

Original Article

Postcopulatory mechanisms of inbreeding avoidance in the island endemic hihi (*Notiomystis cincta*)

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Avoiding genetic incompatibility resulting from inbreeding is thought to be one of the main drivers of mate choice, promiscuity, and sexual conflict. Inbreeding avoidance has been found across a wide range of taxa and is predicted to be adaptive when the costs of inbreeding outweigh the benefits. This study tests the inbreeding avoidance hypothesis at the precopulatory and postcopulatory stages in a natural population of the promiscuous endemic bird, the hihi. This species has high costs associated with inbreeding as it depresses offspring survival. We generate alternative predictions to explain the observed fertilization patterns based on the existence or absence of precopulatory and/or postcopulatory mechanisms of inbreeding avoidance. Nonrandom mating with respect to relatedness is found mainly at the postcopulatory stage. Interestingly, mating patterns appear opposed. There is a trend for females choosing more closely related social males than random, but postcopulatory patterns are biased toward less related extrapair males. This strategy suggests that at the precopulatory stage females may tolerate inbreeding as the costs of developing inbreeding avoidance may be high, especially in light of forced copulations, if natal dispersal is limited or if they gain inclusive fitness. However, as postcopulatory patterns are biased toward less-related individuals inclusive fitness explanations are unlikely. Postcopulatory patterns may arise if there are mechanisms such as sperm ejection or gametic compatibility such as sperm selection or biased fertility/mortality of offspring by related males. The observed patterns are likely to be an optimal compromise between the divergent selection pressures on each sex. **Key words:** extrapair paternity, genetic compatibility, inbreeding avoidance, kin recognition, random mating, stitchbird. [*Behav Ecol* 23:278–284 (2012)]

INTRODUCTION

Mating between relatives can have profound consequences for the fitness of the offspring they produce. Inbred individuals are often smaller, less viable, and fertile than outbred individuals; a phenomenon known as inbreeding depression (Charlesworth and Charlesworth 1999). Inbreeding depression can be substantial under natural conditions, has been found across a number of taxa (Crnokrak and Roff 1999), and is predicted to be a contributing factor in the extinction of wild populations (Frankham 1998; Frankham et al. 2002). Where costly inbreeding has the potential to occur, natural selection is predicted to favor mechanisms to avoid mating between close relatives (Keller and Waller 2002).

Inbreeding avoidance can take place at the precopulatory or postcopulatory stage. Previous studies have suggested 2 main mechanisms of precopulatory inbreeding avoidance: natal dispersal and kin recognition (Pusey and Wolf 1996; Keller and Arcese 1998). Dispersal is generally sex biased and physically

separates close relatives to prevent them from mating (Keller and Arcese 1998). This mechanism of inbreeding avoidance has been extensively studied in vertebrates (Johnson and Gaines 1990; Perrin and Mazalov 1999). In instances where dispersal is not possible, and encountering relatives is relatively common, such as can occur on islands, inbreeding avoidance may rely on kin recognition (Pusey and Wolf 1996; Frankham 1998). Kin recognition can occur in a variety of ways, for example, through early life experience of nest-mates and parents, familiarity or delayed breeding in the presence of relatives (Pusey and Wolf 1996), or signals independent of early life experience, such as phenotypic traits that are correlated with cues of genetic similarity (Tregenza and Wedell 2000; Jamieson et al. 2009).

In many species, social monogamy restricts mate choice, which may lead to suboptimal pairings (Kruuk et al. 2002); however, females may circumvent this by pursuing extrapair copulations (EPCs) (Foerster et al. 2006), which can promote postcopulatory mechanisms of inbreeding avoidance. A number of these have been suggested, including mediation of the sperm competition process by females (cryptic female choice), differential sperm investment by males, or differential mortality of embryos of related individuals (Tregenza and Wedell 2002; Pizzari et al. 2004). However, our understanding of these mechanisms is still limited in internal fertilizing species (Pizzari et al. 2004).

