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Original Article

A hierarchical analysis of incest avoidance in a cooperative breeder

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When animals live near family members, this creates potential for incest and inbreeding depression, especially with unfamiliar kin. We examined incest avoidance when birds paired in kin groups and after dispersal in western bluebirds, *Sialia mexicana*, a cooperative breeder with a persistent, but low frequency of adult males helping at the nest. During their first winter, sons usually live in family groups comprised of parents, brothers, immigrant females, and more rarely, immigrant males and philopatric sisters. Sons usually pair with females that have joined their winter group, although some pair with females they encounter after dispersal. Incestuous pairing among relatives with relatedness ≥0.25 rarely occurred in either context, even considering extrapair fertilizations and other sources of unfamiliar kin. Sons pairing in their winter groups preferentially mated with immigrant females and actively avoided pairing with relatives. After dispersal into kin neighborhoods in spring, active incest avoidance was still required to explain low levels of incest with females within 600 m (2–3 territories) of where sons first bred, whereas absence of incest over larger distances could be explained by random mating. The probability of encountering a female relative within 600 m of where a male settled declined rapidly with dispersal distance to near zero for males dispersing 2 km from home. Although recognition is required to avoid incest when pairing in winter groups or settling near home, female-biased dispersal reduces likelihood of incest to near zero, even when males disperse relatively short distances (e.g., 2 km) from where they were born.

Key words: **inbreeding avoidance, incest avoidance, kin discrimination, kin recognition, outbreeding, outcrossing.**

INTRODUCTION

Breeding with close relatives can lead to inbreeding depression, which manifests as reduced survival and reproductive success of offspring of inbred pairs. Although inbreeding depression is difficult to assess in wild populations of birds, mainly because incest is not very common, empirical support has materialized slowly, mainly from long-term studies (Greenwood et al. 1978; Daniels and Walters 2000; Keller et al. 2002; Kruuk et al. 2002; Townsend et al. 2009). The expectation is that natural selection will lead to behaviors that minimize the risk of incest, especially in cooperative breeders, which often live in small groups and mate locally in situations where they are likely to encounter kin.

The evolutionary pressure to avoid inbreeding is one of the primary explanations for sex-biased dispersal, in which one sex moves farther than the other to breed (Greenwood and Harvey 1982). In birds, females tend to move farther than males, whereas in mammals, females are generally the more philopatric sex (Greenwood 1980). Although sex-biased dispersal can lead to lower encounter rates with close kin, acting as a passive defense against incest, kin-based cooperative breeders often show reduced dispersal distances for both sexes (Zack 1990; Nelson-Flower et al. 2012; Van Dijk et al. 2015), such that spatial separation from kin may not be sufficient to avoid incest.

Some of the best evidence for incest avoidance in animals comes from cooperative breeders in which relatedness to the opposite sex breeder dictates whether younger birds avoid incest and help, or attempt to breed within the group (Koenig and Pitelka 1979; Piper and Slater 1993). Incest is rare in acorn woodpeckers, *Melanerpes formicivorus*, even though they regularly live in groups with close relatives of the opposite sex (Koenig et al. 1999). Whole groups will refrain from breeding for up to 4 years rather than breed with close relatives, suggesting that costs of incest are high. In such cases, avoiding incest means making the transition from helping to breeding status at the right time with the right partners, which requires the ability to discriminate immigrants from close relatives. Even in

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species with a low percentage of helpers, both male and female off-spring can remain in the family group until the start of the first breeding season, forming pairs before settlement and settling close to their natal group (Kraaijeveld and Dickinson 2001). Offspring choosing mates in winter groups can potentially base their choice on familiarity, avoiding pairing with birds that were present at their natal nest. Local dispersal after winter is more problematic, and leads to the spatial genetic structure described as "kin neighborhoods," a life-history trajectory that is also somewhat common in species that do not live in family groups (Foerster et al. 2006; Hatchwell 2010). Apart from work done on islands (Keller et al. 1994, 2002), little is known about incest avoidance, especially for low-frequency cooperative breeders and noncooperative species that breed in kin neighborhoods, rather than in a family group. Whether or not such animals benefit from kin-directed helping or more cryptic forms of cooperation, they will still likely benefit from having the ability to recognize and avoid mating with close kin.

In central, coastal California, kin-based cooperation in western bluebirds (*Sialia mexicana*) usually involves adult males helping at nests of parents or brothers (Dickinson et al. 1996) as well as other more cryptic forms of cooperation, such as cross-territorial assistance in defending against conspecific intruders or predators (Stern 2012). We studied incest avoidance in a population with highly developed winter sociality in which offspring interact in stable, territorial kin groups (Kraaijeveld and Dickinson 2001). Adult males can remain with or rejoin relatives to help at the nest during the breeding season, and even sometimes engage in cross-territorial helping (Dickinson and Akre 1998). In spite of evidence that they maintain lifelong relationships with close relatives and preferentially aid close over more distant kin (Dickinson et al. 1996), western bluebirds appear to use the social relationship, rather than actual kinship (determined with paternity testing), when deciding whom to help, how much aid to give, or even how much parental care to give (Dickinson et al. 1996; Dickinson 2003, 2004). Given that they do not discriminate genetic kin in these fitness-relevant situations, it is also unlikely that they use genetically based cues to avoid incest with unfamiliar kin.

