of mtDNA control region sequence for all 21 individuals revealed six unique haplotypes (Table 3), only one of which was common to both the east and south Abaco study sites.

Fourteen of the 21 sampled males were members of the 10 alliances that met all three association criteria, and six alliances were completely sampled. Each of the six fully sampled alliances was composed of two males with the same control region haplotype (Table 3). The proportion of allied pairs with identical mtDNA haplotypes (100%) was significantly greater than the proportion of all other pairs of males (i.e. pairs of males that were not allies) that shared a common haplotype (Fisher's exact test: P=0.0003; Fig. 2). Furthermore, given the frequency distribution of all resolved control region haplotypes in the Abaco bottlenose dolphin population (Parsons 2002), the probability of six alliances composed of two males sharing a common mtDNA haplotype occurring by chance alone is extremely low (randomization test: P=0.0048).

Estimates of genetic relatedness were calculated for all possible pairs of males (N=210) from the microsatellite genotyping data (Table 3). Despite the significance associated with maternal relatedness, within alliances, the strength of association was not significantly affected by the degree of relatedness ($F_{1,5}$=3.80, P=0.12), and the estimate of relatedness within alliances ($\bar{X}$ ± SD= 0.13 ± 0.07, N=6) only approached significance when compared to all pairs of nonallied males ($\bar{X}$ ± SD=0.01 ± 0.12, N=204; Mann–Whitney U test: U=338, P=0.062). However, the degree of genetic relatedness (r) was significantly correlated with the strength of association (HWI) calculated for all pairs of males (Mantel test: r=0.208, N=210 pairs, P<0.001; Table 4, Fig. 3). Furthermore, examination of strength of association with respect to mtDNA haplotypes revealed a highly significant correlation between haplotype identity and HWI (Mantel test: r=0.176, N=210 pairs, P<0.01; Table 4).

Table 3. Measures of pairwise relatedness (Lynch & Ritland 1999) between genotyped male bottlenose dolphins

			Dolphin identifier																				
mt	Alliance		Tt56	Tt58	Tt213	Tt69	Tt76	Tt53	Tt230	Tt42	Tt72	Tt64	Tt65	Tt157	Tt48	Tt52	Tt23	Tt533	Tt505	Tt536	Tt516	Tt514	Tt591
B	I	Tt56																					
B	I	Tt58	**0.26**																				
B		Tt213	0.07	0.13																			
B	III	Tt69	0.11	0.08	−0.05																		
B	IV	Tt76	0.14	0.32	0.17	0.02																	
B	IV	Tt53	−0.02	0.16	−0.08	0.23	**−0.02**																
A	V	Tt230	−0.13	−0.20	−0.08	−0.06	−0.11	−0.06															
A	V&XI	Tt42	−0.04	0.29	0.07	0.59	0.21	0.33	**0.01**														
A	XI	Tt72	−0.03	−0.06	0.00	0.11	−0.04	0.10	0.03	**0.10**													
D	VI	Tt64	0.06	0.07	−0.11	0.04	0.07	0.22	−0.19	−0.04	−0.04												
D	VI	Tt65	−0.01	0.17	0.00	0.00	0.22	0.09	0.26	0.11	0.12	**0.43**											
E		Tt157	−0.16	−0.12	0.03	−0.19	0.23	−0.09	−0.05	−0.15	−0.13	−0.12	−0.04										
B		Tt48	−0.02	−0.07	−0.06	0.00	−0.08	0.06	0.04	−0.04	0.08	−0.03	0.07	−0.02									
A	VII	Tt52	−0.02	−0.03	−0.13	−0.05	−0.07	0.02	0.02	0.08	0.25	0.13	0.11	−0.06	0.04								
B		Tt23	−0.04	0.15	0.11	0.18	−0.01	0.24	−0.20	0.05	−0.10	0.13	−0.21	−0.04	0.00	0.03							
I	VIII	Tt533	−0.02	−0.03	−0.08	−0.15	−0.07	−0.05	0.09	−0.13	−0.12	0.03	0.03	−0.04	0.00	−0.02	−0.18						
B		Tt505	0.06	0.23	0.06	−0.05	−0.06	−0.05	−0.12	−0.03	0.00	−0.07	−0.04	−0.10	−0.01	−0.02	0.14	−0.13					
H	IX	Tt536	−0.14	−0.09	0.05	−0.14	−0.08	−0.09	−0.09	−0.08	−0.21	−0.06	−0.14	−0.06	0.10	0.06	0.09	0.00	0.14				
H	X	Tt516	0.06	−0.09	−0.08	−0.04	−0.02	−0.11	−0.12	0.01	−0.08	0.00	−0.04	−0.11	−0.02	0.00	−0.09	0.07	0.08	0.20			
H	X	Tt514	−0.05	0.22	0.06	−0.07	0.15	0.08	−0.15	0.13	−0.04	−0.16	0.05	−0.12	0.09	−0.09	0.08	−0.08	0.01	0.35	**−0.02**		
B		Tt591	0.01	0.16	0.23	−0.04	−0.09	−0.17	−0.17	−0.05	0.20	−0.08	−0.12	−0.12	−0.05	0.07	−0.01	−0.02	0.30	0.05	0.01	0.01	

The 'mt' column denotes mtDNA control region haplotypes (GenBank accession numbers. AF155160–AF155162, AF378176–AF378178). Roman numerals in column 2 are alliance identifiers. Relatedness values within alliances are in bold.