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The use of extrapair paternity (EPP) as a mechanism of inbreeding avoidance is believed to arise when genetic similarity with the social male is high (Birkhead and Møller 1998). Inbreeding avoidance is generally tested by comparing whether the frequency of close inbreeding is consistent with the frequency expected from a random mating model (Keller and Arcese 1998; Hansson et al. 2007; Jamieson et al. 2009; Szulkin et al. 2009). Evidence for the use of EPP as an inbreeding avoidance mechanism in birds has been limited to mostly cooperative breeding species (e.g. Daniels and Walters 2000; Foerster et al. 2006; see review Jamieson et al. 2009), with some exceptions (e.g. Blomqvist et al. 2002; Freeman-Gallant et al. 2006). This is most likely because encounter rates with relatives in cooperative breeding species are substantially higher than in noncooperative breeding species (e.g. single pair breeders). High encounter rates with relatives mean individuals have more opportunity to learn to recognize relatives by the time it comes to choosing a mate (Jamieson et al. 2009).

Differences in the likelihood of inbreeding avoidance/tolerance could also be due to a number of other key life-history factors including variation in survival, natal dispersal, mating system, mate fidelity, constraints on breeding, and the cost of avoiding inbreeding (Parker 1979; Kokko and Ots 2006). Most studies have found that inbreeding avoidance is more adaptive; however, there have been a few examples in which inbreeding is not avoided but actively pursued (e.g. Cohen and Dearborn 2004; Thünken et al. 2007; Sherman et al. 2008). In theory, inbreeding may be adaptive if it increases inclusive fitness (Parker 1979; Kokko and Ots 2006).

Inbreeding avoidance is dependent on the cost of inbreeding for each sex (Lehmann and Perrin 2003; Pizzari et al. 2004). Inbred matings may benefit males, increasing their reproductive success, as the cost is lower if they have invested less without forfeiting other mating opportunities (Pizzari et al. 2004). However, in females, it may incur drastic reductions in their lifetime reproductive output (Lehmann and Perrin 2003). Males may also avoid inbreeding under certain circumstances, for example, if they are related to the female by more than two-thirds, and inbreeding costs are very high (Parker 1979). Alternatively, species with strong seasonal or short life span constraints on reproduction should be more tolerant of inbreeding given the potential cost of missing breeding opportunities if only relatives are encountered (Parker 1979; Lehmann and Perrin 2003; Kokko and Ots 2006).

The aim of this study was to test for inbreeding avoidance at the precopulatory stage through social mate selection and at the postcopulatory stage through extrapair offspring in a closely monitored island population of an endangered passerine bird, the hihi (*Notiomystis cincta*). This species has high inbreeding costs associated to hatching and nestling failure (Brekke et al. 2010) and is therefore likely to evolve inbreeding avoidance mechanisms. Hihi are socially monogamous but highly promiscuous with EPC typically being forced on females by males (Castro et al. 1996; Ewen et al. 1999; Ewen and Armstrong 2000; Low 2005). Males can display 2 different, but not mutually exclusive, reproductive strategies within a breeding season; they can be territorial or floaters. Due to these mixed reproductive strategies, males can father offspring within their territory with their social mate and/or further afield with extrapair partners or alternatively remain as floater male seeking copulations with otherwise paired females (Ewen et al. 1999). Territorial males have higher mating opportunities than floater males from their social partner and females in adjacent territories. Therefore, the likelihood of extrapair matings varies considerably among females (Ewen et al. 1999; Ewen and Armstrong 2000). Encounter rates are relatively high due to their mating system, communal feeding

areas, overlapping generations, relatively long life expectancy, and the spatial restrictions on a small island (220 ha) with no immigration or emigration (Armstrong et al. 2002). Sex-biased dispersal is known to occur (mean dispersal distance = 411 m for males and 498 m for females [Richardson et al. 2010]), but it is dependent on population density and sex ratio (56% males:46% females in 2006/2007) and limited by the island size and territory availability (Richardson et al. 2010).

We have assumed that forced copulations by males are random with respect to relatedness. We justify this assumption because forced copulations are frequent and observation has often shown large proportions of the male population can be involved during any particular female's fertile period (Low 2005) and the prediction that in promiscuous systems males do not avoid inbreeding because the costs to their fitness are low (Lehmann and Perrin 2003). However, there may be postcopulation mechanisms for selecting sperm from unrelated males. This generates 4 alternative predictions for the observed patterns of fertilization.