Unfamiliar kin are likely to occur among broods raised in different years in long-lived species and in species with extrapair paternity; both of these circumstances occur in western blue-birds. Although pairs often form within winter groups comprised of familiar kin, including nest mates and parents (Kraaijeveld and Dickinson 2001), unfamiliar kin can arise in 3 ways: 1) when off-spring disperse and do not visit their natal group after dispersal, they will not be familiar with nestlings born into their natal group after they left; 2) similarly, offspring born on a territory will not be familiar with older siblings that dispersed before they were born; 3) extrapair paternity, which occurs in 20% of offspring (Dickinson and Akre 1998), will produce cryptic paternal half-sibs (and cryptic fathers/daughters) that cannot be recognized based on prior experience (Hatchwell 2010). In the absence of genetically based cues for recognition, these individuals would not be recognized as kin. Yet the vast majority of studies on kin recognition in birds indicate that offspring recognition and kin recognition, where they occur, are based on socially learned, vocal cues (e.g., Beecher 1982, 1991; Sharp et al. 2005). Although a few recent studies suggest a potential role for genetically based olfactory cues in recognition, even in these instances the possibility of learning cannot be entirely ruled out (Bonadonna and Nevitt 2004; Krause et al. 2012). If western bluebirds learn who their relatives are as nestlings and juveniles—as is evidenced by studies of vocal kin recognition (Akçay et al. 2013, 2014)—this could reduce significantly the likelihood that they will pair with familiar kin. In the absence of genetically based recognition, western bluebirds would have to rely on spatial separation to avoid mating with unfamiliar kin.

Western bluebirds help familiar kin (Dickinson et al. 2016) and thus far appear to use learned vocal cues to discriminate kin based on individual vocal differences (Akçay et al. 2014), suggesting a possible role for social experience in kin recognition. We therefore examined the potential for a 2-stage process to reduce the likelihood of incest, one relying on kin recognition to avoid pairing with familiar kin in the winter group, or in the kin neighborhood in spring, and another relying on female-biased dispersal to provide enough spatial separation of kin to reduce mating with unfamiliar or cryptic kin on settlement. As area increases exponentially with distance, female-biased dispersal can help reduce the probability of interacting with close relatives, not only within years but also across years, where lack of prior experience with some close relatives means that information on identity and relationship is lacking. Alternatively, if spatial separation is not sufficient, genetically based mechanisms would be required to avoid the costs of incest with unfamiliar kin on dispersal.

In western bluebirds, stable winter groups form during a first phase of dispersal in late summer and early fall and these groups are highly stable until late winter (Figure 1). In our earlier study, we found that 94% of first-winter males and 48% of first-winter females in winter groups were born on the study area and that 70% of sons and only 25% of daughters wintered in their natal group (Kraaijeveld and Dickinson 2001). Daughters more often left their natal group and dispersed off the study area in the fall, or they dispersed into other groups on the study area. The departing females
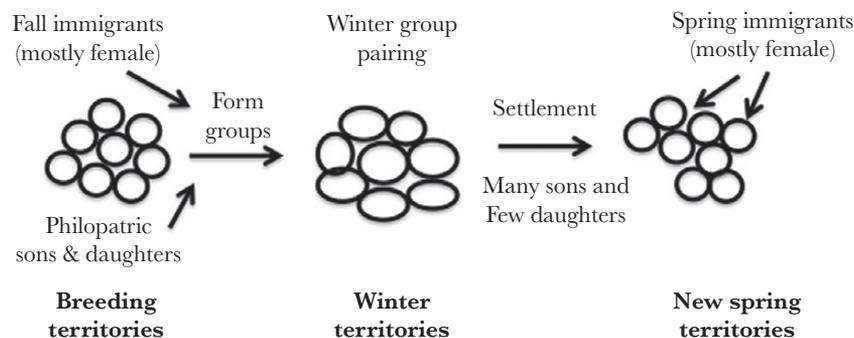

Figure 1
The process of natal dispersal in western bluebirds, showing 2 steps of dispersal, first into winter groups (circles represent territories) and second when pairs split off from winter territories onto spring territories.

were replaced by immigrant females, which comprised 51% of first-winter females in winter groups (compared with 6% immigrants among first-winter males). Overall, nearly twice as many first-winter males as females were local. Ninety-one percentage of local males and 69% of local females wintered with at least 1 individual to which their expected relatedness was ≤0.25, and philopatric daughters were frequently observed in winter groups with their brothers. Pairs formed within winter groups and then settled onto nearby territories. Avoiding incest when pairing up in winter groups would necessarily require active kin discrimination. After winter, during a second phase of dispersal, when yearlings usually leave their winter groups to breed, some individuals (mostly daughters) dispersed away from the 7 km² study area, whereas pairs that had already formed settled onto local breeding territories for spring and summer (Kraaijeveld and Dickinson 2001). Experienced, widowed females and new immigrants present a second opportunity for young males to find a mate. Because of the geometric increase in area with distance, the spring phase of dispersal should lead to spatial separation of kin, which can reduce opportunities for incest. Spatial separation could thus help reduce incest with unfamiliar kin, including siblings born in different years and paternal half-sibs arising from extrapair fertilization.

