



## Great frigatebirds, *Fregata minor*, choose mates that are genetically similar

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(Received 15 November 2002; initial acceptance 7 April 2003;  
final acceptance 8 December 2003; published online 29 September 2004; MS. number: A9489R)

Inbreeding occurs infrequently in most natural populations, with the level of relatedness between mates potentially being influenced by behavioural mechanisms of mate choice and spatial genetic structure of the population. We studied mate relatedness in a Hawaiian population of great frigatebirds with the expectation, based on frigatebird ecology, that mated pairs were unlikely to be more genetically similar than a random draw of adults from the breeding population. We used a paired analysis to compare the DNA fingerprint similarity of a female and her mate versus the same female and a random male that was breeding elsewhere on the same island. Overall, band-sharing scores between mates were higher than band-sharing scores between nonmates, and the mean coefficient of relatedness between mates was 0.082. Relatedness between mates was not a consequence of strong natal philopatry coupled with random mate choice: historical data revealed several recent between-island shifts in the location of the breeding colony, and the degree of genetic similarity between nonmates was not well predicted by the physical distance between their nest sites on Tern Island. Instead, females may deliberately choose related individuals as mates. Tests for a relationship between the genetic similarity of mates and the size and ectoparasite load of their 1-week-old chick were equivocal. The question of how and why females may be choosing genetically similar mates is unresolved, but it appears to be a consequence of active choice rather than spatial genetic structure or limited availability of unrelated mates.

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Inbreeding results from mating between related individuals more often than expected, given the average relatedness between individuals in the population (Shields 1993; Waser 1993). Inbreeding may be costly because it can decrease heterosis and increase the expression of deleterious recessive alleles in offspring (Greenwood 1987; Charlesworth & Charlesworth 1999; Crnokrak & Roff 1999). Consequently, many animals and plants appear to have evolved inbreeding avoidance mechanisms (Darwin 1876; Charlesworth & Charlesworth 1987; Pusey & Wolf 1996). For example, some animals avoid mating with individuals with whom they associated as juveniles, because such individuals have a high probability of being kin (Boyd & Blaustein 1985; Clarke & Faulkes 1999; Fadao et al. 2000). In cases where there is a high likelihood of breeding-age contact with kin and where kin cannot be recognized by any reliable mechanism, females may

copulate with multiple males to ensure that not all of their offspring will be sired by a closely related mate (Stockley et al. 1993).

Despite such inbreeding avoidance mechanisms, inbreeding does occur occasionally in many taxa (Crnokrak & Roff 1999; Keller & Waller 2002), often as a consequence of limited dispersal (Bohonak 1999) in conjunction with a mate choice process that may be random with respect to relatedness. Dispersal distance is typically sex biased in birds and mammals, ostensibly to reduce the likelihood of inbreeding (Greenwood 1980, 1987; but see Part 1996), but there are many species in which neither sex disperses very far. Seabirds, for example, often show strong natal site fidelity (e.g. Schreiber & Schreiber 1993; Austin et al. 1994; Schørring 2001), despite being capable of travelling great distances. In the Laysan albatross, *Diomedea immutabilis*, males and females both typically breed within 30 m of their natal nest site (Fisher 1976) even though they routinely fly more than 2000 km to forage (Fernández et al. 2001). Unless individuals actively avoid mating with relatives, such natal site fidelity could give rise to some level of inbreeding.

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Alternatively, inbreeding might result from a more active process of mate choice, in which an individual deliberately chooses a mate that is likely to be related (e.g. as inferred by natal association) or that is phenotypically similar (i.e. phenotype matching). Such behaviour could be adaptive if outbreeding depression occurs; that is, if mating with individuals that are too dissimilar is costly in terms of genetic incompatibility or disruption of locally adaptive genotype combinations (Bateson 1978; Shields 1982).

Great frigatebirds are interesting in this context of expected levels of inbreeding. They are long-lived seabirds that breed colonially on remote islands in the Pacific and Indian Oceans. Both sexes show some natal site fidelity (Metz & Schreiber 2002; D. Dearborn & A. Anders, unpublished data), and genetic differentiation exists among distant islands (Dearborn et al. 2003). Thus, kin have the potential to come in contact with each other as adults at breeding colonies. If breeding densities are low (i.e. few mates available) and behavioural avoidance of kin is absent, inbreeding could result.

However, several characteristics of the breeding ecology of great frigatebirds make inbreeding unlikely in this species. During each breeding season, female great frigatebirds assess a wide array of prospective mates. In our Tern Island study population in the Northwestern Hawaiian Islands, unpaired females repeatedly fly over the entire 14-ha island during mate choice, evaluating as many as several hundred males that are performing elaborate courtship displays (Dearborn & Ryan 2002). Females have a wide range of local options for mates, as the operational sex ratio is strongly male biased during the pair-formation time of the breeding season (Dearborn et al. 2001). Additionally, individual males often shift the location of their courtship displays, sometimes doing so repeatedly in the course of a single day, and a few males have been shown to move hundreds of kilometres between islands in the course of mate seeking (Dearborn et al. 2003). One consequence of these movements could be an increase in the variety of males locally available to females. Finally, the history of Tern Island (Amerson 1971) and adjacent islands in the same atoll may have disrupted any spatial structure that would have facilitated 'passive' inbreeding, as changes in island structure and vegetation may have forced birds to shift their breeding locations over time.

