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*Proc. R. Soc. B* published online 14 April 2010  
doi: 10.1098/rspb.2010.0039

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# Inbreeding tolerance and fitness costs in wild bottlenose dolphins

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In wild populations, inbreeding tolerance is expected to evolve where the cost of avoidance exceeds that of tolerance. We show that in a wild population of bottlenose dolphins found in East Shark Bay, Western Australia, levels of inbreeding are higher than expected by chance alone, and demonstrate that inbreeding is deleterious to female fitness in two independent ways. We found that inbred females, and females with inbred calves, have reduced fitness (lower calving success). We further show that one of the costs of inbreeding is extended weaning age, and that females' earlier calves are more likely to be inbred. While the exact causes of inbreeding remain obscure, our results indicate that one factor is female age, and thus experience. Any inbreeding avoidance mechanisms such as female evasion of kin, or male dispersal, do not seem to be completely effective in this population, which supports the view that inbreeding avoidance does not always evolve wherever inbreeding incurs a cost.

**Keywords:** inbreeding; inbreeding depression; fitness; mammals; dolphins

## 1. INTRODUCTION

Inbreeding tolerance might evolve where the cost of inbreeding avoidance exceeds that of tolerance (Waser *et al.* 1986; Kokko & Ots 2006). Where inbreeding is more costly than avoidance, natural selection is expected to favour the choice of mates with maximum genetic dissimilarity (Amos *et al.* 2001; Hoffman *et al.* 2007), dispersal from the natal site (Greenwood 1980) or kin discrimination (Blouin & Blouin 1988). Otherwise, inbreeding tolerance (Waser *et al.* 1986) should evolve, for example, when dispersal costs are high (Packer 1979), when subordinate males in polygynous mating systems benefit from inbreeding (Bateson 1983), or when male sexual coercion is strong.

Inbreeding depression (e.g. reduced fitness of inbred matings) has been extensively investigated in captive populations (e.g. Ballou & Ralls 1982; Charlesworth & Charlesworth 1987; Zschokke & Baur 2002; Cassinello 2005) and recently also in wild populations (e.g. Acevedo-Whitehouse *et al.* 2003; Charpentier *et al.* 2005; Da Silva *et al.* 2006; Cohas *et al.* 2009). Some species, however, seem to tolerate certain levels of inbreeding. For instance, species such as the song sparrow *Melospiza melodia* (Keller 1998), the dwarf mongoose *Helogale parvula* (Keane *et al.* 2004) and the naked mole rat *Heterocephalus glaber* (Reeve *et al.* 1990) show high levels of inbreeding, but inbreeding depression was only documented in the song sparrow (Keller 1998). Other studies have shown that moderate levels of

inbreeding may be beneficial. Indeed, theory predicts that in some cases the advantages of mating with close kin can override the effects of inbreeding depression (Lehmann & Perrin 2003; Kokko & Ots 2006; Parker 2006; Thunken *et al.* 2007). For instance, both sexes of African cichlid (*Pelvicachromis taeniatus*) prefer mating with unfamiliar close kin over non-kin, suggesting the presence of inclusive fitness benefits to inbreeding (Thunken *et al.* 2007). A study conducted on a population of Icelanders (*Homo sapiens*) born between 1800 and 1965 showed a nonlinear interaction between kinship and fertility, with the greatest reproductive success found between couples showing degrees of kinship in the order of third and fourth cousins (Helgason *et al.* 2008). In short, it appears that the relationship between inbreeding and Darwinian fitness favours an optimal degree of inbreeding.

The wild population of bottlenose dolphins (*Tursiops* sp.) found in Shark Bay, Western Australia may be vulnerable to inbreeding. Complexities of their social system include sexual coercion (Connor *et al.* 1992a, 1999), male alliance formation (Connor *et al.* 1992a,b, 2000, 2001), extensive maternal care (Mann *et al.* 2000), philopatry of both sexes (Connor *et al.* 2000), kin association (Krützen *et al.* 2004a) and an open fission–fusion social system (Smolker *et al.* 1992; Connor *et al.* 2000). In this population, inbreeding might be more costly to females than males, given the prolonged period of nursing and maternal investment (Mann *et al.* 2000), and the lack of any paternal investment beyond access to mating. Males form long-term alliances of up to 14 animals (Connor *et al.* 1992a,b, 2000, 2001) that use sexual coercion towards females during their oestrus cycle, as well as

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to prevent other alliances from accessing the female (Scott *et al.* 2005; Connor *et al.* 2006). Therefore, inbreeding avoidance could occur through either mate evasion or choice, but the mechanisms involved for either case are not understood. Additionally, the presence of sex-segregation in adult association patterns might also allow females to avoid related males. Although both males and females are philopatric, males and females are generally found in separate groups (Smolker *et al.* 1992; Gibson & Mann 2008*a,b*). For example, Gibson & Mann (2008*b*) show that adult females associate with juvenile and adult males far less than expected.

