

Host-Parasite Relatedness in Wood Ducks: Patterns of Kinship and Parasite Success

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ABSTRACT: We investigated the role of kinship in intraspecific nest parasitism of wood ducks (*Aix sponsa*). Among waterfowl, female philopatry creates the potential for female relatives to nest in proximity. Costs of intraspecific nest parasitism to host females may be reduced if parasites lay eggs with kin. However, previous observations of marked wood ducks indicated that females avoided parasitizing clutch mates or the female that incubated them. To further examine the role of kinship, we determined the genotypes of 27 host-parasite pairs at five microsatellite loci. Average relatedness between hosts and all females laying parasitic eggs was only 0.04 ± 0.03 . Parasites appeared to choose hosts randomly with respect to kinship from among females with nests in the neighborhood and those within the entire study area. However, host relatedness to the parasite with the greatest number of young leaving the nest was 0.11 ± 0.03 , which was greater than expected if eggs were accepted randomly from neighboring females or from females present on the entire study area ($p = .03$ and $p = .02$, respectively). These patterns may reflect parasitism of randomly selected nests followed by differential acceptance by hosts, differential hatching success of related parasites (e.g., due to greater laying synchrony), or a mixture of parasitic strategies, one with a focus on related hosts and the other on unrelated hosts. Genetic data revealed that social relationships did not always reflect true relatedness and that success of primary parasites was associated with kinship to hosts.

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Alternative reproductive behaviors are common in male vertebrates and invertebrates (Alcock, 2005; Dominey, 1984; Gross, 1996). Some examples of alternative behaviors exist in females as well but are generally less well studied. Competition among females for scarce reproductive resources appears to be the basis for most cases. A classic example is the golden digger wasp (*Spex ichneumoneus*) in which some females dig and provision a nest burrow, whereas other females usurp burrows built by conspecifics (Brockmann et al., 1979). In this example, the two tactics have equal fitness and are part of a mixed evolutionarily stable strategy. However, in most cases, the behavioral options are part of a conditional strategy in which individuals behave in one way under one set of ecological or social circumstances and another way under different conditions.

In some birds, females occasionally lay eggs in the nests of conspecifics but neither incubate them nor care for the young. Waterfowl (Anseriformes) have an unusually high incidence of this intraspecific nest parasitism (Eadie et al., 1998). Also unusual among birds, female waterfowl are more philopatric than males (Anderson et al., 1992; Greenwood, 1980). Female philopatry has the potential to elevate relatedness among females in local populations, and kin selection could potentially help explain the prevalence of intraspecific nest parasitism among waterfowl (Andersson, 1984; Hamilton, 1964a,b). If so, then intraspecific nest parasitism may be more appropriately described as providing parental care to related young (i.e., a form of cooperative breeding, Andersson, 1984, 2001; Zink, 2000), and relatedness between hosts and parasites could alter the expression of parasitism.

Behavioral observations of wood ducks (*Aix sponsa*) suggested that females (especially adults) avoid parasitizing close kin (Semel and Sherman, 2001). In contrast, among goldeneyes (*Bucephala clangula*), behavioral observations combined with protein fingerprinting revealed that parasites that laid the most eggs in host nests were more closely related on average to the host female than expected by chance (Andersson and Ahlund, 2000). Combining behavioral observations with DNA or protein analyses can provide a more comprehensive understanding of the role of kinship in social behavior. Indeed, this approach has revealed that social monogamy is not always synonymous with genetic monogamy due to extrapair mating and fertilization (reviewed in Griffith et al., 2002; Hasselquist and Sherman, 2001; Westneat and Sherman, 1997; Westneat and Stewart, 2003). Applying genetic tools to behavioral studies of nest parasitism in wood ducks may reveal the extent to which parasitic behaviors go undetected in observational studies or how pedigree relationships among females are obscured by mixed maternity among clutches.