1. If females choose social partners based on relatedness and there are also postcopulatory mechanisms of sperm selection then social mate choice should be nonrandom with respect to relatedness and all (within and extrapair) fertilizations should be from less related males than expected if fertilizations were random.
2. If there is female choice for social partner but no postcopulatory mechanisms of sperm selection then social pair fertilizations should be nonrandom with respect to relatedness and extrapair fertilizations should be random with respect to relatedness. Under the assumption that extrapair fertilizations are the result of forced copulations and that these copulations are random with respect to relatedness.
3. Alternatively, if female mate choice is random with respect to relatedness but there is postcopulatory sperm selection then all fertilizations should be nonrandom with respect to relatedness and any social pairing to a related male will result in a reduced proportion of social male sired offspring when less closely related extrapair males have copulated with the female.
4. Finally with no female mate choice or postcopulatory mechanisms of sperm selection then all fertilizations should be random with respect to relatedness.

MATERIALS AND METHODS

Study site and species background

The hihi is a sole representative of an endemic New Zealand bird family, Notiomystidae (Driskell et al. 2007). Hihi were originally found throughout the northern half of New Zealand but following European colonization declined to a single remnant population on (3020 ha) Little Barrier Island (by about 1885; Brekke et al. 2011). The conservation of this species focuses on the establishment of new populations (Brekke et al. 2011). The reintroduced population of Tiritiri Matangi Island was established in 1995 and 1996 from 51 individuals originating from Little Barrier Island (Armstrong et al. 2002) and has expanded to around 150 individuals. All nesting events are monitored, and individuals are identifiable by a numbered metal band and a selection of color bands attached prior to fledging (no living founders remain).

Hihi are highly mobile, wide ranging, and strong fliers. Annual survival probability of adult hihi is about 64% (Ewen et al. 2007), and they can live up to 9 years on Tiritiri Matangi although they show a reproductive peak around 3–4 years of age (Low and Pärt 2009). The breeding season spans from

September to February and hihi have the potential to reproduce in their first year of life, which means that generations overlap. Females can lay up to 3 clutches during each breeding season of on average 4 eggs, but normally can only rear a maximum of 2 clutches successfully per breeding season.

Microsatellite analysis

In the 2006–2007 Austral breeding season (September to February), blood samples from adults (males = 75; females = 50 [86% of individuals seen alive in the population]) caught in feeding cages, and fledglings ($n = 143$ [91% of total number of fledglings]) sampled at the nest (clutches, $n = 76$ [76% of total number of clutches]) before fledging, were collected via brachial venipuncture (ca. 70 μ l per hihi) and preserved in 1.5 ml microfuge tubes filled with absolute ethanol. Offspring that died before hatching ($n = 37$ [95% of developed embryos]) and fledgling ($n = 54$ [59% of dead nestlings]) were also collected from in and around the nest and 15 g of tissue preserved as above. Genomic DNA was extracted from whole blood using an ammonium acetate precipitation method (Nicholls et al. 2000). All samples were screened using a set of 19 selectively neutral microsatellite loci (15 species specific and 4 isolated from other passerines) following methods detailed in Brekke et al. (2009). The markers had an observed heterozygosity of 0.64 and 199 alleles across all loci. Samples were individually amplified twice, and if the allele calls were not consistent they were repeated until they were or were excluded from the analysis to decrease the effect of genotyping errors due to allelic drop out or false alleles (Taberlet et al. 1996).

Measuring relatedness

Testing inbreeding avoidance mechanisms requires the calculation of relatedness between individuals. Relatedness between 2 individuals can be defined as the probability that 2 alleles at a locus, 1 taken at random from each individual, are identical by descent (IBD) (Malécot 1948). If pedigree data is not complete, or reliable, an alternative approach to estimating relatedness can be exploited. The similarities (dissimilarities) between the multilocus marker genotypes of 2 individuals provide information of the genetic relatedness between the 2 individuals (Glémin et al. 2006). Marker-based pairwise relatedness methods can statistically infer IBD from alleles that are identical in state (IIS) (Blouin 2003). We used the program COANCESTRY v1.0 (Wang 2010a) which implements a moment estimator, developed by Wang (2002), to produce relatedness estimates (for details, see Wang 2002) for male and female pairs with the potential to breed in the 2006–2007 breeding season.