In this study, we investigated the degree of inbreeding of 58 calves. We show that levels of inbreeding are higher than expected if mating was random. We document the effect of inbreeding on the mothers' fitness measured as calving success (number of calves successfully reared to 3 years or older). We show that one of the costs of this inbreeding is in extended weaning age, and that this cost is disproportionately allocated to females' earlier calves.

## 2. MATERIALS AND METHODS

### (a) *Study site*

Shark Bay is situated 850 km north of Perth in Western Australia (25°47' S, 113°43' E). The population of bottlenose dolphins (*Tursiops* spp.) has been the focus of extensive study since the mid-1980s (Connor & Smolker 1985; Smolker *et al.* 1992), making it, along with the Sarasota Florida population (Wells *et al.* 1987; Wells & Scott 1999) one of the most comprehensive and detailed studies of bottlenose dolphins.

### (b) *DNA sampling of mother–calf pairs*

A total of 58 mother–calf pairs from 41 mothers were used in this analysis. Since 1994, skin biopsy samples have been collected during boat surveys using a system especially designed for small cetaceans (Krützen *et al.* 2002). Biopsy tissues were stored in a saturated solution of 5 M NaCl/20 per cent (v/v) dimethyl sulphoxide solution (Amos & Hoezel 1991). Most calves were not sampled until they were at least 3 years old, but 2 years was the youngest age at which calves could be sampled. Maternity was determined behaviourally by observing consistent association between calf and presumed mother, as the calf regularly swims in 'infant position' under the mother, where all nursing takes place (Mann & Smuts 1998). Allo-nursing has never been observed and there are a few (five) instances in 22 years of observation where a calf swam in infant position (lasting only for very brief seconds typically) with a non-mother (Mann & Smuts 1998). The maternity of the mother–calf pairs used in this study was also genetically confirmed.

### (c) *Molecular measures of inbreeding*

Genomic DNA was extracted and genotyped using a total of 16 microsatellites: 14 tetranucleotide loci (E12, Tur4\_66, 87, 91, 98, 105, 108, 111, 117, 128, 138, 141, D8, F10; 55, (Nater *et al.* 2009)), and two dinucleotide microsatellite loci (D22 (Shinohara *et al.* 1997), and MK6 (Krützen *et al.* 2004*b*)). Out of those 16 loci, four were initially not found to be in Hardy–Weinberg equilibrium (observed heterozygosity was smaller than expected) when the 430 East Shark Bay sampled dolphins ( $n = 430$ ) were pooled into one population. However, when those 430 dolphins were divided into the five subpopulations proposed in Krützen *et al.* (2004*b*) and after sequential Bonferroni correction

(Rice 1989), all loci were in Hardy–Weinberg equilibrium (Kopps 2007; Nater *et al.* 2009). We took several measures to control problems related to insufficient DNA concentration such as allelic drop-out and scoring errors. First, we used only microsatellite loci cloned from the population under investigation, except D22, thus lowering the chance of null-allele occurrence owing to origin of microsatellites from phylogenetically distant species. Second, polymerase chain reaction was carried out only in individuals where DNA concentration was higher than  $10 \text{ ng } \mu\text{l}^{-1}$ , as there is a strong negative correlation between the amount of template DNA used and the occurrence of allelic dropout (Morin *et al.* 2001). The overall scoring error among all 16 loci used in this study was estimated to be 0.121 per cent (Nater *et al.* 2009), based on the formula provided by Hoffman & Amos (2005) for mother–calf pairs, and is unlikely to have a significant influence on the overall results, because internal relatedness is averaged over 16 loci. Finally, only individuals with no missing data were included in the analysis.