Using simulations, we tested whether first-winter birds avoided incest with siblings in their winter groups and under what conditions, once males dispersed, separation by distance was sufficient to allow them to avoid incest with cryptic kin, even if mating was random with respect to relatedness, as is the case when kin are unfamiliar and thus probably cannot be recognized as such. Together these simulations provide a means of understanding how temporal shifts in social system and hierarchical shifts in the extent of spatial separation of kin (Wright 1943) can influence the potential for incest and selection for active incest avoidance for organisms living in kin groups and dispersing into kin neighborhoods.

MATERIALS AND METHODS
Field methods and use of long-term data

Western bluebirds are small ~25-g passerines that live year round at Hastings Reserve in upper Carmel Valley, CA. The species is socially monogamous with long-term pair bonds; females mate outside their pair bond with the result that ~20% of offspring are sired by a male other than the social father. Nearly half of females (45%) have extrapair young in their nests, but genetic parentage has no influence on whether helpers help or how much they help (Dickinson and Akre 1998; Dickinson 2004) nor do fathers discriminate against extrapair young when feeding at the nest (Dickinson 2003). As a consequence, most of our analyses focused on social genealogies.

This study is based on continuous monitoring of nests and color-banding of all nestlings and adults using nest-boxes at Hastings Reserve and the adjacent Oak Ridge Ranch from 1 March to 15 July in the years from 1983 through 2001 and on a smaller share of the study area from winter of 2001–2006. Because of the importance of knowing the social parents of philopatric individuals, we only used pairing data collected after 1985. Breeding adults were identified as they initiated nests and were banded (if new to the study area) by day 9 of the nestling phase. Nestlings were banded on day 14. Blood samples were routinely taken from nestlings born on the study area and from immigrant adults. More detailed methods can be found in Dickinson et al. (1996).

Relational data tables were managed in Microsoft Access. We used 2 separate data sets for the analyses in this article, one for incest based on pairing in territorial winter groups (2001–2005; year represents fall leading into winter) and the other for incest based on first breeding attempt and settlement location in spring of 1986–2001. During all but one of the years when groups were monitored in winter, we had both social genealogies and genealogies based on use of microsatellite data to assign genetic parentage (Ferree et al. 2010). Relatedness was calculated based on the social genealogies for the simulations. For the winter data, both social genealogies and genealogies based on parentage assignment were used to determine whether incest had occurred.

Data set for simulation of incestuous pair formation in winter

A large share of philopatric males will become first-time breeders with a female from their winter group, where they are likely to encounter both immigrant females and female relatives (Kraaijeveld and Dickinson 2001). The data set on potential for incest during pair formation in winter groups comes from fall 2001 to spring 2006 when funding allowed year-round field work to census winter groups and test hypotheses about winter sociality (Dickinson et al. 2014). These data were collected after we lost access to a privately owned portion of the main study area at the end of the 2001 breeding season. The data thus comprise a smaller extent and a higher ratio of edge to area than did the long term (1986–2001) breeding data. Although the extent of the study area and the relative amount of edge will have significant influence on opportunities for incest population-wide, they should not affect analysis of pairing in winter groups, each of which is a discrete unit.

Winter group composition was determined based on 3–4 censuses per month, 1–2 h in length (for roughly 1 h of actual observation per census), from October to March (2001–2006). New birds entering winter groups were banded for individual recognition as they were captured on the roost, using baited Potter's traps or by using mist nets placed near decoys, water sources, or mealworm feeders. The frequency of incestuous pairing was evaluated based on social relationships and also based on genealogies inferred using microsatellite typing to detect potential cryptic incests.

Data set for simulation of incest after settlement

We used the larger, long-term data set from the entire 700 ha study area on Hastings Reserve and Oak Ridge Ranch to examine the spatial potential for incest based on 279 yearling males' locations *after* they dispersed to breed. This allowed us to include in the simulation both the yearling males that paired in winter groups and the yearling males that paired during settlement, after winter. Such males could pair with females that wintered elsewhere on the study area, females that had previously bred, but were widowed, and post-winter immigrants. This analysis tested for incest avoidance with any kin related by at least 0.25, including unfamiliar kin, using data gathered during the breeding season from 1986 to 2001; this was the period when we monitored the largest extent of the study area (700 ha of Hastings Reserve and Oak Ridge Ranch) and thus had the best chance of detecting pairings of offspring with both familiar and unfamiliar relatives based on their social pedigrees. Observed incest rates were based only on the social pedigree due to a lack of molecular pedigree for these years.

Calculation of incest rates based on social genealogies and microsatellite typing

Incest rates based on social genealogies were used for both types of simulations. Incest rates based on paternity assignment were only available for 107 individuals born in 2000–2005 and were based on genotyping at 11 microsatellite loci with a mean of 12.82 alleles per locus and no null alleles. Detailed methods on microsatellite analysis are found in Ferree et al. (2010). Microsatellite-based genealogies were used to assess rates of cryptic incest for the winter data. Individual heterozygosity estimates based on 6–11 loci from a larger sample of 1454 offspring (mean 10.88 loci, standard deviation [SD] 0.49 loci) were 0.70 ± 0.01 (standard error of the mean).

