

Original Article

No evidence for inbreeding avoidance through active mate choice in red-billed gulls

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Except for cooperative breeders, most studies on wild birds have failed to find evidence for inbreeding avoidance via kin discriminative mate choice. This, together with evidence for kin avoidance through dispersal, has led to the general view that dispersal is often a sufficient inbreeding avoidance mechanism and active discrimination through mate choice is unnecessary. Yet, the study of inbreeding avoidance in the wild is difficult and long-term studies of pedigreed wild populations can provide important insights. We studied the occurrence of inbreeding avoidance in a highly philopatric red-billed gull (*Larus novaehollandiae scopulinus*) population subject to an individual-based field study since 1958 in Kaikoura, New Zealand. Despite a wealth of breeding and pedigree data, we did not observe a single inbred pair. This observation was a small but significant deviation from the expectation under the null hypothesis of random mating when we looked at annual breeding attempts, suggesting inbreeding avoidance. However, the difference disappeared when we examined pair bonds rather than annual breeding attempts. Our results are consistent with the expectation that close inbreeding occurs rarely in large random-mating populations. They also demonstrate how mating systems, in this case long-term monogamous pair bonds with sex differences both in the age of first breeding and breeding dispersal at natal subcolonies, can reduce the likelihood of inbreeding. *Key words:* aves, inbreeding, inbreeding avoidance, pedigree, relatedness. [*Behav Ecol* 23:672–675 (2012)]

INTRODUCTION

The negative effects of inbreeding on fitness have been well documented in nature (Keller and Waller 2002; Frankham 2010), and inbreeding depression has been shown to be a threat to the persistence of populations in the wild (Newman and Pilon 1997; Saccheri et al. 1998; Vilas et al. 2006). Because of its often severe consequences to fitness, inbreeding has been thought to impose strong selection for kin avoidance mechanisms (see, e.g., Pusey and Wolf 1996). However, inbreeding avoidance is not expected to evolve universally because of the fitness costs of its mechanisms (e.g., Waser et al. 1986) and because of the potential benefits of mating between relatives for their inclusive fitness (e.g., Kokko and Ots 2006). When present, inbreeding avoidance can operate via several distinct mechanisms, including dispersal and kin-discriminative mate choice (see, e.g., Blouin SF and Blouin M 1988; Pusey and Wolf 1996).

Kin avoidance through active mate choice is known to exist in several species in the wild (Pusey and Wolf 1996). However, the pattern is not uniform (e.g., Holand et al. 2007). In birds, the phenomenon seems to be restricted to cooperative breeders which have high levels of encounter rates with close kin (Jamieson et al. 2009). One of the rare examples in a non-cooperative breeder is the savannah sparrow (*Passerculus sandwichensis*), in which individuals avoid mating with close relatives and for which there is also some evidence of inbreeding

depression (Wheelwright et al. 2006). Most studies that have looked for active mate choice—for example in the great tit (*Parus major*; Greenwood et al. 1978; van Tienderen and van Noordwijk 1988; Szulkin et al. 2009), medium ground finch (*Geospiza fortis*; Gibbs and Grant 1989), collared flycatcher (*Ficedula albicollis*; Pärt 1996), song sparrow (*Melospiza melodia*; Keller and Arcese 1998), great reed warbler (*Acrocephalus arundinaceus*; Hansson et al. 2007), and New Zealand saddleback (*Philesturnus carunculatus*) and robin (*Petroica australis*) (Jamieson et al. 2009)—have failed to detect deviations from what would be expected under random mating or even indicated that individuals favor their kin in mate choice (Szulkin et al. 2009). It has been suggested that this together with evidence for kin avoidance through dispersal (e.g., Szulkin and Sheldon 2008) supports the conclusion that dispersal is frequently an adequate inbreeding avoidance mechanism in the wild and that active mate choice plays a relatively minor role (Hansson et al. 2007; Szulkin and Sheldon 2008). However, this lack of evidence might also at least in part be due to the difficulty of studying inbreeding avoidance through mate choice in natural populations (Pärt 1996). Despite the difficulties, long-term studies on wild animal populations with sufficient pedigree information can provide important insights on inbreeding avoidance in the wild (Pemberton 2008).

Here, we studied the levels of inbreeding and the occurrence of inbreeding avoidance through kin-discriminative mate choice in a wild population of red-billed gulls (*Larus novaehollandiae scopulinus*) in Kaikoura, New Zealand. Many of the previous studies on inbreeding avoidance have been performed on species with relatively high emigration rates. The red-billed gull population, however, is essentially a closed population that is highly philopatric with extremely low levels of

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immigration and emigration (Mills et al. 2008). The breeding population in Kaikoura has been the subject of an individual-based long-term field study since 1958 (see, e.g., Mills 1973), but this is the first study to look at the levels of inbreeding in this declining (Mills et al. 2008) population. We were interested in whether the observed levels of inbreeding, as calculated from the pedigree, could be explained by random mating, life history and the spatial, temporal and pedigree structure of the population, or whether behavioral kin-avoidance mechanisms should be invoked to explain the observed patterns.