Inbreeding occurs via two distinct mechanisms: inbreeding as a result of choice of relatives or inbreeding as a result of restricted population size (Templeton & Read 1994; Keller & Waller 2002). In this study, we are concerned with inbreeding resulting from a choice of relatives. While parentage analyses and pedigree reconstruction are better methods to assess inbreeding (choice of relatives) (Keller & Waller 2002), both methods require extensive datasets and long-term studies (Pemberton 2004), which are difficult to achieve in species with slow life histories, such as bottlenose dolphins. Thus, we chose to investigate the extent of within-individual genetic diversity of 58 successfully reared calves (3 years or older) and their mothers using three molecular metrics of inbreeding (internal relatedness (IR), Amos *et al.* 2001); homozygosity by loci (Aparicio *et al.* 2006) and standardized heterozygosity (Coltman *et al.* 1999). Although there has been a great deal of debate around which molecular metrics best measure true inbreeding (e.g. Aparicio *et al.* 2006), we found that, in our study, and as predicted by Chapman *et al.* (2009), all three molecular metrics strongly correlate with each other (all  $r$ 's  $> 0.9$ , data not shown). Thus, we present only the results obtained with IR. We calculated IR for each calf (c-IR) and mother (m-IR).

### (d) *Measurement of calving success*

Out of the 41 mothers available in this study, calving success data were available for a total of 32. Assessment of inbreeding effects must be made for all individuals at the same stage of the life cycle. Ideally, we would be able to follow inbred calves throughout their breeding lives, analysing their survival and reproduction. However, because calves do not reach sexual maturity until the age of 11–12, it was not feasible to follow the lifetime survival and reproduction of the calves. Therefore, other approaches were taken to make partial estimates of the effect of inbreeding on calving success. Female calving success (Cs) was defined as the number of offspring surviving to 3 years divided by the number of years of reproductive data available for that particular female. As stated above, inbreeding depression must always be assessed at some defined point in the life cycle, and we choose to assess it at the age of 3 years because although most calves nurse beyond 3 years, no unweaned nursing calves older than 3 years of age have died before weaning (of 317 calves with known survival outcomes), indicating that calves surviving until the age of 3 will usually

reach juvenile (weaned) status. If a female was of known age, her total reproductive years were counted from her twelfth year (Mann *et al.* 2008). Otherwise, reproductive years were counted from her first known birth.

#### (e) Measurement of weaning age

To investigate the relationship between the age at which dolphins were weaned and their internal relatedness (c-IR), we used 53 calves for which we had precise weaning ages (known birth and weaning month, accurate  $\pm$  six months). Weaning age was estimated using information on three major behaviour changes: first, the last sighting of the calf in infant position swimming with the mother; second, a significant decrease in mother–calf association (to less than 50%); and third, birth of a sibling (for full description see Mann *et al.* 2000). Based on these two measures alone, we found that all calves were weaned several months before the birth of the next sibling. If there was a gap in sightings, then weaning date was assigned three months prior to sibling birth.

#### (f) Does there appear to be inbreeding in East Shark Bay?

Inbreeding tolerance or avoidance would, respectively, lead to calves having lower (inbreeding tolerance) or higher (inbreeding avoidance) levels of genetic variability when compared with levels expected if mating is random. To test this, we used a Monte Carlo simulation implemented in STORM v. 1.0 (Frasier 2008). This analysis, which is described in Frasier (2008), generates c-IR values expected from the gene pool if mating is random with respect to parental relatedness. To do so, we generated simulated c-IR measures by sampling random males ( $n = 31$ ) and females ( $n = 93$ ) with replacement. Each random mating pair produces a simulated offspring whose internal relatedness can be measured (c-IR). Fifty-eight simulated offspring were generated and their simulated c-IR values were then averaged. This average of simulated c-IR was recalculated 1000 times. In order to test significance, the observed mean c-IR was compared with the distribution of average simulated c-IR.

To test the hypothesis of significant inbreeding further, we tested the covariance in heterozygosity across markers, using the ‘heterozygosity–heterozygosity correlation’ method (Balloux *et al.* 2004; Chapman *et al.* 2009). This test is based on the assumption that a positive ‘heterozygosity–heterozygosity correlation’ indicates a genome-wide effect that is probably caused by inbreeding (Pemberton 2004). Hence, we randomly divided our sample of 16 loci into two groups of eight loci, and investigated whether the IR of the first group correlates with the IR of the second group. This procedure was repeated 100 times to obtain a mean and standard error for the correlation as suggested by Balloux *et al.* (2004).