In this study, we combined previous behavioral observations with molecular genetic techniques to investigate relatedness between hosts and parasites in wood ducks. Our primary objective was to determine whether nesting females and their nest parasites were more likely to be kin than expected by chance. We explicitly examined the possibility that kin may interact simply because female philopatry places relatives in proximity, rather than because such interactions provide inclusive fitness benefits. If parasitism and incubation occur in the natal neighborhood, then nest mates and their social mothers may be more likely to interact. If parasitism

occurs in natal neighborhoods, but nest sites for incubation are secured outside the natal neighborhood, then individuals may be more likely to parasitize relatives but would rarely interact with relatives in the vicinity of their nests. Alternatively, if both parasitism and incubation occur outside the natal neighborhood, relatives probably interact rarely.

METHODS

Field methods

Semel and Sherman (2001) studied wood ducks nesting in artificial nest-boxes at Moraine Hills State Park in McHenry County, Illinois from 1989 to 1995. We used blood samples they collected in 1991–1994 for this study. Briefly, nest-boxes were placed to mimic the positions and locations of natural cavities (i.e., on trees, .6 m high, at densities of < 0.09 boxes/ ha). Adults were captured using decoy traps and by blocking nest-box entrances when the box was occupied. We placed uniquely numbered colored nasal saddles on adults to allow individual identification during behavioral observations. Before releasing the bird, we collected a blood sample and stored it in Queen's lysis buffer (Seutin et al., 1991) for later genetic analyses.

The contents of each nest-box in the study area were examined every 3–4 days in 1991–1992 and every day in 1993–1994 throughout the nesting season. When more than one new egg was added to a nest per day, Semel and Sherman (2001) began focal observations of the box the following morning. This method increased the likelihood of observing a parasitic event because all nests could not be observed simultaneously and parasites commonly returned to the same host again in subsequent days. The identity of all females that entered the box (and accompanying males when possible) was recorded, and the presence of any new eggs was confirmed. When the clutch hatched, ducklings were web-tagged and blood samples were collected from each and stored in lysis buffer.

Laboratory methods

We extracted DNA from blood samples using a standard phenol: chloroform extraction protocol, followed by ethanol precipitation (Maniatis et al., 1982). We screened .30 microsatellite loci developed in other species. The polymerase chain reaction (PCR) was performed in a Biometra Trio Thermoblock 48 using conditions slightly modified from those reported by Fields and Scribner (1997), Buchholz et al. (1998), and Stai and Hughes (2003), to optimize amplification. PCR products were separated on 7.5% polyacrylamide gels, stained with SYBR green, and visualized on a Kodak Digital Science ID 3.0.2 Imaging System. Individuals with homozygous genotypes were amplified twice to minimize errors due to allelic dropout. Two to four individuals per locus were repeated on every gel for use as size standards to facilitate comparisons between gels. Eventually, we settled on five microsatellite loci that showed sufficient polymorphism for estimating relatedness: Sfil4, Sfil5 (Fields

and Scribner, 1997), Bcal11 (Buchholz et al., 1998), cmaat28, and cmaat35 (Stai and Hughes, 2003). These loci produced 4, 8, 5, 20, and 18 alleles, respectively.

Data analysis

Genetic analysis

Population allele frequencies were estimated from all genotyped females ($n = 53$). We tested deviations from Hardy Weinberg expectations and linkage equilibria with randomization and permutation tests in FSTAT (version 2.9.3; Goudet, 2001). The relatedness of the host and parasite, r , was calculated with the program Kinship (version 1.2; Goodnight and Queller, 1999), according to equations described in Queller and Goodnight (1989). Besides estimating relatedness, Kinship also determines the likelihood of the observed combination of genotypes based on a specified relationship (i.e., parent-offspring or full sibling pairs) and identifies significant relationships through the use of a simulation routine that determines the likelihood ratio values corresponding to different levels of significance. A series of pairs is generated that matches the null hypothesis (i.e., that pairs are unrelated), and another series of pairs is generated that matches the primary hypothesis (i.e., that pairs are related) using allele frequencies in the population. The ratio of these values is then compared to the observed ratio.