Simulation based on winter group composition

Using social genealogies, we tested the idea that western bluebirds avoid incest when forming pairs in winter groups; we used a simulation that paired first-winter males at random with first-winter females in their winter group to generate a distribution of the frequency of incests that would occur if there was random within-group mating. We considered as incestuous any potential pairings between individuals that were related by at least 0.25. This cutoff matches the level at which prior experience would allow first-winter birds to recognize relatives, and excludes first-cousin incest, which is controversial and varies in terms of whether it is considered incest across human cultures and geographies (Bittles 2009). The simulation was based on social relationships because multiple lines of evidence indicate that western bluebirds do not recognize or alter their behaviors in response to individuals that are cryptic extrapair kin.

The simulation was based on pairing yearling males ($N = 247$) and females ($N = 173$) from 107 winter groups monitored over 5 winters from 2001 to 2005 (with year 2001 representing winter 2001–2002 and so forth ending spring 2006). The rationale for this simulation is earlier work showing that most yearling males in winter groups (57–59%) settled with yearling females from their winter groups (Kraaijeveld and Dickinson 2001). We included in the simulation only males and females that fledged the prior spring and thus would each be mating for the first time. We asked whether siblings living in the same winter group avoided pairing with each other.

The mean group size was 7.47 ± 0.32 individuals and the total number of individuals involved in the simulation was 420 (only the first-winter birds) of a total of 799 birds. Each first-winter male or female had its own probability of breeding with a relative if it paired at random with an opposite sex yearling in its winter group. The ratio of natal males to immigrant, and thus unrelated, females in winter groups was 1.70, and the ratio of natal males to related sisters born the same year was 2.63. The simulation paired eligible males and females at random, without replacement, for each of the 107 winter groups, and repeated the random mating trial 1000 times for all groups, using relationships based on social parentage to determine whether incestuous mating occurred or not. This generated a distribution of 1000 iterations calculating the combined number of cases of incest ($r \geq 0.25$ between members of a mated pair) for all groups. The number of actual cases of incest in all groups was then compared with this distribution to determine whether first-winter birds avoided incest when forming pairs in their winter groups.

Simulating expected incest rates on dispersions after winter groups dissolve

We developed a set of simulations to test whether separation by distance was sufficient to reduce the likelihood of incest with unfamiliar kin after dispersal and over what radius spatial separation was sufficient. The simulation looked only at yearling males and used as their location the box in which they first bred. First, we found all females that bred within a specified distance of each yearling breeder male; we removed from this set any females that were breeding with the same male they bred with the previous year, assuming that such females were not available to mate. We then allowed each yearling male to mate at random with 1 eligible female within a specified radius of his nest-box (including the female he did mate with), tabulating whether that mating involved incest. We conducted 5 simulations using radii of 200, 400, 600, 800, and 1000 m around a male's first breeding location.

In these simulations, incest was defined as mating with first- or second-order relatives ($r \geq 0.25$), including mothers and sisters or half-sisters (maternal or paternal), aunts, and grandmothers. Daughters were not included as they do not exist for first-time breeders. The simulation was based on social parentage, not genetic parentage, again, because evidence indicates that it is conservative to assume that western bluebirds respond behaviorally based on social relationships and recognize only familiar kin. Even where social relationships are concerned, siblings and half-siblings can be familiar based on prior association or they can be unfamiliar due to having been born a different year.

We ran each of the 5 simulations 1000 times and calculated for each iteration the number of males mating incestuously. We then plotted the means and the lower 95% confidence intervals from the simulations against the radius of inclusion around the males' nests and compared these with the observed frequency of incest in the sample. Due to lack of data on winter pairings over this period, males that paired with females in their winter groups could not be distinguished and thus were included in the analysis along with males that paired after winter. These data were further analyzed using a generalized linear mixed model (GLMM) (binomial distribution) to examine how the probability of incest with females within 600 m of where a male first bred attenuated with natal dispersal distance. We chose 600 m because it was the largest radius for which active incest avoidance was observed.

RESULTS

Description of kin structure in winter and spring based on social parentage

The resighting rates of nestlings banded in years 1986–2000 were consistent with female-biased dispersal: 21.3% of males born on the study area were observed on the study area as yearlings, whereas only 6.0% of females were retained in the population. The natal dispersal distributions for sons (including helpers staying home with their parents) and daughters within the study area are shown in Figure 2, which indicates that females dispersed farther and were more likely to leave the study area than were males, but that within 1 km of the natal site, dispersal distances frequently overlapped for the 2 sexes.

Western bluebirds were in close proximity to opposite sex kin both in winter groups and on their spring territories. Young of the year in winter groups (for winters starting in fall 2001–2005) were often retained offspring from the prior spring; there were 2.6 times as many sons as daughters in winter groups and 1.5 times as many non-natal females as non-natal males joining groups for winter (Figure 3). When pairs settled after the second step of dispersal, they formed kin neighborhoods. Figure 4 shows that the mean frequency with which 279 first-time breeder males in our sample

(1986–2001) had different kinds of female relatives within 600 m of where they first settled to breed. The per capita number of relatives first-time breeder males had within 600 m was 0.484. They were far more likely to have their mother nearby than they were to have full-sibs, half-sibs, aunts, or grandmothers (Figure 4).