Overall, inbreeding in our study population of great frigatebirds seems unlikely. If it were to occur, we would expect it to be the result of either active mate choice or random mating (with respect to relatedness) in the presence of strong spatial genetic structure. If inbreeding occurred actively, we might expect a fitness benefit to mating with genetically similar individuals; if inbreeding occurred passively due to spatial structure, we might expect it to be selectively neutral or perhaps costly. In this study, we address three issues. First, we compare the genetic similarity of mated pairs to the genetic similarity of nonmates in the Tern Island population of great frigatebirds. Second, we use current genetic data and historical demographic data to test for the presence of spatial structure that could affect mate relatedness in this population. Third, we examine components of

initial offspring performance to make a very preliminary test for a fitness consequence of inbreeding in this population.

## METHODS

### DNA Fingerprinting and Band Scoring

We studied the genetic similarity of great frigatebird mates on Tern Island (23°45'N, 166°17'W), a 14-ha island in the French Frigate Shoals atoll, northwestern Hawaiian Islands. Blood samples were collected from 92 family groups (the female and male and their single chick) when the chicks were 2–5 weeks old. Subsequent DNA fingerprinting of these family groups revealed one extrapair fertilization (Dearborn et al. 2001). In the analyses presented here, we use data only from the 91 family groups in which both the male and female were the genetic parents of the chick. Samples in this study were collected from 61 families in 1998 and 30 families in 1999. In the 1998 sample, 20 families were from nests that were already being monitored for a study of parental effort (Dearborn 2001); an additional 41 families were chosen randomly from throughout the colony and across the breeding season. This sampling was achieved by randomly selecting a location from a grid of the island and then sampling the closest nest of appropriate age. In the 1999 sample, because of a shorter field season, we used the 30 earliest nests of the season that survived to the nestling stage. Blood samples were taken from the leg or foot and stored in lysis buffer (Longmire et al. 1988). The location of each nest was plotted on a detailed map of the island, using a combination of landmarks and a long-axis grid.

We used multilocus minisatellite fingerprinting to assess genetic similarity of individuals (see Dearborn et al. 2001 for details; for similar approaches see Bensch et al. 1994; Kempenaers et al. 1996). We used a standard phenol/chloroform extraction, followed by digestion of genomic DNA with *Hae*III. Fragments were separated on agarose gels, transferred to nylon, and hybridized with Jeffreys' probe 33.15 (Jeffreys et al. 1985a, b).

Samples were grouped on gels by social family, each consisting of a male, a female and a single chick. Families were generally grouped on gels by date of nest initiation. Because mate choice is made strictly by females in this species (Nelson 1975; Dearborn et al. 2001), we assessed the genetic similarity between each female and her mate and compared this to the genetic similarity between that same female and the male from an adjacent family on the gel. Distance between individuals being compared on the gel was never more than four lanes. Band sharing between birds was calculated as  $2S/(A + B + 2S)$ , where  $S$  = number of bands shared by the pair of birds,  $A$  = number of bands unique to bird A, and  $B$  = number of bands unique to bird B (Wetton et al. 1987). We did not deliberately adjust our definition or categorization of bands to take into account any expectations about relationships. We used a paired  $t$  test to compare the band-sharing score between a female and her mate versus the band-sharing score between that same female and a male to whom she

was not mated. Three pieces of evidence support the notion that the nonmate comparison male can legitimately be viewed as a male with whom a particular female could have mated but did not: (1) all birds re-pair for each breeding attempt (Nelson 1975), (2) mate choice is conducted by females at the spatial scale of the entire breeding colony (Nelson 1975; D. Dearborn & A. Anders, unpublished data), and (3) the mean  $\pm$  SE difference in date of nest initiation between each female and her nonmate comparison male in this study was  $2.2 \pm 1.31$  days, meaning that in most cases the nonmate male was likely to be displaying at the time that the female was evaluating mates.

To interpret the DNA fingerprint similarities between individuals, we used the method of Reeve et al. (1992) to estimate mean coefficients of relatedness between individuals with three types of relationships: mates, nonmates, and females and their offspring. This approach measures band sharing within and between members of groups to estimate a relatedness coefficient  $r'$  that is analogous to the relatedness estimate of Queller & Goodnight (1989). Because this method accurately assesses only mean relatedness within groups rather than exact relatedness for particular pairs of individuals, correlational analyses of the occurrence of genetic similarity between mates (e.g. versus date of nest initiation) were based on actual band-sharing scores rather than relatedness coefficients.