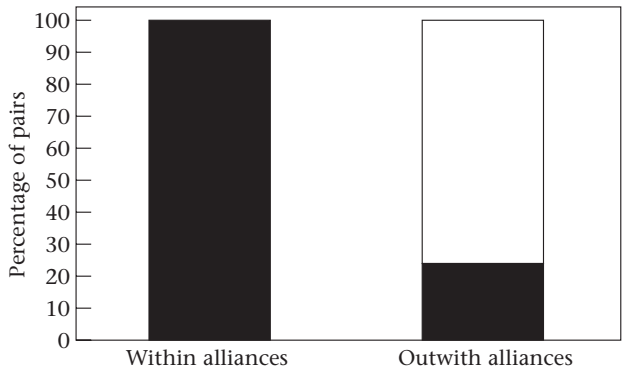

Figure 2. The frequency of shared mtDNA control region haplotypes between pairs of male bottlenose dolphins within alliances (N=6) and between males not in alliances (N=204). ■: Male pairs with the same mtDNA haplotype; □: male pairs with different haplotypes.

DISCUSSION

Our results provide evidence that kin selection may be important in the formation of male alliances between bottlenose dolphins. The formation of alliances is a striking feature of the social structure of bottlenose dolphins that has been similarly described in several geographical regions (Sarasota Bay, Florida, U.S.A., Wells et al. 1987; Shark Bay, Western Australia, Connor et al. 1992; Abaco, Bahamas). Such alliances appear to function within a reproductive context (Wells 1991; Connor et al. 1992, 2001) and, as such, relatedness between alliance members could markedly affect the benefits accrued by cooperating males.

The integration of both behavioural and molecular methods allowed us to identify and characterize 11 male alliances in this study. Although the strength of association varied between alliances, the patterns of preferential association were consistently significant. Unlike other regions that have documented dyads, triplets and quadruplets of bottlenose dolphins operating as coalitions (Wells 1991; Connor et al. 1992; Möller et al. 2001), all of the alliances we identified were made up of pairs of males. Both mtDNA haplotype identity and microsatellite-based relatedness were significantly correlated with the strength of association between pairs of males, suggesting that males spend more time associating with conspecifics to whom they are more closely related. Furthermore, for each allied pair identified and genetically typed there was a single mtDNA control region haplotype. The probability of all six alliances comprising two random males of the same haplotype forging an alliance by chance alone is extremely low, indicating that maternal relatedness is an important factor in determining alliance membership. Together, these data suggest that alliances between male bottlenose dolphins in the Bahamas are formed between maternal relatives.

Evidence from long-term studies suggest that bottlenose dolphins are philopatric, with adult males occupying larger home ranges than females (Scott et al. 1990; Connor et al. 2000). As such, it is possible that closely related males would forge strong associations simply because of frequent chance encounters. However, because our data suggest that the probability of all alliances comprising two maternally related males affiliating by chance is extremely low, it is likely that some other factor facilitating kin recognition is operating. Related males may be able to distinguish one another as kin on the basis of a selection criterion, such as acoustic identification of conspecifics. The unique signature whistles of male calves are remarkably similar to that of their mother (Sayigh et al. 1990), and this acoustic signature may function as a method of evaluating the matrilineal origin of potential allies. Because male alliances appear to function as a mating strategy through agonistic interactions with other alliances, and consortships with receptive females (Connor et al. 1992, 1999), kin-based evaluation of long-term allies would enhance the inclusive fitness of allied dolphins.

Kin selection theory is often used to explain a variety of social interactions and cooperative behaviours. However, recent work suggests that the influence of genetic relatedness on patterns of affiliation and cooperation is not constant across taxa, nor within the genus *Tursiops*. A parallel study in southeastern Australia (Möller et al. 2001) has revealed a lack of kinship within male *T. aduncus* alliances, suggesting the existence of multiple mechanisms for the evolution of coalitions. The absence of kin selection within social groups may be caused by morphological differences between the two *Tursiops* species (Möller et al. 2001). Alternatively, a lack of kin-biased behaviours in some populations may occur where there is a high probability of incorrectly identifying kin (Keller 1997). However, this would seem an unlikely reason for differences between bottlenose dolphin populations, where individually unique signature whistles appear to offer a reliable method of individual recognition and kin discrimination (Smolker et al. 1993; Janik & Slater 1998).