Parentage assignment

Social parentage for each clutch was determined from behavioral observations at the nest for all nesting attempts. Parents were identified by observing nest building, incubation, and nestling feeding behavior. Males are extremely territorial during breeding and guard their female mates especially closely whilst they are fertile (Castro et al. 1996; Ewen et al. 2004; Low 2005). A maximum likelihood method implemented in the program COLONY v2.0 (Wang and Santure 2009) was used to resolve genetic paternity. This program allows estimation of parentage under a promiscuous mating system and incorporates full and half-sibship relationships to increase the statistical power (Wang 2004). It also accounts for mutations and genotyping errors (Wang and Santure 2009) to avoid/reduce misassignments. The combined exclusion probability of the

markers used in this study for parental assignment was calculated in COANCESTRY v1.0 for one known parent (0.99) (Wang 2010a). COLONY v2.0 provides a posterior probability value for each maternal and paternal assignment, which usually increases when behavioral information on potential parentage or clutch is incorporated (Wang and Santure 2009). Only individuals assigned with 95% confidence were included in this analysis. There was a high congruence between social and genetic maternity assignments (99.2%), which confirmed the power of the parentage analysis using this set of markers and method. Therefore, social maternity was included as a known parameter in subsequent paternity assignment analyses. An offspring was assigned to an extrapair male when its social father's genotype was present in the candidate father pool, but paternity was not genetically assigned to the social father. Extrapair males thus identified may have genotypes or may not be sampled or genotyped. Only the former was used in calculating relatedness between males and females ($n = 53$).

Removing potential bias

When the same markers are used to assign paternity and estimate relatedness, and the estimated paternity and relatedness are used jointly in a downstream analysis (such as in a mate choice study as the one presented here), the analysis results may be biased because a subset of genotypes may be favored when assigning paternity (Wetzel and Westneat 2009). The magnitude and direction of the bias is dependent on the methods used (for paternity assignment and/or relatedness estimation), the allele frequency distribution, and the number of markers used (Wetzel and Westneat 2009; Wang 2010b). To reduce this bias, we followed Wang (2010b) and split our markers into 2 nonoverlapping subsets in determining the relatedness of extrapair mates. The first subset contained 18 markers of the 19 available and was used to assign paternity. The second subset contained the one remaining marker and was used to calculate relatedness. There are in total 19 possible partitions, and paternity and relatedness were inferred for each partition. The relatedness for extrapair mates was obtained by averaging single locus relatedness estimates of all inferred pairs in each partition and by averaging over the 19 partitions. The relatedness for extrapair mates was then tested against that of random mates by permutation (see below). The relatedness for social mates is calculated directly using the entire set of 19 markers, as social males are determined from behavioral rather than marker data.

Mate choice as an inbreeding avoidance mechanism

To test whether mate choice was used to avoid inbreeding, pairwise relatedness values were calculated for all possible matings between sampled adult males ($n = 75$) and females ($n = 50$) known to be alive in the 2006–2007 breeding season. From these 75×50 relatedness values, a subsample of 105 values, equal to the number of inferred extrapair matings genetically resolved from offspring, was taken at random without replacement and were used to calculate the mean relatedness. This resampling was conducted 100 000 times to produce a null distribution of mean relatedness. The observed mean of extrapair relatedness was then compared with this distribution to determine whether extrapair mates were chosen at random or not with respect to relatedness. The significance of the difference from random mate choice was determined from the position of the observed mean of extrapair relatedness in the null distribution using a 2-tailed significance test ($\alpha = 0.05$). Whether social mates are more or less related than at random was tested using a subsample of 42 values, equal to the number of social matings and following the same

procedure. Statistical tests and comparisons were performed with MATHEMATICA v4.0.

Null model

Choosing the correct null model to compare with observed behavior is difficult, in particular to produce an expected frequency distribution that is independent of inbreeding avoidance behavior (Pärt 1996; Keller and Arcese 1998). To test whether the use of random mating as a null model is correct requires mate availability to be specified. Given hihi have very high levels of EPP and female promiscuity, we have restricted the random pool of males to those that were alive (territorial or floaters) in the 2006–2007 breeding season. This is further justified because hihi are highly mobile, moving across the whole island looking for food and EPC (Ewen et al. 1999) and hence the likelihood of encountering every other individual in the population is high. We therefore assume that the analysis is unaffected by temporal (e.g. migration or arrival time; Pärt 1996; Kruuk et al. 2002), spatial (e.g. limited breeding opportunities to the physically closest individuals; Keller and Arcese 1998), or social structuring of available mates (e.g. mating not socially limited; Van de Castele and Matthysen 2006).