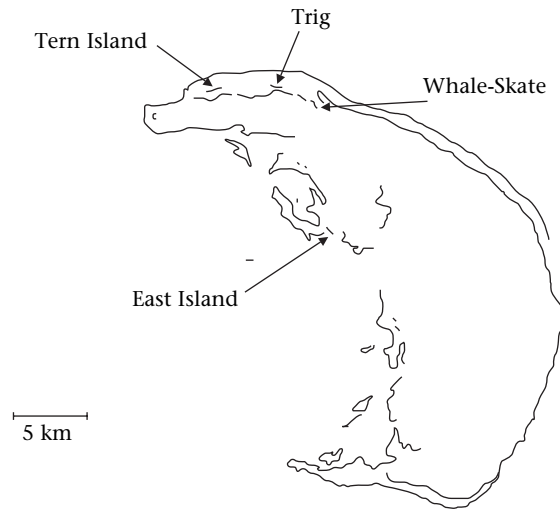
### Spatial Structure of the Population

Natal site fidelity and the resulting spatial structure of a population can lead to genetic similarity between mates even when mate choice is random with respect to relatedness. To test whether this mechanism could underlie inbreeding in this population of frigatebirds, first we used a linear regression to determine whether band-sharing scores between nonmates declined with physical distance between their nest locations on Tern Island.

Second, we used data from the Pacific Ocean Biological Survey Program (Amerson 1971) and the U.S. Fish and Wildlife Service (unpublished data) to explore the spatial distribution of this breeding colony over a span of approximately 40 years. At various points in this time period, four of the islands in French Frigate Shoals have had sufficient vegetation to support frigatebird nests: Tern Island, East Island, Trig and Whale-Skate (Fig. 1). Nest counts have been conducted at the approximate peak of the breeding season on all islands in the atoll since 1964, and historical records were used to assess human impacts on breeding colony location. If these data indicate that the locations of breeding colonies are stable, the potential exists for spatial demographic structure to create genetic 'neighbourhoods', which could facilitate passive inbreeding.

### Mate Relatedness and Offspring Performance

To make a preliminary test of whether relatedness between mates has consequences for offspring performance, we measured structural size, body mass and



**Figure 1.** French Frigate Shoals is a crescent-shaped atoll bounded by active coral reef. Of the 12 small islands in the atoll, Tern Island, Trig, Whale-Skate and East Island have been occupied by nesting frigatebirds at various points in the past 50 years. Our study of mate relatedness was conducted on Tern Island, which is the current location of nearly all frigatebird breeding in the atoll.

ectoparasite load of 20 nestlings when they were 7 days old. To assess structural size, we measured wing length and culmen length with calipers. We expected chick size to indicate performance (i.e. better chicks would grow and develop faster), which could lead to either a greater likelihood of surviving the nestling period or larger adult size (which in turn might be related to fecundity or mating success). Because older nestlings are sexually size dimorphic, we used a genetic marker (Griffiths et al. 1998) to identify the sex of the chicks; we used the P2 and P8 primers, modifying the Griffiths et al. (1998) protocol by using these cycle parameters: 2 min at 95°C, followed by 35 cycles of 30 s at 95°C, 1 min at 48°C, 2 min at 72°C, followed by a final extension period of 5 min at 72°C. Because nestling gender (12 males, 8 females) did not explain any variation in 7-day-old chick size in any analyses, it was not included in final models.

Although adult frigatebirds in this population harbour a variety of ectoparasites (D. Dearborn & A. Anders, unpublished data), young chicks are parasitized only by lice. Thus, ectoparasite load was assessed by counting all lice on each chick's body; chicks are still unfeathered at 7 days, and these lice are typically 4 mm long and slow-moving, so the lice are easily detected. We used regression analysis to test for relationships between offspring performance and genetic similarity of parents, using a Bonferroni-adjusted alpha of  $0.05/4 = 0.0125$ . Following these analyses, we performed post hoc calculations of confidence intervals for the effect sizes observed in our study (Steidl & Thomas 2001). Such confidence intervals represent approximate bounds on the likely value of the true (but unknown) effect size and may span either biological or statistical significance, both, or neither. In our regressions of chick performance versus parent relatedness, the

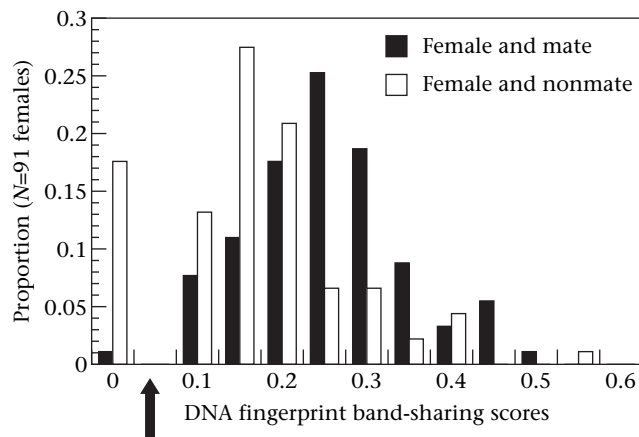
absolute effect size is the difference between the observed estimate of  $B_1$  (the slope of the line) and the null hypothesis slope against which we wished to compare it, in this case, a slope of zero. Thus, the confidence interval on the observed absolute effect size is the same as the confidence interval on the estimate of  $B_1$ , which is  $B_1 \pm t_{0.0125(2), 18} s_{b1}$  (Zar 1996), with a  $t$  value that reflects the Bonferroni adjustment to alpha.