Another explanation concerns the underlying amount of genetic relatedness within the community. An apparent lack of nepotism, or kin selection, may be caused by the benefits of such behaviours being offset by the cost incurred by other group members (Keller 1997). As such, selection against kin-biased behaviours is expected where there is little variance in relatedness between community members. Direct comparison of variance in estimated genetic relatedness between the Australian and Bahamas dolphin populations is not possible, because of the different number of microsatellite loci used in the two studies (Port Stephens, N=9 loci; Abaco, N=17 loci). However, if mitochondrial genetic variability is considered, we see a lower variance in mtDNA relatedness in the Australian dolphins. In a comparable number of dolphins, six unique mtDNA haplotypes were identified among the males sampled in the Bahamas population (N=21), whereas only three haplotypes were identified among the Australian males (N=20; Möller et al. 2001). Furthermore, all sampled male alliances in the Bahamas comprised two dolphins with identical mtDNA haplotypes, suggesting that alliances form largely, if not exclusively, between maternally related males. This pattern appears to support the hypothesis that kin selection is favoured within groups where greater

Table 4. The correlation between pairwise levels of association between males and measures of genetic relatedness (N=210 pairs)

	Strength of association (HWI)	Relatedness (r)	MtDNA haplotype identity
Strength of association (HWI)	—	0.001 (0.001)	0.010 (0.015)
Relatedness (r)	0.222**	—	0.0005 (0.002)
MtDNA haplotype identity	0.176*	0.237**	—

Correlation coefficients (Mantel test) are displayed in the lower matrix (*P<0.025; **P<0.01). Values in the upper matrix are significance values based on the standard normal variate, and, in parentheses, based on 1000 random permutations of the original matrix.

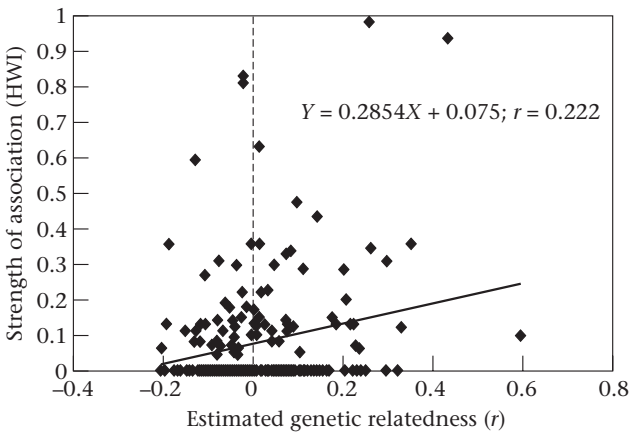

Figure 3. Strength of association (HWI) as a function of estimated genetic relatedness for all possible pairs (N=210) of male bottlenose dolphins in the Bahamas ($F_{1,209}$=10.76, P=0.0012).

differences in relatedness exist between community members. Consequently, altruism directed at one close relative should not occur at the cost of an equally close relative (Keller 1997; Griffin & West 2002). This may also explain the existence of larger (triplet and quadruplet) alliance groupings in Port Stephens, southeastern Australia. If a male is more or less equally related to all other males in his population, cooperation with multiple allies could overcome the cost incurred by choosing one relative over another. Estimation of relatedness between males in other regions such as Shark Bay, western Australia, where both triplet and second-order alliances have been documented (Connor et al. 1992, 1999), would prove valuable for evaluating this hypothesis further.

Finally, differences in the role of kinship in the establishment of male alliances may be attributed to interpopulation differences in dispersion and the ratio of reproductive males to females (operational sex ratio). Observations from societies of social insects indicate that levels of violence among kin are positively correlated with the importance of winning a fight (Griffin & West 2002). Similarly, the prevalence of alliances within a dolphin population and the importance of kinship to alliance membership may be inversely correlated with the importance of forming an alliance. If alliance membership markedly enhances a male's reproductive fitness, then joining an alliance may prove beneficial whether or not other alliance members are close relatives. The contrasting patterns of kinship in Port Stephens and Abaco may be caused by differences in the number of receptive females available, or their spatial distribution and defensibility. In a recent review, Connor et al. (2000) addressed some of these possible scenarios, but further empirical studies with directly comparable methodologies are required to evaluate hypotheses concerning the effect of such interpopulation differences on bottlenose dolphin social organization.

Despite the pattern of genetic relatedness observed within male alliances in the Bahamas, Hamilton's theory of inclusive fitness (Hamilton 1963) cannot be unequivocally invoked to explain these patterns. As Griffin & West (2002) showed in a review, a high degree of relatedness between cooperating individuals is not, in itself, sufficient evidence that kin selection is operating. Direct evaluation of the relative reproductive success of male bottlenose dolphins using contrasting mating strategies would further our understanding of the potential contributions of both direct and indirect fitness benefits. Although the mechanism and reproductive benefits of alliance formation remain to be tested, the patterns of genetic relatedness we observed suggest that kin selection may play a role in the development of long-term alliances between male bottlenose dolphins in certain populations, further emphasizing the cultural and ecological diversity of *Tursiops* populations globally.

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