Patterns of EPP within mixed broods

A generalized linear model was used to test whether the number of extrapair males that sired offspring at each clutch was related to the level of relatedness (continuous variable) between the female and her social mate. The number of EPP males was used as the response variable with the relatedness values between the female and her social partner and clutch size as the potential explanatory variables. To test whether the number of extrapair offspring in the clutch was related to the female and social male relatedness a generalized linear mixed model was implemented with a binomial response variable (EPP/not EPP) and relatedness between the female and social male and clutch size as potential explanatory variables. Mother identification was set as the random factor to control for nest and other potential maternal effects. Models were constructed in the R v2.11.1 statistical programming environment (R Development Core Team 2007).

RESULTS

Degree of EPP in relation to social pair relatedness level

Parentage was successfully assigned to 199 (86%) offspring of the 232 available. One hundred and nineteen of these individuals were from extrapair matings (60%) and 80 from within pair matings (40%). Seventy-six complete clutches were sampled successfully with 87% (standard deviation [SD] 7.02) containing EPP offspring with an average EPP offspring per clutch of 61% (SD 3.05). In the remaining 33 offspring, paternity could not be assigned due to lack of social father's genotype to check against paternity assignment or lower than 95% likelihood of correct paternity assignment. During this breeding season territorial males fathered on average 1.41 offspring with their social female and 1.50 offspring with non-social females, whereas floater males fathered 1.05 offspring through extrapair matings. Therefore, territorial males have considerably higher reproductive success overall.

From the models, we found that social pair relatedness was not a useful predictor of the number of extrapair offspring within a nest (EPP offspring: slope = -0.076 , standard error

[SE] = 1.952, z value = -0.039 ; P value = 0.969) or the number of extrapair males that sired these offspring within a nest (EPP males: slope = 0.049, SE = 0.123, z value = 0.396; P value = 0.692).

Mate choice as an inbreeding avoidance mechanism

Relatedness levels were derived from observed social pairings, or from genetic paternal assignment, in the 2006–2007 breeding season on Tiritiri Matangi Island (observed mean = 0.022; n = 125). The mean observed social pair relatedness was 0.072 (n = 42; Figure 1) and extrapair mean relatedness was -0.020 (n = 105; Figure 1). The observed mean relatedness value of social pairwise relatedness was not higher than random (expected mean = 0.028; 2.5 percentile = -0.035 ; 97.5 percentile = 0.073; P value = 0.05, for a 2-tailed test, see Figure 2a). Therefore, under the null hypothesis of random mate choice there was no evidence of inbreeding avoidance through social mate choice. However, social mates do have a strong trend toward being more closely related than expected by random. Under the same conditions, observed mean pairwise relatedness between females and extrapair mates that attained fertilizations was significantly different from random (expected mean = 0.023; 2.5 percentile = -0.013 ; 97.5 percentile = 0.053; P value = 0.022, for a 2-tailed test, see Figure 2b). Relatedness values for social pairs were also significantly higher than for extrapair pairs that attained fertilizations (P value = 0.006, for a 2-tailed test, see Figure 2a,b). Therefore, under the null hypothesis of random mate choice, we concluded that inbreeding was avoided through postcopulatory mate choice enabled by EPC.

DISCUSSION

Our results indicate that hihi rely largely on postcopulatory mechanisms of inbreeding avoidance. EPCs occur frequently in hihi and are mostly forced on females by males (Ewen and Armstrong 2000; Low 2005) and result in successful extrapair fertilizations (Ewen et al. 1999; see Results). While we are unable to determine whether EPC is more likely between less-related individuals (unlikely if any convenience polyandry is operating), we do reveal that fertilizations result from copulations between individuals less related than expected under random mating. This is important because our results suggest inbreeding avoidance based on genetic compatibility at the postcopulation stage, a pattern most commonly found in invertebrate species and

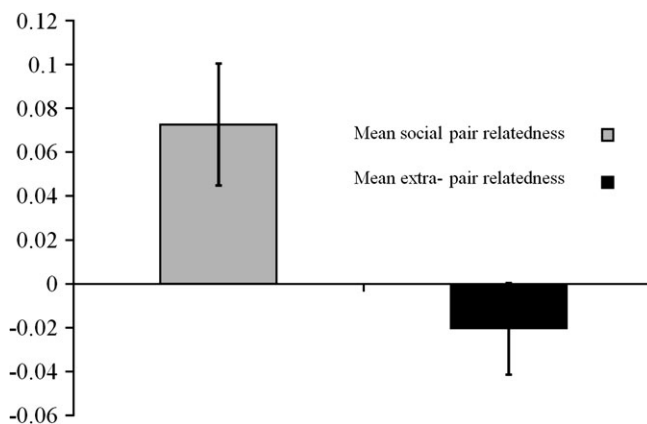


Figure 1
Average pairwise relatedness of observed social and extrapair matings with vertical bars indicating 1 SE around the mean.