## RESULTS

### DNA Fingerprinting and Band Scoring

Band-sharing scores were higher between mates ( $\bar{X} \pm SE = 0.234 \pm 0.010$ ,  $N = 91$ ) than between nonmates ( $0.144 \pm 0.011$ ; paired  $t$  test:  $t_{90} = 5.85$ ,  $P < 0.0001$ ; Fig. 2). Band-sharing for both of these groups was lower than band-sharing between first-order relatives (females and offspring:  $0.618 \pm 0.009$ ; versus mates, paired  $t$  test:  $t_{90} = 30.47$ ,  $P < 0.0001$ ; versus nonmates,  $t_{90} = 34.59$ ,  $P < 0.0001$ ). The mean relatedness,  $r$ , between individuals was  $0.082 \pm 0.013$  for mates,  $-0.044 \pm 0.022$  for nonmates, and  $0.533 \pm 0.011$  for females and offspring. Because the Reeve et al. (1992) estimator can have a downward bias relative to true pedigree relatedness, 0.082 may be a slight underestimate of actual relatedness between mates.

For 20 nests in 1998 with known initiation dates, there was not a significant relationship between the genetic similarity of mates and the date of pair formation (regression:  $t_{18} = -2.01$ ,  $P = 0.060$ ,  $r^2 = 0.183$ ).



**Figure 2.** A comparison of DNA fingerprint band-sharing scores of female frigatebirds and their mates versus band-sharing scores of females and nonmate comparison males who were also breeding on Tern Island during the same time period. Mean relatedness based on the DNA fingerprints was  $0.082 \pm 0.013$  SE for mated pairs. Arrow indicates the band-sharing score between social mates for the only sampled pair in which the female engaged in an extrapair fertilization (Dearborn et al. 2001); this female was not included in these analyses but is shown here to highlight the low genetic similarity between this female and her social mate, which suggests that extrapair fertilization does not serve as an inbreeding avoidance mechanism in this species.

### Spatial Structure of the Population

The current spatial pattern of relatedness and the historical shifts in location of the breeding colony did not support the hypothesis that inbreeding is a passive consequence of spatial genetic structure. There was only a weak negative relationship between band-sharing scores of nonmates and the locations of nests (regression:  $t_{87} = -2.21$ ,  $P = 0.030$ ,  $r^2 = 0.053$ ): adults whose nests were located near each other were slightly more genetically similar than adults whose nests were at opposite ends of the island, but distance explained less than 6% of the variation in band sharing between nonmates.

The history of French Frigate Shoals revealed shifts in the location of the frigatebird breeding colony that would have disrupted any spatial structure in the population. Seabird breeding habitat was disrupted or eliminated on Tern Island from 1942 to 1979 and on East Island from 1935 to 1952 due to the activities of a Naval Air Facility, a commercial fisheries operation, and a Coast Guard LORAN station. Although the islands of Whale-Skate and Trig were never extensively altered by human activity, in the 1980s and 1990s they began to erode in a series of wash-over events that stripped away the vegetation; Trig still persists as a sand spit, but Whale-Skate washed away altogether in 1998. In accord with these changes, nest counts conducted over the past 40 years revealed repeated shifts in the location of the French Frigate Shoals great frigatebird breeding colony (Fig. 3). In the 1960s, all nests were located on Whale-Skate. In the late 1970s, there was an expansion to East Island and later to Trig. Frigatebirds began breeding on Tern Island in the mid-1980s and subsequently stopped breeding on Trig, East Island and Whale-Skate. Since 1997, some individuals have shifted back to the few available bushes on East Island, but over 95% of nests are currently built on Tern Island.

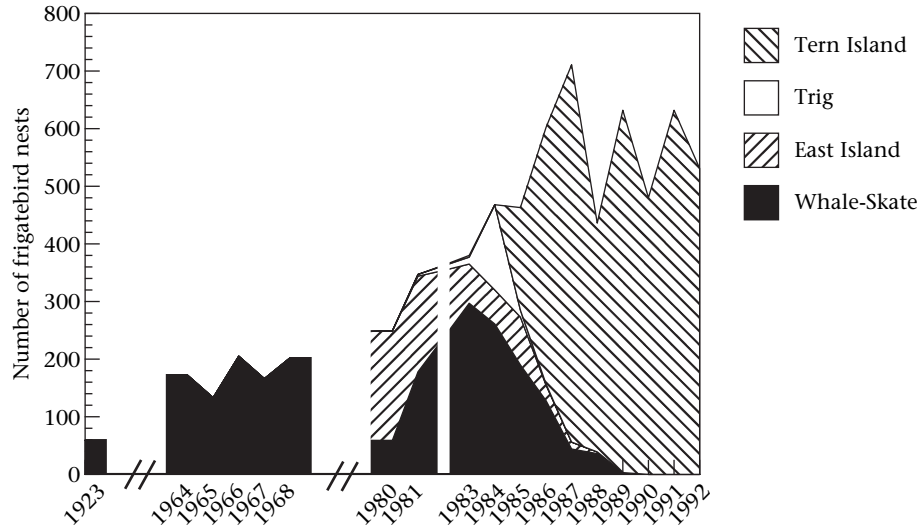
### Mate Relatedness and Offspring Performance