Observations of incest

Based on winter group data, after wintering on the study area, 41 of 107 natal males (38%) bred on the study area in spring. Of these, only 1 bred with a local, banded female, and this female was from 8 km away; the rest bred with immigrant females whose parents were not known for a rate of zero incests. We examined rates of incest in a larger sample of all natal males settling on the study area as yearlings in 1986–2001; we detected 4 cases of incest in 279 nesting attempts of yearling males born and breeding on the study area for an overall incest rate of 1.4% for males pairing for the first time. Three of these were cases where males paired with

their mother and one was a case in which the male paired with his full-sister born the same year, but not in the same nest (the male was from an egg laid in early April, whereas the female was from a different nest attempt with a lay date of early June). In one case where 2 sons stayed with their mother, and the father was absent, early molecular data (minisatellite DNA) indicated that their father had sired all offspring in the nest (Dickinson and Akre 1998). No molecular data were available for the remaining cases of apparent mother–son incest. Taken together, these data sets show an incest rate of between 0% and 1.4%; the rate of incest was not statistically different between winter and spring pairings (Fisher $P = 0.58$).

Incest rates were also low based on assignment of parentage for all yearling males that bred in the population in 2001–2005, the years for which both winter group composition and population-wide paternity analysis were available. Paternity data covered 6 years, but only overlapped with winter group monitoring by 5 years. In this sample, we had 120 unique first-time breeding pairs for which both the breeder male and breeder female were
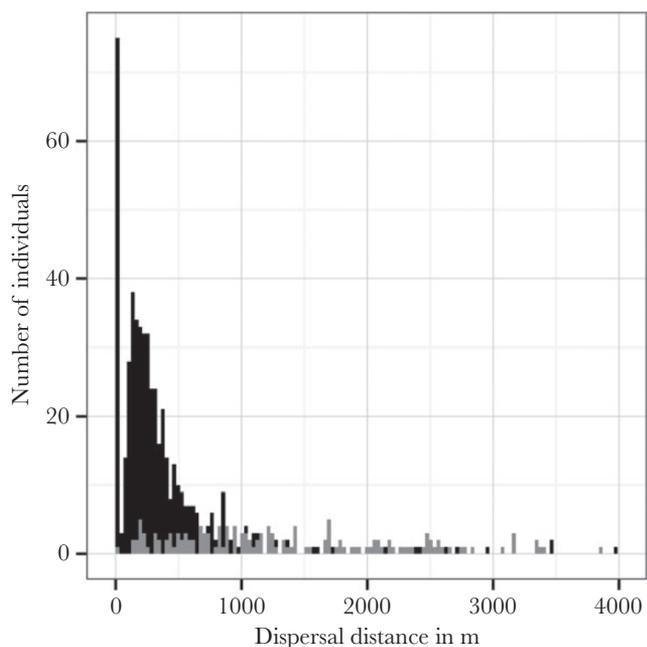

Figure 2
Distribution of dispersal distances for male (black bars) and female (gray bars) western bluebirds born on the study area (note that fledging sex ratios are even, Dickinson et al. 2016).
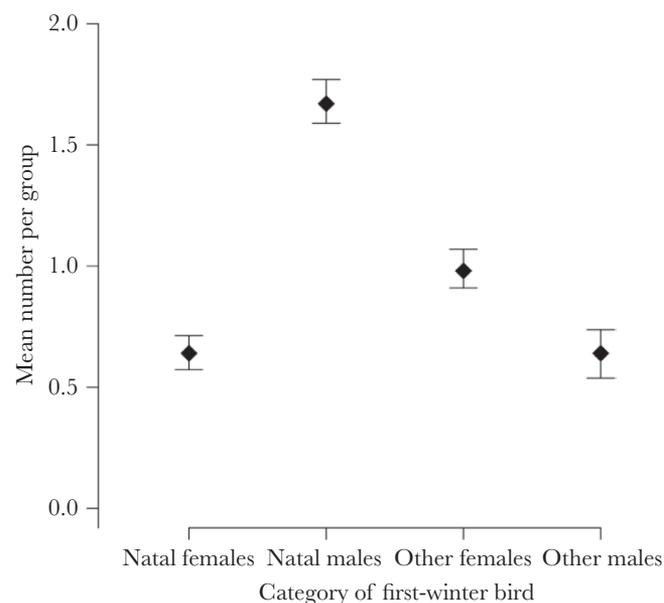

Figure 3
The mean ± SEM number of natal females, natal males, and other first-winter females and males, which are comprised of fall immigrants (most) and individuals joining winter groups from other territories within the study area.
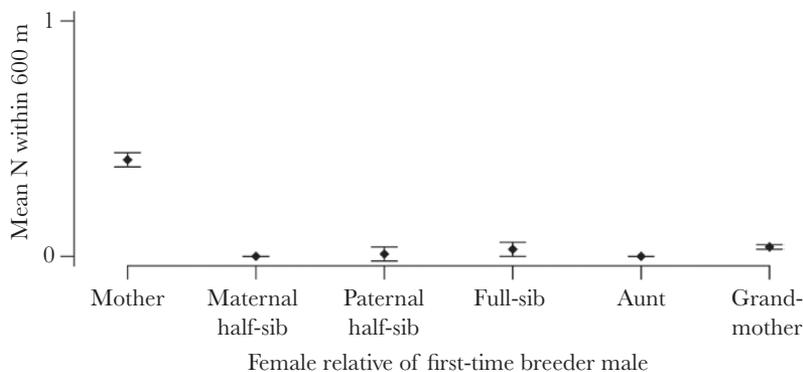