MATERIALS AND METHODS

Study species, population, and data

The red-billed gull is a predominantly coastal breeding, long-lived native bird of New Zealand (e.g., Mills 1973, 1989, 1994). They form long-term, monogamous pair bonds, and extra-pair copulation attempts are common but rarely successful (Mills 1994)—an analysis of 1025 family groups for paternity determination has revealed that only 2.6% of the genotyped offspring are extra-pair young (Cloutier A, Mills JA, Baker AJ, unpublished results). Based on previous studies, on average 83% of pairs retain mates from one season to the next and a substantial share of the mate changes that do occur is caused by death, causing 39% of breakups of females and 27% of males (Mills et al. 1996). The differences between the sexes arise because there are more females in the population and over their life span they tend to breed less frequently than males (Mills 1989, 1991). The probability of divorce when both members survive from 1 year to the next is 10.5%. Young individuals and pairs breeding for the first time are more likely to divorce than older individuals and longer term pair bonds (Mills et al. 1996). The number of pairs formed by individuals over their lifetime varies from 1 to 9, with 75% having no more than 2 partners.

We studied the species at the Kaikoura Peninsula (42°26'S, 173°42'E), where the breeding population has been under an individual-based long-term field study since 1958 (see, e.g., Mills 1973). There are several breeding subcolonies, some of which are permanent sites and others temporary from year to year, within a 3 kilometer radius on the headland of the peninsula and the population is highly philopatric (Mills et al. 2008). Based on records of individuals banded in 35 localities in New Zealand and in the outlying islands, immigrants are rare at the peninsula, averaging just 1.3% of breeding individuals in 1964–1969 (Mills 1973).

We used annual breeding data collected from 1975 to 2004—the period of the long-term study for which we had the most complete breeding records—and a pedigree using information from all available years with 6 generations and 7137 individuals with relatives known to have attempted to breed during that period, 1111 for which both parents were known. The breeding data consisted of 4870 pair bonds between individually marked birds and 12 454 annual breeding attempts, reflecting the large size of the population that varied between 8300 and 19 400 individuals (Mills et al. 2008). We excluded from the analysis female–female pairs that annually average about 7% of nesting pairs (Mills 1989; Mills et al. 2008).

Statistical analysis

We used coancestry coefficient F calculated from the pedigree to measure inbreeding levels of pairs. The coancestry coefficient of a pair is equivalent to the inbreeding coefficient of its offspring (Lynch and Walsh 1998). All individuals with both parents unknown were considered founders for which $F = 0$.

Individuals with one unknown parent also had $F = 0$ but were potentially informative on the F of their offspring.

We tested whether the observed number of inbreeding events, defined as pairs with $F \geq 0.0625$, was different from expectations under random mating. The inbreeding level of $F \geq 0.0625$ corresponds to the inbreeding coefficient of the offspring of first cousins and was judged to be substantial enough for the expression of the deleterious effects of inbreeding and thus for the need for inbreeding avoidance. To rule out the effect of our arbitrary definition of the inbreeding event ($F \geq 0.0625$), we reran the analysis using different definitions ($F > 0$ and $F \geq 0.25$) and average coancestry coefficient instead of the number of inbreeding events. The analysis with $F \geq 0.25$ in which inbreeding events consisted of pairs of a parent and an offspring and of full-sibs also provided information on inbreeding avoidance through active mate choice between closely related individuals.

For the test, we generated 10 000 random permutations of the pair data by randomly assigning a mate for each female from all the males available in a given year. For each permutation, we calculated F for each pair from the pedigree and then the number of inbreeding events, thus obtaining a distribution for the expected F under random mating. This was done for 4 different scenarios: for 1) breeding attempts and 2) pair bonds ignoring the colony structure and for 3) breeding attempts and 4) pair bonds incorporating the colony structure. At the pair bond level ($N = 4870$), the individuals were deemed to be available for mating only in the first year of an observed pair bond, whereas at the breeding attempt level ($N = 12 454$) in all years of a pair bond. Comparison of different scenarios provides information on the effect of mating system and colony structure on the expected frequency of inbreeding. For example, the comparison between scenarios (1) and (2) allows testing for the effect of long-term pair bonds, whereas the comparison between scenarios (1) and (3) allows testing for the impact of colony structure.