There was no relationship between the wing length, culmen length, or mass of 7-day-old nestlings and the genetic similarity between the parents (wing regression:  $t_{18} = -1.06$ ,  $P = 0.305$ ,  $r^2 = 0.058$ ; culmen regression:  $t_{18} = -2.18$ ,  $P = 0.043$ ,  $r^2 = 0.208$ ; mass regression:  $t_{18} = -1.03$ ,  $P = 0.317$ ,  $r^2 = 0.056$ ).

Nestling ectoparasite loads ranged from 0 to 47 lice, all of which were *Fregatiella aurifasciata* (Suborder Amblycera, Family Menoponidae). A chick's ectoparasite load was not related to the genetic similarity between its parents (regression:  $t_{18} = 1.60$ ,  $P = 0.127$ ,  $r^2 = 0.125$ ) or to date of nest initiation for these 20 nests (regression:  $t_{18} = 0.166$ ,  $P = 0.870$ ,  $r^2 = 0.002$ ).

As estimated across the observed range of band-sharing values between mates (0–0.444), the lower and upper confidence limits of the chick fitness measures spanned a wide range of values (confidence intervals, CI, for difference in chick culmen length across observed band-sharing scores: 0.81 to 6.7 mm; CI for difference in mass across observed band-sharing scores:  $-90.5$  to  $+41.5$  g; CI for difference in wing length:  $-6.1$  to  $+2.7$  mm; CI for





**Figure 3.** The location of the frigatebird breeding colony at the atoll of French Frigate Shoals has shifted between four islands over the past 30 years, in conjunction with changes in island vegetation and human activity. Data are from Wetmore (1923 cited in Amerson 1971), Amerson (1971) and U.S. Fish and Wildlife Service, Honolulu, Hawaii.

difference in number of lice:  $-13.6$  to  $+50.8$ ). Although the slopes of chick performance versus parent relatedness were not significantly different from zero, the confidence intervals on these slopes spanned not only zero but also values that were large enough to be biologically meaningful. Thus, our tests for effects of inbreeding on chick size and parasite load are not conclusive.

## DISCUSSION

Contrary to our expectations, DNA fingerprinting revealed that female frigatebirds were more genetically similar to their mates than to males that were nesting with other females on the same 14-ha island at the same time of year ('nonmates'). The mean coefficient of relatedness between mates was 0.082, somewhat less than the theoretical value for first cousins (0.125), while the relatedness between nonmates was not significantly different from zero. Relatedness between females and their offspring (0.533) was slightly higher than the expected value of 0.5, probably as a result of the relatedness between mates.

The mean relatedness between frigatebird mates is fairly high when viewed in the context of other wild bird populations. Studies that document inbreeding depression frequently are focused on the fitness effects of rare matings between close relatives, while the average level of inbreeding in these populations overall is often very close to zero (Kempnaers et al. 1996; Brown & Brown 1998; Keller 1998; Keller et al. 2002; Kruuk et al. 2002).

Our second key finding was that the observed genetic similarity between mates did not appear to be strongly driven by spatial genetic structure. If females acquired genetically similar mates as a consequence of strong natal philopatry and mate choice that was random with respect to relatedness, one would expect a strong negative relationship between genetic similarity of nonmates and the physical distance between their breeding locations.

However, distance between breeding locations on the island accounted for less than 6% of variation in the genetic similarity scores of nonmates. This poor fit is not a reflection of limited sampling scale: distances between breeding locations in our data set ranged from less than 3 m to 672 m (the island is only 970 m long, and the western-most 200 m have no frigatebird nests).

Additional evidence against strong spatial structure comes from historical data, which show that this breeding population has shifted between islands several times in the past 30 years, moving from Whale-Skate to East Island, then back to Whale-Skate, then to Trig, and then to Tern Island. These shifts correspond partially with changes in the availability of vegetation for displaying and nesting. Although each transition occurred fairly gradually over the course of several years, birds in this population can live up to 42 years or more (D. Dearborn & F. Juola, unpublished data) and probably take 7 years to reach sexual maturity, meaning that each population shift between islands was due to a shift by breeding adults rather than to natal dispersal. In addition, frigatebirds re-pair for each breeding attempt between and within seasons (Nelson 1975; Reville 1988), so pairs that might have formed during any circumstance of spatial structure in the past would be paired with different individuals now. Lastly, these islands vary in size, shape, and the amount and location of shrubs (*Scaevola taccada* and *Tournefortia argentea*) used for courtship displays and nest sites. If spatial genetic structure did persist through these extraordinary shifts in colony location, it would suggest that settlement patterns during colony establishment are influenced by cues tied to relatedness.