Figure 4
The mean ± SE number of observations of different kinds of female relatives nesting within 600 m of 279 first-time breeder males.

identified. In order to look for incest, however, their parents had to be identifiable, meaning they had to be born on the study area. In 45% of cases, the male's parents were known, but the female's parents were not (male mated with immigrant), whereas in only 1 case (<1%), were the female's parents known, but the male's not. In 48% of pairs both were immigrants. This left only 6 pairs where parents of both breeders were identified; only one member of these 6 pairs (a male) had an extrapair sire. We found no cases of cryptic incest, meaning incest between paternal half-sibs or a father and his extrapair daughter.

Is there incest avoidance within winter groups?

Figure 5 shows the results of a simulation of random mating of first-winter males to first-winter females within their winter groups, sampling without replacement ($n = 1000$ times across all groups). Given that some daughters remained in their natal groups for winter, the expected frequency of incest was tightly distributed around 40 cases of incest over the 5 winters when winter groups were monitored (with censuses beginning in fall of each year, including 2001–2005) (Figure 5). We observed no actual cases of incest in this sample of 107 groups, indicating that sons pairing in their winter groups were more likely to pair with immigrant females than expected by chance.

Is there incest avoidance among birds present after dispersal?

We simulated the passive effect of dispersal on the frequency of incest by asking what this frequency would be if first-time breeder males of known social parentage mated at random with females within 200–1000 m of where they themselves bred. We include in the simulation the females with which the yearling males actually bred. Because divorce is rare (Dickinson et al. 1996), we excluded from the simulation any females that were paired with the male they bred with the previous year, but we did allow yearling males to breed with older females whose prior mate was not their current mate. The expected number of incestuous matings among new pairs for this spatially constrained randomization set is presented in Figure 6a. The dotted line shows the actual observed incest rate for this data set (4 incests). As Figure 6a shows, the number of incests
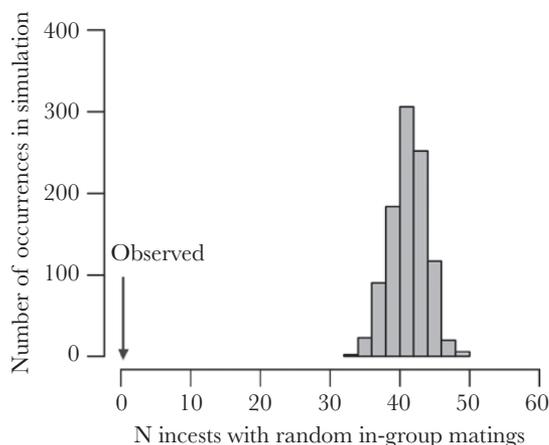

Figure 5
Histogram of expected number of incestuous pairings (full-sib and half-sib) among yearling males and females pairing at random within their winter groups in 2001–2006 for 1000 iterations. The arrow shows the observed level of incest to be zero.

observed was statistically lower than expected with random mating within 200–600 m of where a male first bred, whereas a count of 4 incests was consistent with males mating at random over a larger radius (800–1000 m). In addition, the greatest number of cases of incest observed in the simulation was 18, indicating that the upper bound on the rate of incest with random mating was 6.5% of pairings. As Figure 6b shows, the probability of encountering a female of $r \geq 0.25$ within 600 m of a male's breeding location declined with his natal dispersal distance to near zero at 2 km from his natal nest (GLMM: -0.002 ± 0.0004, $z = -3.86$, $P < 0.001$).

DISCUSSION

Western bluebirds provide an important example of incest avoidance in a highly social species because kin live in close proximity over the period of time when social pairing first occurs. Related males and females co-occur in winter groups, and in an earlier study up to 59% of males that settled locally did so with a female from their winter group (Kraaijeveld and Dickinson 2001). Over the 6 years when winter groups were monitored for this study, there were no cases of incest observed; the rate of actual incest fell far below the range expected with random mating among first-winter males and females living together in winter groups (Figure 5). When we examined all yearling males pairing on the study area after winter dispersal, incest rates were also low (1.4%), but in this case, the rate was lower than expected if males mated at random with available females breeding within 600 m of their first settlement location and within the range expected if they mated at random with available females breeding within 800–1000 m of their first breeding site.

Our results thus show that incest avoidance in western bluebirds is a 2-stage process in which 1) familiar kin are actively avoided as mates when pairing occurs in winter groups and 2) sex-biased dispersal reduces the likelihood of incest within the population at large after dispersal onto breeding territories, where unfamiliar kin may be encountered. The latter has 3 main influences in that low incest rates with unfamiliar kin can be achieved through the combined effects of removal of females from the population via sex-biased dispersal, increasing a male's encounter radius through exploration, and increasing a male's own dispersal distance (Figure 6a,b). The resulting spatial separation is likely sufficient to keep incest rates low even when kin are unfamiliar with each other, such as will always be true with paternal half-sibs arising from extrapair fertilization and will sometimes be true for full- and half-siblings that fledged in years when the male of interest was not yet born or was no longer on his natal territory. We found no evidence that cryptic paternal half-sibs (where at least one arises from extrapair paternity) mate with each other.