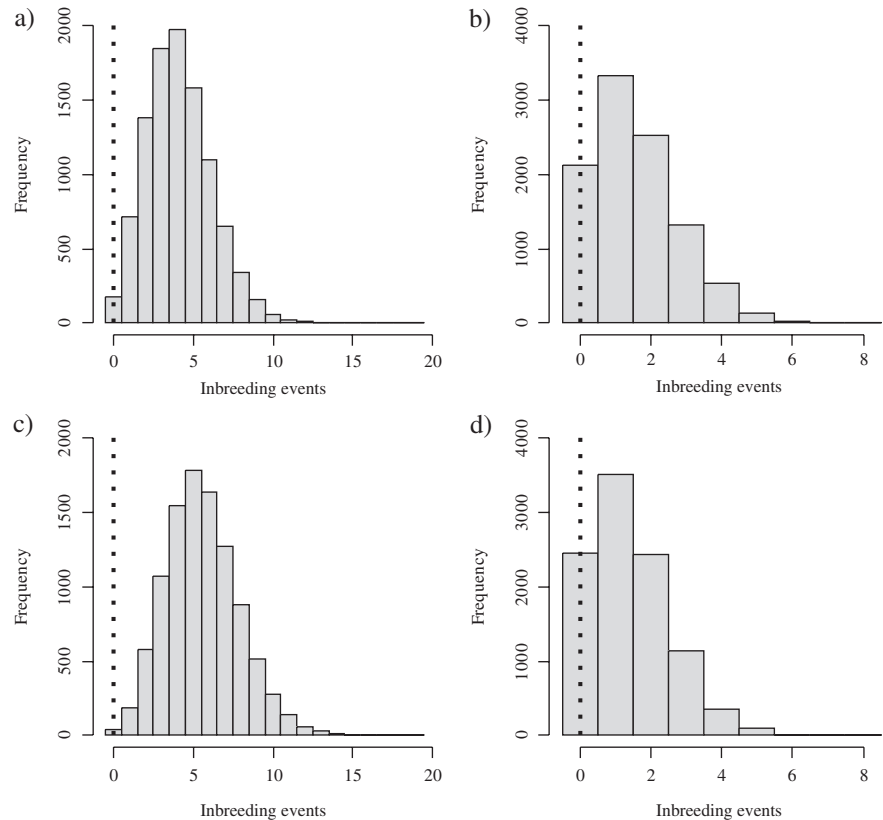
Each of the 10 000 random permutations of the pair data consisted of either 12 454 or 4870 randomly assigned pairs, depending on whether the annual breeding attempts or pair bonds were analyzed. At the breeding attempt level, there were 3 observed cases in which a female had 2 partners during a year and one case in which a male had 2 partners during a year. Because these were extremely rare, the cases were simply ignored and these individuals were assigned 2 random mates within a year. When applicable, colony structure was incorporated by limiting random permutation to occur within each of the 32 subcolonies from which we had observations in the data set. The analysis was done with the statistical software R (<http://www.r-project.org>). F was calculated using the function “inbreeding” of the extension package “pedigreemm” (available through CRAN; <http://cran.r-project.org>).

RESULTS

The observed number of inbreeding events was zero. At the breeding attempt level ($N = 12 454$), this was significantly lower than would be expected under random mating, both ignoring the colony structure (median = 4; $P = 0.017$; Figure 1a) and taking it into account (median = 5; $P = 0.003$; Figure 1c). However, at the pair bond level ($N = 4870$), there was no significant difference when the colony structure was ignored (median = 1; $P = 0.215$; Figure 1b) or when it was accounted for (median = 1; $P = 0.243$; Figure 1d). Overall, the expected proportion of inbred pairs both at the breeding attempt and at the pair bond level was very low (range based on the medians: $0.21\text{--}0.40 \times 10^{-3}$). The analyses with different definitions for the inbreeding event ($F > 0$ and $F \geq 0.25$) and the analysis based on average coancestry coefficient produced similar results and

Figure 1

The expected number of inbreeding events ($F \geq 0.0625$) in the study population under the null hypothesis of random mating for (a) breeding attempts and (b) pair bonds ignoring the colony structure and for (c) breeding attempts and (d) pair bonds taking the colony structure into account. The observed number of inbreeding events in the wild at both breeding attempt and pair bond level was zero, and it is marked by a vertical dashed line for comparison. The difference between the observed and the expected number of inbreeding events was significant for (a) and (c) ($P = 0.017$ and $P = 0.003$, respectively). Distributions are based on 10 000 randomizations.



identical conclusions, the single exception being that with $F \geq 0.25$ the difference between the observed and expected number of inbreeding events at the breeding attempt level, ignoring colony structure, dropped below the threshold of statistical significance (median number of expected inbreeding events = 3; $P = 0.069$; other results not shown).