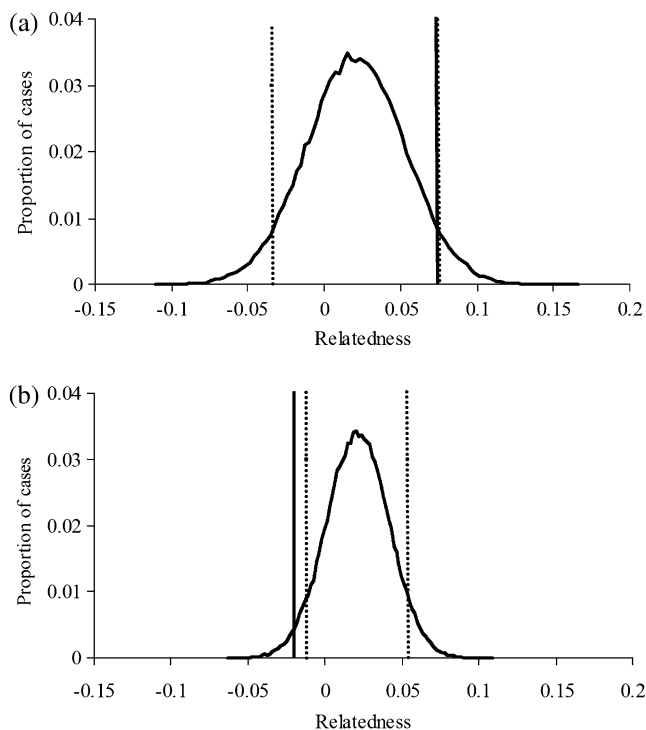


Figure 2
Distribution of the 100 000 randomized mean relatedness values of (a) social and (b) EPP matings under the random mating null hypothesis shown as solid black curves. The vertical solid black lines refer to the mean observed pairwise relatedness level of (a) social and (b) EPP matings. The vertical broken lines refer to the 5% critical values.

rarely addressed in wild vertebrates (Tregenza and Wedell 2000; Simmons et al. 2006; Welke and Schneider 2009; but for vertebrate, see e.g. Firman and Simmons 2008; Ala-Honkola et al. 2010; Gasparini and Pilastro 2011).

Interestingly, there is also a trend toward female precopulatory mate choice for more-related partners, which can theoretically evolve to increase inclusive fitness (Parker 1979; Kokko and Ots 2006). Furthermore, this evidence comes from a small population restricted to a small island with limited possibility for natal dispersal (Richardson et al. 2010), and where inbreeding depression is known to affect fitness (Brekke et al. 2010). Our results are discussed in light of these 2 apparent opposing strategies in relation to inbreeding.

Relatedness to extrapair males

Evidence for nonrandom mate choice for less genetically related mates has been found mostly in cooperative matings systems (see review in Jamieson et al. 2009). Our study is rare in that it shows inbreeding is being avoided through postcopulatory mechanisms (EPP) enabled by EPCs in a socially monogamous species. EPCs may vary among individuals (Ewen and Armstrong 2000) but tend to be random as is shown by social mate choice and as may be expected under convenience polyandry. However, some studies have found comparable results, for example, in nonpasserines such as shorebirds (Blomqvist et al. 2002) that tend to have lower levels of EPP. In passerines, examples include the savannah sparrow (Freeman-Gallant et al. 2006) and house finch (Oh and Badyaev 2006) both of which have higher levels of EPP than shorebirds but lower than this study. Inbreeding avoidance at the postcopulatory stage could be adaptive in socially monogamous species with extrapair matings as they can cir-

cumvent social mate choice, by choosing more genetically complementary individuals through cryptic female choice (Birkhead and Møller 1998; Tregenza and Wedell 2002).