What is surprising is that 3 of the 4 incests that occurred from 1986 to 2001 were between familiar kin. For one of these cases, the father had died after siring the entire clutch and thus the apparent breeders were sons helping their mother. For the other two, genetic data are not available. Given that helping is driven by a shortage of adult females in the population (despite a fledging sex ratio of 1:1), there are 2 possibilities: 1) where sons appear to pair with their mothers, nestlings are sired by dead fathers or by extrapair males as occurs in some fairy-wrens (Cockburn 2016) or 2) the shortage of females creates a situation in which—at least for sons—paying the cost of incest is making the best of a bad job. Given that females control extrapair copulations in this population of western bluebirds (Dickinson 2001), the former hypothesis—that sons that
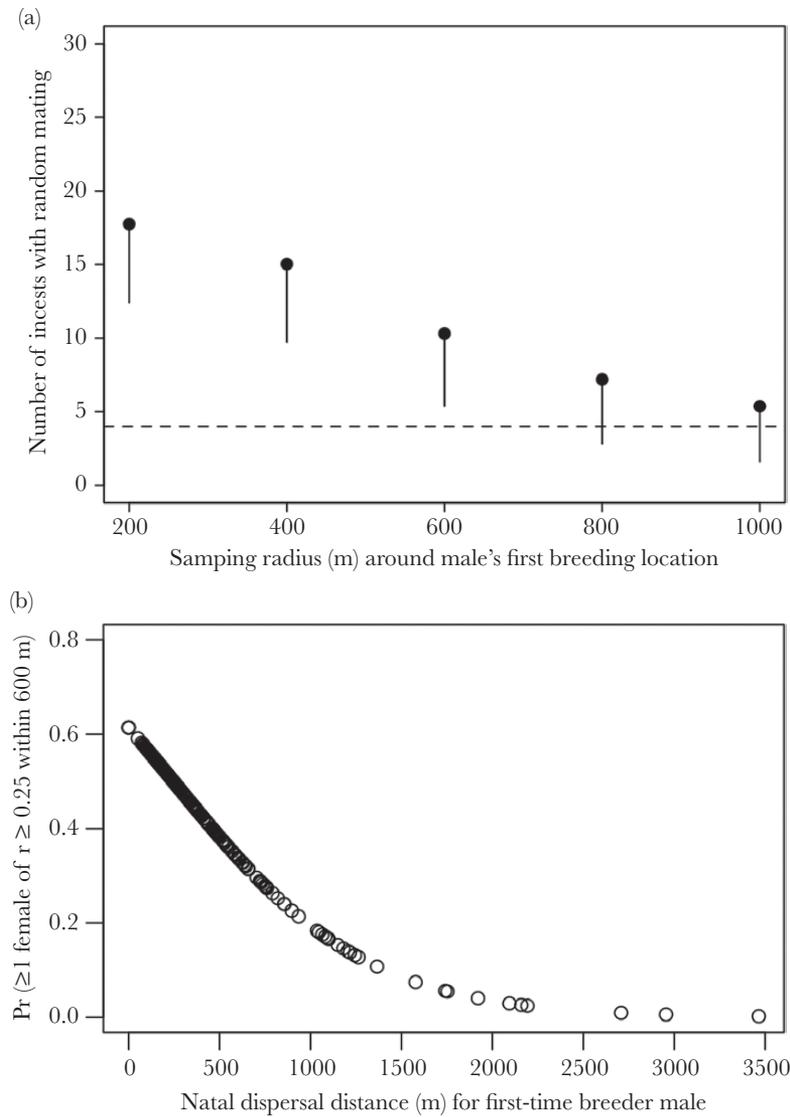
Figure 6

(a) Mean and lower 95% confidence interval for the number of incests ($r \geq 0.25$ between members of a mated pair) for yearling males mating at random with females that settled within 200–1000 m of where the male first settled to breed. Females were considered to have been available to mate at the start of the season if they were not paired to a male they had nested with the prior year. The dashed line represents the number of observed incests over all years considered. (b) Fitted probability that a yearling male had at least 1 female of $r \geq 0.25$ within 600 m of the first bred as a function of the male's natal dispersal distance (based on binomial response variable).

appear to be committing incest are not actually siring offspring—seems more likely.

It is important to note that this study examined incest avoidance at the level of pairs whose coefficient of relationship was 0.25 or greater; this does not mean that inbreeding does not occur in the population, rather it means that a combination of incest avoidance based on familiarity and spatial separation via female-biased dispersal is, together, sufficient to reduce incest with close family members (parents, sibs, and half-sibs) to very low levels. Examination of inbreeding (including matings between individuals whose relatedness is less than 0.25) requires multigenerational parentage analysis using deep, molecular pedigrees extending back to great-great-grandparents (Szulkin et al. 2013; Reid et al. 2015). With such data, it would be possible to use similar simulations to those we have used here to determine whether there is evidence of inbreeding at different spatial scales. Although the strong tendency for locally

dispersing sons to mate with immigrants in this study suggests that inbreeding, measured in this way, could potentially be quite low, a deep pedigree analysis would provide definitive measures of the genetic consequences (inbreeding) of the 2 mechanisms of incest avoidance we have identified in this study.