DISCUSSION

Surprisingly, there was not a single observation of inbreeding despite the fact that the study spanned 30 breeding seasons, 6 generations, more than 7000 banded individuals and over 10 000 recorded breeding attempts. As a comparison, in previous studies in the collared flycatcher and savannah sparrow, inbred pairs have been very few but nonzero (22 inbred pairs out of 2107 [Kruuk et al. 2002] and 9 inbred pairs out of 1110 [Wheelwright et al. 2006], respectively). At the level of breeding attempts, there was a small but significant difference between the observed and expected number of inbreeding events. However, the difference disappeared when pair bonds instead of annual breeding attempts were examined, indicating that the long monogamous pair bonds reduce the probability of inbreeding in red-billed gulls. Furthermore, the expected number of inbreeding events, and hence the expected proportion of inbred pairs, was very low. The total lack of observed inbreeding was thus adequately explained by random mating, which together with long pair bonds negated the need to invoke behavioral kin avoidance.

The low expected proportion of inbred pairs was caused by the low number of relatives available to mate, compared with the large pool of available unrelated individuals. The significant difference between the breeding attempt and pair bond level, that is, between what would be expected if the gulls would form pairs annually and their actual mating system, has a number of potential causes. The difference probably results in part

because males commence breeding at an earlier age than females (Mills et al. 1996) causing a reduced likelihood of breeding with a same-aged sibling. Since 83% of individuals retain their partner in subsequent years, this would lower the probability of incestuous pair bonds. However, long-term pair bonds (up to 17 years) likely would not entirely prevent inbreeding because same-aged siblings could be available following the death or divorce of a partner, and in any case, there would be different aged full or half siblings within the population. This results because future generations are maintained by only a small proportion of the population, with 20% of males in the population producing 58% of all fledglings and 15% of females producing 52% (Mills 1989). However, the maximum number of fledglings produced by an individual in its lifetime was 19 (Mills JA, unpublished data). Assuming that all the progeny survived to breeding age, there would only be 9 or 10 of the opposite sex to breed with provided they were unmated at the time. Overall, the chances of siblings pairing together in a large population with 8000–19 000 individuals would be small.

Another factor which might also initially reduce inbreeding is that males returning to breed for the first time are more likely to breed at the natal subcolony than females (Mills 1973). In any one season, there were between 4 and 23 subcolonies on the peninsula where individuals could breed. This can be viewed as a limited form of sex-specific dispersal, a factor thought to help with kin avoidance in other species (e.g., Szulkin and Sheldon 2008). However, this effect can also be limited because if the pair is unsuccessful at the first breeding site or in a subsequent season, they are highly likely to shift to a different subcolony and often divorce (Mills et al. 1996 and Mills JA, unpublished data), therefore increasing the chance of coming in contact with siblings of the opposite sex. In the analyses of the present study, colony structure did not have an apparent effect on probability of inbreeding.

Our results are in line with the expectation that close inbreeding will occur only rarely in a random-mating population with a large population size (e.g., Shields 1993; Jamieson et al. 2009). Furthermore, the results demonstrate how the mating system of a species can influence its propensity for inbreeding. Obviously, since we could not observe the life history of nonexistent inbred individuals in our study, we cannot without experiments address the magnitude of inbreeding depression or whether there would be any selective advantage from inbreeding avoidance in this species in the first place. However, given the rarity of inbreeding events under random mating, any contemporary selection for kin avoidance would be weak, even assuming a high load of deleterious mutations that characterize large outbred populations (Frankham et al. 2002). Future studies can provide further information on the potential for inbreeding depression and the genetic structure of the population.

Pedigrees for wild populations vary in quality and completeness (Pemberton 2008). Despite the considerable size of our pedigree, the expected number of inbreeding events was relatively low and did not differ significantly from zero at the level of pair bonds. This might have been because of the structure of the pedigree or breeding data, or, most likely, a combination of both. It is thus reasonable to question whether our failure to find evidence for kin discriminative mate choice was due to lack of statistical power or a true absence of the phenomenon. Unfortunately, the question cannot be conclusively answered with the current data. Final resolution of the issue rests in potential mate choice experiments or in analyses employing molecular genetic approaches to assess whether mate choice involves maximizing heterozygosity and whether there are preferences for dissimilar major histocompatibility complex genes among social mates. What is clear now is that in some species and populations we—something counter intuitively—do not expect to find any inbred individuals based on pedigree data even when we have monitored the population for decades. Current evidence in the red-billed gull is consistent with previous reports (Hansson et al. 2007; Szulkin and Sheldon 2008; Jamieson et al. 2009) concluding that random mating in combination with other factors, such as dispersal or long-term pair bonds, should render inbreeding avoidance via kin discriminative mate choice in many cases unnecessary.

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