We also examined the maternity of 211 ducklings from 17 clutches. We compared behavioral and genetic estimates of the number of parasites per nest and the number of parasitic eggs per nest using Wilcoxon signed-ranks tests ($\alpha = 0.05$ throughout). Because related individuals were likely to be similar across multiple loci, we used a mismatch at one locus as our criterion for maternal exclusion. This maximized identification of parasite offspring and ensured that parasite success in the nests of related hosts would not be underestimated. To minimize false exclusions, we followed host exclusion with a comparison between ducklings and parasites observed at the nest. The probability that an unrelated parasite would be excluded as the mother was .99. Likewise, the probability that a related parasite would be excluded when the host was the actual mother was .80. Therefore, the probability of correctly estimating the success of parasites in the nests of related hosts was high.

A “primary parasite” was designated for each clutch. This was the female with the greatest number of ducklings leaving the nest or the only parasite detected. We analyzed data from both the host and parasite perspectives. From the host perspective, we determined the average relatedness of hosts to primary parasites, even if hosts were parasitized by multiple parasites. From the parasite perspective, relatedness values of parasites to hosts were averaged across hosts when females parasitized multiple nests. This avoided pseudoreplication when hosts were parasitized by multiple females or when females parasitized multiple nests because each host or parasite was included only once in the analysis. Standard errors were calculated by jackknifing over host-parasite pairs, as recommended by Queller and Goodnight (1989) and according to methods described in Manly (1997). For each nest, the average relatedness of the host and parasite was determined using allele

frequencies in the population of females. We also tested whether neighbors were a random sample of the population of females or close kin in two ways: (1) by comparing estimates of r based on allele frequencies of neighborhoods to estimates based on allele frequencies in the population (Queller, 1994; West et al., 2001) with Wilcoxon signed-rank tests and (2) by comparing the relatedness of 25 pairs selected randomly from neighborhoods to 25 pairs drawn from the population (using t tests).

Determining neighborhoods of potentially interacting females

We estimated the nesting area used by each female within a season by calculating the distance between boxes in which she was observed laying. Distances were estimated using ArcView (version 3.2; Environmental Systems Research Institute, Redlands, California, USA). The frequency distribution of the average distances between boxes for each female was plotted, and the longest distance was identified. This distance is thus an estimate of how far females might travel to lay eggs. We used this distance as a radius to describe a circular area centered on a focal nest-box. All sampled females that laid eggs in this "neighborhood" were considered potential parasites of the focal box, and all females that nested in the neighborhood were considered potential hosts to parasites of the focal box. We reasoned that females laying eggs in boxes outside the neighborhood were unlikely to lay eggs in the focal box, based on the distance between these boxes and the focal box. The greatest distance between any two boxes on the study area was 7445 m.

To determine if females used nest sites within or outside their natal neighborhoods, we compared the distances between natal sites and sites of parasitism in subsequent years to the distances between natal sites and sites of incubation in subsequent years using the Mann-Whitney test. We also examined the potential for future overlap in nest site use by females and their social mothers by comparing the distance between nests of the same females in different years to the distance between a female's natal site and the site where she incubated a clutch.

Randomization tests

To determine whether the average r between host-parasite pairs differed from those expected under random host selection, we generated a null distribution by randomizing r values from possible combinations of parasites and hosts 5000 times. Candidate parasites were randomly chosen from the set of females either in the local neighborhood or from the entire population. Candidate hosts were randomly selected for each parasite from females with nests either in the neighborhood or from the entire population. The average relatedness between observed hosts and parasites was then compared to the two distributions generated under random expectation. If the average host-parasite relatedness was in the upper 5% of the distribution, then the null hypothesis of random choice was rejected. This method incorporated the possibility that females parasitize relatives because of spatial overlap by restricting potential hosts and parasites to females within a neighborhood. If relatives were clustered spatially, the distribution generated under

random expectation would reflect the high proportion of relatives locally. In effect, the null distribution would shift to reflect the proportion of relatives among neighbors and the critical value of relatedness necessary to reject the null hypothesis would be automatically corrected.

Seasonal patterns in host-parasite relatedness

To examine temporal variation in host-parasite relatedness, we used linear regression to examine the relationship between relatedness and the date the female began incubating the nest. We tested normality using the Shapiro-Wilk test. We tested the assumption of homogeneity of the variance with the Levene test.