And finally, as a third test, we investigated the correlation between the internal relatedness of calves and the relatedness of their parents ( $n = 28$ ). Paternities were assigned using CERVUS 3.0 (Kalinowski *et al.* 2007). Eighty-seven per cent of paternities were assigned on a 95% confidence level and the others on 80% confidence level (Kopps 2007). Biparental relatedness between mothers and assigned fathers was measured using the Queller & Goodnight’s relatedness estimate (Queller & Goodnight 1989) based on its high level of precision, accuracy and ease of computation (Krützen *et al.* 2003).

Table 1. GLMM predictors of mothers’ calving success (Cs,  $n = 31$ ) following a binomial distribution. (Mothers’ identity was added to the model as random effect.)

predictors	average effect	s.e.	<i>z</i> -value	<i>p</i>
m-IR	−0.64181	0.250	−2.56	0.01
c-IR	−1.39663	0.310	−4.50	0.00001
m-IR $\times$ c-IR	−3.07254	2.121	−1.45	1

#### (g) Does inbreeding have a cost in East Shark Bay?

To analyse potential costs associated with inbreeding, we investigated the interactions between mothers’ Cs and both their m-IR and the mean internal relatedness of their calves (c-IR), fitting a generalized linear mixed model (GLMM). GLMMs incorporate two statistical frameworks; linear mixed models (which allows for random effects) and generalized linear models, which deal with non-normal data by using link functions and the exponential family (Bolker *et al.* 2009). In our GLMM model, mother’s calving success was treated as the response variable, and to account for heterogeneity, we weighted the model by the number of years each female was followed and the total number of calves recorded during that period. As fixed factors, we initially included m-IR, c-IR, calves’ weaning age and the mother’s age at calf’s birth, but only significant interactions are reported. We incorporated the mothers’ identity as a random factor, in order to control for individual effects. Because calving success is a ratio, the error structure was fitted with a binomial distribution (Bolker *et al.* 2009). Subsequent analyses were analysed using linear mixed models (LMM). Both GLMM and LMM analyses were conducted using the Lme4 package in R.

### 3. RESULTS

We investigated the degree of inbreeding in this population. The mean internal relatedness of calves (c-IR) was significantly higher than that expected under random mating (Monte Carlo randomization procedure ( $\times 1000$ ): mean c-IR  $\pm$  95% confidence interval =  $0.05 \pm 0.045$  and mean simulated c-IR  $\pm$  95% confidence interval =  $-0.012 \pm 0.007$ ,  $p = 0.002$ ). Additionally, in populations subjected to inbreeding, heterozygosity is expected to be positively correlated across neutral markers (Aparicio *et al.* 2007). To test this hypothesis, we randomly divided the 16 markers into two sets of eight markers and calculated the correlation in the IR values between the two random sets (heterozygosity–heterozygosity correlation). This procedure was repeated 100 times as suggested by Balloux *et al.* (2004). We found that heterozygosity was positively correlated among loci (mean  $r = 0.172 \pm 0.17$ , minimum = 0.02, maximum = 0.56,  $n = 219$ ). Furthermore, we found that the IR of calves were positively correlated to the pairwise relatedness of their parents ( $r = 0.573$ ,  $n = 28$ ).

To investigate whether female bottlenose dolphins show fitness reduction as a result of inbreeding, we studied the correlation between IR and Cs. We found that the mothers’ Cs was negatively correlated with both m-IR and c-IR (table 1 and figure 1). The m-IR and the c-IR did not interact in their effects on Cs (table 1). There was no correlation between the m-IR and c-IR (table 2). However, a calf’s weaning age was positively



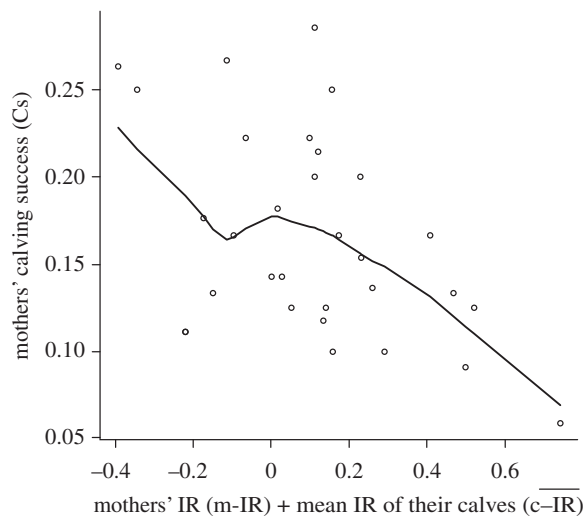


Figure 1. Significant relationship between mothers' calving success (Cs) and their internal relatedness (m-IR) + the mean internal relatedness of their calves (c-IR). Significance was assessed using GLMM (see table 1 for details).