If the genetic similarity between mates is not primarily a consequence of spatial structure, it may be the product of mate choice. In this context, two components of the mate choice process warrant discussion: the mechanism, and the consequences. Possible mechanisms for inbreeding in this population include sexual imprinting

(Bateson 1978), self-referent phenotype matching (Hauber & Sherman 2001) and heritable microhabitat preference (Petrie et al. 1999). A role of heritable microhabitat preference seems unlikely in our population of frigatebirds, as individual females evaluate mates over the entire island, and individual males display from multiple sites on the island (D. Dearborn & A. Anders, unpublished data) or even on multiple islands hundreds of kilometres apart in a few cases (Dearborn et al. 2003). In contrast, both sexual imprinting and self-referent phenotype matching are plausible mechanisms for inbreeding in this population, and both of these mechanisms have been shown to affect mate choice in other species (Penn & Potts 1998; Alberts 1999; Plenge et al. 2000; Zelano & Edwards 2002). Because great frigatebird chicks are fed by both parents for up to 10 months, and because extrapair fertilizations are rare (Dearborn et al. 2001), ample opportunity exists for accurate sexual imprinting in this species. Self-referencing would be possible but presumably unnecessary, as its main advantages are in situations of uncertain parentage (e.g. absence of parental care, frequent extrapair fertilizations, or brood parasitism; Hauber & Sherman 2001).

The second issue is whether there are fitness consequences to the level of inbreeding that we observed. Three general possibilities exist: choice of genetically similar mates is maintained by a benefit to low-level inbreeding; choice of genetically similar mates persists despite being costly (because of constraint, trade-off, etc.); or the observed level of inbreeding is selectively neutral. Given that the widespread low level of inbreeding observed in this population appears to be the result of active choice during mate selection, we initially hypothesized that this level of inbreeding would be beneficial. Our small sample of chick performance data did not detect a benefit: 1-week-old chicks of more genetically similar parents had no fewer ectoparasites and were not larger in body size than those of less genetically similar parents. However, these tests are inconclusive because the confidence intervals on the effect sizes in these analyses suggest that undetected benefits (or even costs) could exist in our measures of 7-day-old chick performance. Even if there are no fitness effects at this stage, offspring performance could be impacted at a later point in life (e.g. adult fecundity: Slate et al. 2000; Amos et al. 2001), a particularly important point for a species in which individuals can live for more than 40 years. Deleterious effects of inbreeding can also impact fitness-related traits at a variety of life stages (Bensch et al. 1994; Crnokrak & Roff 1999; Slate et al. 2000; Keller & Waller 2002) and may be most detectable under conditions of environmental stress (Keller et al. 1994, 2002). A final possibility is that the observed level of inbreeding is selectively neutral, perhaps existing solely as a by-product of sexual imprinting.

Although the potential for fitness consequences needs to be explored further, the inbreeding observed in this study remains interesting because it appears to be the result of neither spatial genetic structure nor constraint. Because many male frigatebirds are displaying when a female chooses a mate in this population (Dearborn et al. 2001) and because frigatebirds re-pair for each

breeding attempt (Nelson 1975; Reville 1988), females are not constrained to either become or remain socially paired with genetically similar individuals. And lastly, female frigatebirds do not appear to use extrapair fertilizations to circumvent social pairings with relatives, as hypothesized by Bensch et al. (1994) for females who are constrained in their choice of social mate. In fact, the one female in our sample that had an extrapair young was less genetically similar to her social mate than were 94% of the females we sampled, raising the intriguing possibility that EPCs in this species might even be used to increase, rather than decrease, genetic similarity to one's mate.

Overall, we have demonstrated an unusually high level of average relatedness between mates in a population of great frigatebirds. Because relatedness was greater between mates than between nonmates, the observed inbreeding is not an effect of high overall genetic similarity in the population, nor does it appear to be explained by spatial structure or constrained availability of mates. Although the potential for fitness consequences of this inbreeding remains unclear, the occurrence of inbreeding in this population is intriguing because it is likely to be the result of an active mate choice process.

### Acknowledgments

We thank Angela Anders for assistance in collecting data in the field and in the laboratory and for discussions of inbreeding. We thank Patty Parker for extensive help with DNA fingerprinting. Mark Hauber provided thought-provoking ideas about mate choice, and Martyn Kennedy and Isabel Marshall kindly identified the ectoparasites from the chicks. Thanks to Beth Flint, of the U.S. Fish and Wildlife Service, for providing permits, logistical support, and access to unpublished data. Angela Anders, Frans Juola and two anonymous referees provided helpful comments on this manuscript.