It has previously been suggested that avoiding inbreeding depends on the costs of this behavior (Keller and Arcese 1998). Therefore, if the cost of inbreeding is higher than that of tolerating it, inbreeding avoidance would evolve (Keller and Arcese 1998). Inbreeding avoidance is also predicted to be important for females as they invest disproportionately in reproduction in promiscuous systems and would incur heavier costs from offspring mortality due to inbreeding. The cost to females may be magnified in hihi as the social mate also contributes to offspring rearing and his contribution (about one-third that of the female) is further reduced if there has been frequent EPC (Ewen and Armstrong 2000).

Relatedness to social males

Female social mate choice in hihi tended toward mating with closer relatives than random. However, this result was not significant and offers 2 potential alternative explanations: social mating may not be random with respect to relatedness. This pattern has been found in other species, for example, in birds (Mandarte song sparrow, Keller and Arcese 1998; tree swallow, Shutler et al. 2004; and great tit, Szulkin et al. 2009) and other nonavian taxa including Peron's tree frog (Sherman et al. 2008) and cichlid fish (Thünken et al. 2007). Social mate choice for closer relatives may suggest some inclusive fitness benefits from inbreeding (Parker 1979; Kokko and Ots 2006). This could potentially be adaptive in light of forced copulations and paternal care provisioning in this species. Socially pairing with relatives may increase male inclusive fitness as extrapair offspring would also be related to some degree. However, this seems unlikely as pairing with relatives provides few genetic benefits and relatively large costs. Inbreeding strongly depresses hatching and nestling survival, and smaller clutches suggest lower female investment in the reproductive event (Brekke et al. 2010). Territorial males in this population have also been found to have limited dispersal from their natal site as territories are limited (Richardson et al. 2010); this may also increase the likelihood of socially pairing with related females as has been found in other passerine species (Szulkin et al. 2009).

Under the conditions outlined above, social mating may also be random with respect to relatedness, as is thought to occur in many small populations that still suffer from inbreeding (Crow and Kimura 1970). Random precopulatory social mate choice by females could arise due to a lack of a discriminating mechanism or if the costs associated with developing precopulatory inbreeding avoidance outweigh the benefits. In the context of frequent forced EPC, any effort spent in choosing a mate based on relatedness may be futile. Alternatively, females may choose social mates based on nongenetic considerations, for example, based on territory familiarity, quality, and paternal care. EPC could then act by allowing females to achieve increased fitness via postcopulatory mechanisms of choice or genetic compatibility. Our finding that EPPs were by males that were significantly less related to the female than random fit this pattern.

Females do sometimes solicit EPC and may select unrelated males and time these to maximize chances of fertilizations. Equally, males may seek and force copulate unrelated females. Testing how likely this is remains a challenge under field constraints. Given the rarity of female solicited EPC (Low 2005), we suggest a more plausible explanation is that EPC is random with respect to relatedness, but fertilizations are biased toward genetically compatible partners. This would be consistent with the "genetically loaded raffle" model (Ball and Parker 2003) in

which cryptic postcopulatory female choice regulates the sperm competition processes by selecting genetically compatible or unrelated sperm (Griffith and Immler 2009). However, under a model of random mating and postcopulatory sperm selection, we would also expect social pairings between relatives would result in reduced fertilization by the social male. We found no evidence that the proportion of extrapair males or extrapair offspring per nest were linked to relatedness between the female and the social male. It is likely that postcopulatory mechanisms are not perfect because social males retain an advantage of proximity and knowledge about their mates fertility and can time copulations to increase fertilization success (through frequent copulations or last male precedence as has been commonly shown in birds; Birkhead 1990).

In conclusion, none of our 4 alternative predictions were substantiated by the mating patterns, we have revealed in this study. Both precopulatory and postcopulatory mate choice appear to be nonrandom with respect to relatedness but biased in opposite directions with precopulatory choice (of social mates at least) for more-related individuals and postcopulatory choice for less-related individuals. The costs associated with precopulatory inbreeding avoidance mechanisms may be too high or inbreeding may be adaptive if females may gain inclusive fitness from mating with relatives. Postcopulatory mechanisms of inbreeding avoidance may have evolved in light of forced copulations and inbreeding depression costs; for example: female sperm selection, biased fertilization, or offspring mortality or alternatively related males may avoid forcing copulations on related females or invest less sperm when mating with related females. Ultimately, female multiple mating opens the way for postcopulatory mechanisms to avoid inbreeding, and it is likely that the observed patterns will be an optimal compromise between the sometimes divergent selection pressures on the sexes.

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