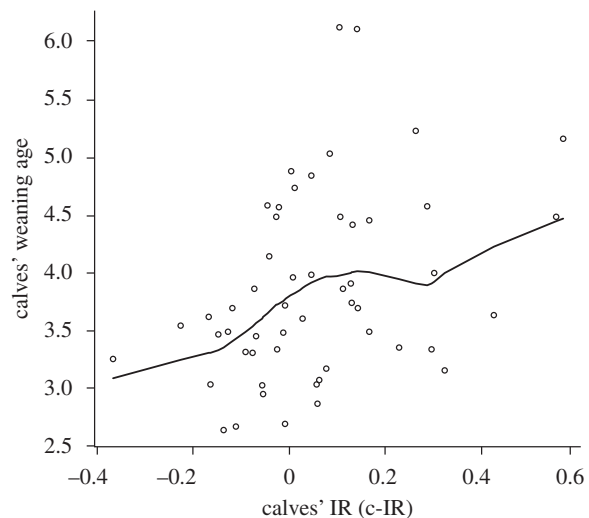


Figure 2. Significant relationship between calves' weaning age in years and their internal relatedness (c-IR). Significance was assessed using LMM (see table 2 for details).

Table 2. LMM predictors of calves internal relatedness (c-IR,  $n = 53$ ). (Calves identity was added to the models as random effect.)

models	average effect	s.e.	<i>t</i> -value	d.f.	<i>p</i>
c-IR ~ weaning age	1.6	0.605	2.81	51	0.007
c-IR ~ order of calves	0.110	0.047	2.34	51	0.0233
c-IR ~ mother's IR	0.087	0.24	0.36	51	0.720

correlated with its c-IR (table 2 and figure 2), and a mother's first calf had a higher c-IR, than subsequent calves (table 2).

#### 4. DISCUSSION

This study provides evidence that the wild bottlenose dolphins in East Shark Bay are prone to inbreeding. First, we found that the mean internal relatedness of calves was significantly higher than expected if mating was random. Fourteen per cent of all calves showed IR larger than 0.25, the value expected for offspring of half-sibling mating, and in 29.5 per cent of the cases IR was larger than 0.125. Second, although non-significant, heterozygosity was positively correlated across the 16 markers as expected in populations subjected to inbreeding (Aparicio *et al.* 2007). And last, four out of the 16 loci used in this study showed heterozygote deficiency, indicating a weak Wahlund effect caused by the presence of subpopulation structure (Krützen *et al.* 2004b). This degree of inbreeding is unusual in a mobile free-living species. It would be less surprising in species restricted by habitat or nesting sites, such as the naked mole rat (Reeve *et al.* 1990). Inbreeding in other mammals has been attributed to population crashes (Randall *et al.* 2007), but Shark Bay is one of the largest documented populations of

inshore bottlenose dolphins and contains a minimum of 2000–3000 dolphins (Preen *et al.* 1997), and there has been no documentation of any population crash in the past five decades.

Fitness reduction as a result of inbreeding has been reported in many species, such as mountain goats (*Oreamnos americanus*, Mainguy *et al.* 2009) and mandrill baboons (*Mandrillus sphinx*, Charpentier *et al.* 2005). For instance, survival of juveniles was found to correlate with individual levels of standardized multilocus heterozygosity in a population of alpine marmots (*Marmota marmota*, Cohas *et al.* 2009). This study provides further evidence for heterozygosity-fitness correlations in the wild. Most importantly, we demonstrate that the cost of inbreeding in female bottlenose dolphins in East Shark bay operates through two independent pathways: first, through the internal relatedness of the mother herself, and second through the internal relatedness of her calves. Specifically, we found that compared with non-inbred females, inbred females have reduced fitness (lower calving success), females with inbred calves also have reduced fitness (lower calving success) and inbred calves wean later. It is important to note that because calves could only be sampled around the age of 3, the heterozygosity-fitness correlation documented in this study might even be stronger if inbred calves had a higher mortality rate at birth relative to non-inbred calves.

The fitness cost of having an inbred calf may be explained by the positive relationship between calf inbreeding and weaning age. This could mean that females compensate for calf condition (inbreeding) by delaying weaning (also see Mann & Watson-Capps 2005), or that inbred calves develop more slowly. Furthermore, delayed weaning might instead reflect lowered genetic conflict between more closely related mother and offspring (Trivers 1974). In either case, overall lifetime female calving rate is decreased, by delaying conception of the next offspring.