### References

- Alberts, S. C. 1999. Paternal kin discrimination in wild baboons. *Proceedings of the Royal Society of London, Series B*, **266**, 1501–1506.
- Amerson, A. B., Jr. 1971. The natural history of French Frigate Shoals, northwestern Hawaiian Islands. *Atoll Research Bulletin*, **150**, 1–383.
- Amos, W., Wilmer, J. W., Fullard, K., Burg, T. M., Croxall, J. P., Bloch, D. & Coulson, T. 2001. The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society of London, Series B*, **268**, 2021–2027.
- Austin, J. J., White, R. W. G. & Ovenden, J. R. 1994. Population-genetic structure of a philopatric, colonially nesting seabird, the short-tailed shearwater (*Puffinus tenuirostris*). *Auk*, **111**, 70–79.
- Bateson, P. P. G. 1978. Sexual imprinting and optimal outbreeding. *Nature*, **273**, 259–260.
- Bensch, S., Hasselquist, D. & Von Schantz, T. 1994. Genetic similarity between parents predicts hatching failure: non-incestuous inbreeding in the great reed warbler? *Evolution*, **48**, 317–326.

- Bohonak, A. J. 1999. Dispersal, gene flow, and population structure. *Quarterly Review of Biology*, **74**, 21–45.
- Boyd, S. K. & Blaustein, A. R. 1985. Familiarity and inbreeding avoidance in the gray-tailed vole *Microtus canicaudus*. *Journal of Mammalogy*, **66**, 348–352.
- Brown, J. L. & Brown, E. R. 1998. Are inbred offspring less fit? Survival in a natural population of Mexican jays. *Behavioral Ecology*, **9**, 60–63.
- Charlesworth, B. & Charlesworth, D. 1999. The genetic basis of inbreeding depression. *Genetical Research*, **74**, 329–340.
- Charlesworth, D. & Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics*, **18**, 237–268.
- Clarke, F. M. & Faulkes, C. G. 1999. Kin discrimination and female mate choice in the naked mole-rat *Heterocephalus glaber*. *Proceedings of the Royal Society of London, Series B*, **266**, 1995–2002.
- Crnokrak, P. & Roff, D. A. 1999. Inbreeding depression in the wild. *Heredity*, **83**, 260–270.
- Darwin, C. 1876. *The Effects of Crossing and Self-fertilisation in the Vegetable Kingdom*. London: J. Murray.
- Dearborn, D. C. 2001. Body condition and retaliation in the parental effort decisions of incubating great frigatebirds (*Fregata minor*). *Behavioral Ecology*, **12**, 200–206.
- Dearborn, D. C. & Ryan, M. J. 2002. A test of the Darwin–Fisher theory for the evolution of male secondary sexual traits in monogamous birds. *Journal of Evolutionary Biology*, **15**, 307–313.
- Dearborn, D. C., Anders, A. D. & Parker, P. G. 2001. Sexual dimorphism, extra-pair fertilizations, and operational sex ratio in great frigatebirds (*Fregata minor*). *Behavioral Ecology*, **12**, 746–752.
- Dearborn, D. C., Anders, A. D., Schreiber, E. A., Adams, R. M. M. & Mueller, U. G. 2003. Inter-island movements and population differentiation in a pelagic seabird. *Molecular Ecology*, **12**, 2835–2843.
- Fadao, T., Tingzheng, W. & Yajun, Z. 2000. Inbreeding avoidance and mate choice in the mandarin vole (*Microtus mandarinus*). *Canadian Journal of Zoology*, **78**, 2119–2125.
- Fernández, P., Anderson, D. J., Sievert, P. R. & Huyvaert, K. P. 2001. Foraging destinations of three low-latitude albatross (*Phoebastria*) species. *Journal of Zoology*, **254**, 391–404.
- Fisher, H. I. 1976. Some dynamics of a breeding colony of Laysan albatrosses. *Wilson Bulletin*, **88**, 121–142.
- Greenwood, P. J. 1980. Mating systems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, **28**, 1140–1162.
- Greenwood, P. J. 1987. Inbreeding, philopatry, and optimal outbreeding in birds. In: *Avian Genetics: a Population and Ecological Approach* (Ed. by F. Cooke & P. A. Buckley), pp. 207–222. London: Academic Press.
- Griffiths, R., Double, M. C., Orr, K. & Dawson, R. J. G. 1998. A DNA test to sex most birds. *Molecular Ecology*, **7**, 1071–1075.
- Hauber, M. E. & Sherman, P. W. 2001. Self-referent phenotype matching: theoretical considerations and empirical evidence. *Trends in Neurosciences*, **24**, 607–614.
- Jeffreys, A. J., Wilson, V. & Thein, S. 1985a. Hypervariable minisatellite regions in human DNA. *Nature*, **314**, 67–73.
- Jeffreys, A. J., Wilson, V. & Thein, S. 1985b. Individual-specific ‘fingerprints’ of human DNA. *Nature*, **314**, 76–79.
- Keller, L. F. 1998. Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). *Evolution*, **52**, 240–250.
- Keller, L. F. & Waller, D. M. 2002. Inbreeding effects in wild populations. *Trends in Ecology and Evolution*, **17**, 230–241.
- Keller, L. F., Arcese, P., Smith, J. N. M., Hochachka, W. M. & Stearns, S. C. 1994. Selection against inbred song sparrows during a natural population bottleneck. *Nature*, **372**, 356–357.