Although western bluebirds appear to avoid mating with kin in their natal groups, an earlier study revealed that some locally born first-winter females on the study area in winter (about one-quarter of all first-winter females, including immigrants) had moved to new groups (Kraaijeveld and Dickinson 2001). All but 15% of local females departed from the study area in spring and those that remained bred more than 1800 m from home. In superb fairy-wrens (*Malurus cyaneus*), females will use a non-natal territory as a staging post that allows them to ascend to breeding status or bud off a portion of the territory to breed with a helper male (Cockburn 2003). Whether they stay home or move to a new group for winter,

western bluebird females disperse to breed 10.9 times as far from their winter group as do immigrant females (Kraaijeveld and Dickinson 2001), suggesting that immigrant females that overwinter in a group have a better chance of gaining a local reproductive vacancy than do females born in a group. This bias favoring immigrant females may play a role in lowering rates of cryptic incest and, more generally, inbreeding.

Theoretical treatments of incest avoidance have led to conflicting conclusions on whether sex-biased dispersal is sufficient to explain low levels of incest, especially with overlapping generations (Blyton et al. 2015; Parreira and Chikhi 2015). In western bluebirds, avoiding incest during pair formation in winter groups requires an active behavioral avoidance mechanism, whereas avoiding incest in the population at large and with unfamiliar kin is sensitive to dispersal distance: A male has a near zero probability of committing incest when mating at random with any eligible female within an intermediate distance (600 m) of where he first breeds, so long as he disperses 2 km from his natal site. Taking into account the possibility of temporal fluctuations in spatial proximity of relatives and mechanisms of incest avoidance across life stages can increase understanding of how animals avoid the costs of close inbreeding (Riehl and Stern 2015).

We found just one case of full-sibling incest and none of half-sibling incest, indicating that sibling incest rates are quite low in the population. How do western bluebird pairs avoid mating with unfamiliar kin? It is possible that philopatric individuals use a simple rule that involves pairing only with immigrants. No male that settled locally paired with a sister from his winter group and virtually all yearling males that were followed through the winter paired with immigrants, whether they paired with a female from their winter group or not. Our previous research on winter sociality showed that although experienced males did not show evidence of nepotism (in the form of reduced aggression toward sons and daughters), experienced females showed reduced aggression toward sons and daughters compared with immigrant first-winter birds that joined their groups (Dickinson et al. 2009). Females are especially aggressive to first-winter females that are not their daughters. Whether or not this nepotism relies on offspring recognition by the female parents is unknown, but it may nevertheless help young birds learn to discriminate between kin and nonkin. Young males and females in natal groups could potentially use nepotism (or aggression) to distinguish between immigrants and relatives, including returning older siblings. Recognition of familiar kin is not required to avoid incest within winter groups in western bluebirds, rather recognition of a class of birds that behave or are treated as immigrants may be sufficient. Immigrants may be tagged as "not co-nestlings" based on the ways they behave on arrival on the study area.

It is also possible the young birds learn their nest mates' identities. Thus far, we have found evidence for kin discrimination in adult males based on levels of aggression against territorial intruders (Akçay et al. 2013). Male breeders are less aggressive to playbacks from equidistant kin than nonkin in living their neighborhoods, but this recognition does not appear to involve a distinctive kin signature (Akçay et al. 2014). Rather, males appear to use individual variation in the combination of notes produced to recognize particular males as kin, a feat that can only be achieved by learning who each individual is based on prior experience. Because our earlier vocal recognition studies involved only males, the mechanism of recognition of opposite sex kin is still an open question. Potential mechanisms may involve individual recognition based on vocalizations, perhaps augmented by other cues such as odor

(Krause et al. 2012) or variable plumage coloration (Budden and Dickinson 2009).

Although learning kin based on a set of cues can provide an opportunity for offspring to avoid incest through recognition of nestmates and parents, loose ties among kin may also help facilitate learning to discriminate kin. Contextual cues can provide opportunities for young of the year to learn the identities of older siblings via a relatively simple rule that involves direct experience (males that rejoin the group for winter and bring their families are relatives). In carrion crows, *Corvus corone corone*, young birds are hypothesized to learn to distinguish unfamiliar kin based on sporadic visits to their natal nest by older relatives, even though they have not "lived together" (Baglione et al. 2003). Such visits also occur in the context of cross-territorial helping in western bluebirds (Dickinson et al. 1996; Stern 2012). Humans use contextual information to discriminate kin within step-families: They identify kin by direct experience during the perinatal period, but in the absence of this information, they discriminate kin based on the length of their coresidence as "siblings" in the family group (Lieberman et al. 2007). Contextual information does appear to be used in helping in western bluebirds, where sons occasionally accompany their fathers when coming to the aid of grandparents, yet never do so on their own (Dickinson JL, unpublished data).

Although the 2-step process of incest avoidance appears sufficient to explain the low levels of incest in western bluebirds, further research on mechanisms of kin discrimination among males and females is required to determine how they avoid mating with kin in their natal groups and whether social interaction and other contextual mechanisms allow them to discriminate kin across different phases of their lives.

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