Inbred females also appear to suffer a fitness reduction (lower calving success), but the proximate causes remain unknown. Unfortunately, the unpopulated coast-line of

Shark Bay and its high shark density makes the recovery of calves' carcasses unlikely (Mann *et al.* 2000). For example, the high calf mortality rate (approx. 42%) observed in Shark Bay (Mann *et al.* 2000) could include additional death owing to inbreeding. While some parameters such as maternal age and water depth have already been found to correlate with calving success (Mann *et al.* 2000), future studies are needed to help us elucidate the mechanisms for reduced fitness of inbred females (e.g. reduction in fertility, increased diseases, increased calf mortality, habitat quality, etc.). This will be investigated as our long-term dataset grows.

The evident female costs of inbreeding lead us to question why inbreeding is tolerated in this population. It seems likely that East Shark Bay dolphins have some ability to identify relatives, because at least some males form alliances based on kinship (Krützen *et al.* 2003). Furthermore, long periods of maternal association for calves increase the chance that both sexes can recognize at least maternal kin. Thus, it might be possible for adult females (or males) to avoid inbred matings. However, younger females may be vulnerable to inadvertent or accidental pairing with relatives because they are more likely to have unknown, older male kin within the population that are reproductively competitive. This is because dolphin males may be more sexually competitive when older (20s–30s), and it is believed that female dolphins have higher calving success when they are younger (less than 20s). In other words, while fathers would only have to wait 12 years to sire offspring with their daughters, a mother would be in her 30s by the time her son was reproductively competitive. This claim is supported by our finding that a female's first calf is more likely to be inbred than subsequent calves. As a result, inbreeding tolerance in bottlenose dolphins is likely to be the product of strong bisexual philopatry and high male sexual coercion, which are notable characteristics of this population (Connor *et al.* 1992*a,b*, 1999, 2000, 2001). Females may be able to identify younger maternal kin by association, but not know who their older half-brothers, fathers or uncles are because of sex-biases in association (Smolker *et al.* 1992; Gibson & Mann 2008*b*). Even with kin recognition, younger females may be more susceptible to coercive consortships than older females. Thus, younger females may be more vulnerable to inbred matings. Alternatively, older females may be more likely to lose calves that are inbred compared with younger females. In addition, the observed mating bias for close relatives may be a deliberate choice by females, but it seems unnecessary to postulate this given the apparent age-bias in the mothers of inbred calves found in this study. Finally, the presence of inbreeding in this species might also represent a conflict between males and females over inbreeding tolerance. It might be possible that inbreeding might occur more often in highly polygynous systems, where males tend to be larger than females (hence better able to coerce females) and/or low-ranking males might be disposed to mate with any female, regardless of relatedness. Males are not larger than females in East Shark Bay, but alliances of males can effectively intimidate females. These hypotheses remain to be tested as our paternity dataset grows.

To conclude, while the causes of inbreeding remain obscure, the inexperience of young females may be

involved. It further highlights that mechanisms of kin recognition (whether based on genotypes or social experience) are not foolproof. In any case, the cost of inbreeding in this population might not have been sufficient to have led to the development of effective avoidance mechanisms, such as female evasion or male dispersal.

Ethics approval was obtained by the University of New South Wales (99\_52) and the University of Zürich. Samples were transferred to Zurich under the cetacean permit 2004-55242 from the Department of Environment and Conservation (Australia) and exchanged under institutional CITES permits (AU069 and CH-019). Biopsy sampling was conducted under the Department of Conservation and Land Management Sampling Permit SF002958 (to M.K.).

Long-term data monitoring was supported by grants to J.M. and R.C.C., NSF grant no. 0316800 and grants to J.M. from Georgetown University and NSF grant no. 9753044. We especially thank Lukas Keller and Peter Prentis for constructive comments on an earlier version of this manuscript, as well as Mark Kirkpatrick and the two anonymous referees who helped improve this paper. This work was supported by all members of the Monkey Mia Dolphin Research Foundation. The Monkey Mia Dolphin Resort and Monkey Mia Wildsights supported the authors during their field studies. The study was funded with grants to W.B.S. and M.K. from the Australian Research Council, WV Scott Foundation, National Geographic Society, Seaworld Research and Rescue Foundation and Schultz-Stiftung.

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