- Keller, L. F., Grant, P. R., Grant, B. R. & Petren, K. 2002. Environmental conditions affect the magnitude of inbreeding depression in survival of Darwin’s finches. *Evolution*, **56**, 1229–1239.
- Kempnaers, B., Adriaensen, F., Van Noordwijk, A. J. & Dhont, A. A. 1996. Genetic similarity, inbreeding and hatching failure in blue tits: are unhatched eggs infertile? *Proceedings of the Royal Society of London, Series B*, **263**, 179–185.
- Kruuk, L. E. B., Sheldon, B. C. & Merilä, J. 2002. Severe inbreeding depression in collared flycatchers (*Ficedula albicollis*). *Proceedings of the Royal Society of London, Series B*, **269**, 1581–1589.
- Longmire, J. L., Lewis, A. K., Brown, N. C., Buckingham, J. M., Clark, L. M., Jones, M. D., Meincke, L. J., Meyne, J. M., Ratliff, R. L., Ray, F. A., Wagner, R. P. & Moyzis, R. K. 1988. Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. *Genomics*, **2**, 14–24.
- Metz, V. & Schreiber, E. A. 2002. Great frigatebird (*Fregata minor*). In: *The Birds of North America*. No. 681 (Ed. by A. Poole & F. Gill). Philadelphia: Academy of Natural Sciences; Washington, D.C.: American Ornithologists’ Union.
- Nelson, J. B. 1975. The breeding biology of frigatebirds: a comparative review. *Living Bird*, **14**, 113–155.
- Part, T. 1996. Problems with testing inbreeding avoidance: the case of the collared flycatcher. *Evolution*, **50**, 1625–1630.
- Penn, D. & Potts, W. 1998. MHC-disassortative mating preferences reversed by cross-fostering. *Proceedings of the Royal Society of London, Series B*, **265**, 1299–1306.
- Petrie, M., Krupa, A. & Burke, T. 1999. Peacocks lek with relatives even in the absence of social and environmental cues. *Nature*, **401**, 155–157.
- Plenge, M., Curio, E. & Witte, K. 2000. Sexual imprinting supports the evolution of novel male traits by transference of a preference for the colour red. *Behaviour*, **137**, 741–758.
- Pusey, A. & Wolf, M. 1996. Inbreeding avoidance in animals. *Trends in Ecology and Evolution*, **11**, 201–206.
- Queller, D. C. & Goodnight, K. F. 1989. Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.
- Reeve, H. K., Westneat, D. F. & Queller, D. C. 1992. Estimating average within-group relatedness from DNA fingerprints. *Molecular Ecology*, **1**, 223–232.
- Reville, B. J. 1988. Effects of spacing and synchrony on breeding success in the great frigatebird (*Fregata minor*). *Auk*, **105**, 252–259.
- Schørring, S. 2001. Ecologically determined natal philopatry within a colony of great cormorants. *Behavioral Ecology*, **12**, 287–294.
- Schreiber, E. A. & Schreiber, R. W. 1993. Red-tailed tropicbird (*Phaethon rubricauda*). In: *The Birds of North America*. No. 43 (Ed. by A. Poole & F. Gill). Philadelphia: Academy of Natural Sciences; Washington, D.C.: American Ornithologists’ Union.
- Shields, W. M. 1982. *Philopatry, Inbreeding, and the Evolution of Sex*. Albany: State University of New York Press.
- Shields, W. M. 1993. The natural and unnatural history of inbreeding and outbreeding. In: *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives on Population Structure* (Ed. by N. W. Thornhill), pp. 143–169. Chicago: University of Chicago Press.
- Slate, J., Kruuk, L. E. B., Marshall, T. C., Pemberton, J. M. & Clutton-Brock, T. H. 2000. Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). *Proceedings of the Royal Society of London, Series B*, **267**, 1657–1662.
- Steidl, R. J. & Thomas, L. 2001. Power analysis and experimental design. In: *Design and Analysis of Ecological Experiments* (Ed. by S. M. Scheiner & J. Gurevitch), pp. 14–36. Oxford: Oxford University Press.

- Stockley, P., Searle, J. B., MacDonald, D. W. & Jones, C. S.** 1993. Female multiple mating behaviour in the common shrew as a strategy to reduce inbreeding. *Proceedings of the Royal Society of London, Series B*, **254**, 173–179.
- Waser, P. M.** 1993. Sex, mating systems, inbreeding, and outbreeding. In: *The Natural History of Inbreeding and Outbreeding* (Ed. by N. W. Thornhill), pp. 1–16. Chicago: University of Chicago Press.
- Wetton, J. H., Carter, R. E., Parkin, D. T. & Walters, D.** 1987. Demographic study of a wild house sparrow population by DNA fingerprinting. *Nature*, **327**, 147–149.
- Zar, J. H.** 1996. *Biostatistical Analysis*. Englewood Cliffs, New Jersey: Prentice Hall.
- Zelano, B. & Edwards, S. V.** 2002. An Mhc component to kin recognition and mate choice in birds: predictions, progress, and prospects. *American Naturalist, Supplement*, **160**, 225–237.