RESULTS

The five microsatellite markers selected for genotyping appeared suitable for analyzing maternity and relatedness. We found no evidence of linkage disequilibria among them, and four loci, *cmaat28*, *cmaat35*, *Bcal11*, *Sfil5*, showed no evidence of departure from Hardy-Weinberg expectations ($p > .01$). The remaining locus, *Sfil4*, displayed a heterozygote excess ($p = .01$). No evidence of null alleles (i.e., heterozygote deficiencies) was detected.

Neighborhood analyses

Nineteen females laid eggs in .1 nest-box in a single season during the study. These females laid eggs in an average of $2.3^6 \pm 0.2$ boxes (range 2–5) that were located < 3750 m from each other (Figure 1). This distance was used as the radius defining a circular neighborhood around each box. Females that laid eggs < 3750 m from the focal box were considered

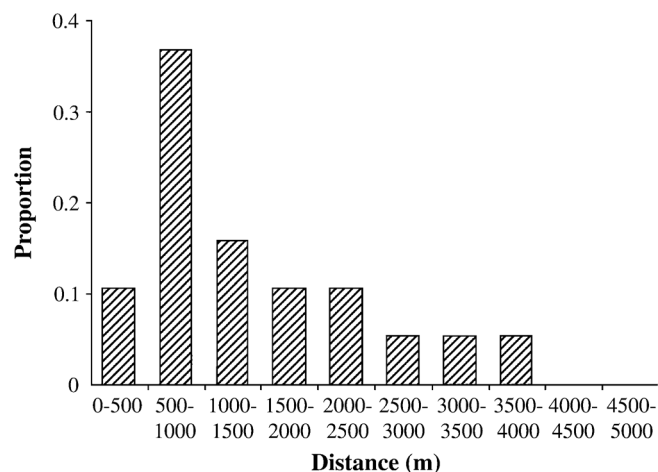


Figure 1

Frequency distribution of the average distances between nest-boxes in which individual female wood ducks laid within a year.

potential hosts and parasites for the focal female. On average, a neighborhood included 14.1 ± 1.3 potential parasites and 14.6 ± 2.3 potential hosts.

Twenty-five web-tagged females returned to the study area and were observed laying eggs in 33 boxes. These females parasitized and incubated nests at similar distances from their natal boxes ($U = 114.5$, $p = .46$, Figure 2). The mean distance between the natal box and the box where females eventually incubated nests (2247 ± 268 m) was much greater than the mean distance between the nests incubated by the same female in different years (790 ± 148 m, $n = 55$, $U = 100.5$, $p < .001$, Figure 2).

Host-parasite relatedness and parasite success

The estimate of relatedness between hosts and parasites based on population allele frequencies was strongly correlated with the estimate using allele frequencies of neighboring females ($n = 25$, $R^2 = .94$, $p < .001$). Relatedness of individual host-parasite pairs evaluated within the context of the neighborhood was similar to the same pairs evaluated within the context

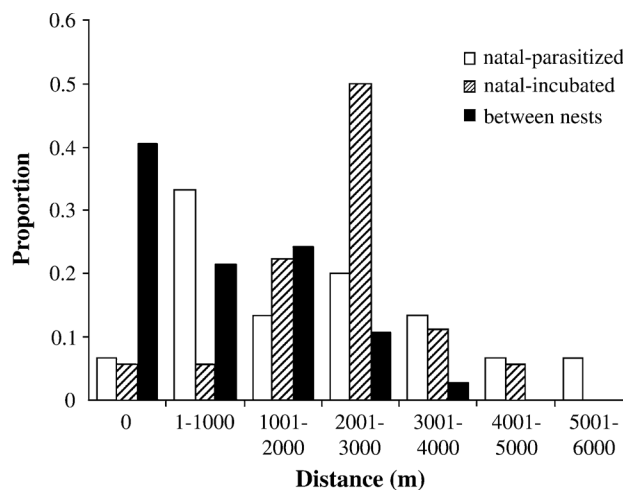


Figure 2

Frequency distribution of average distances between (1) natal boxes and parasitized boxes, (2) natal boxes and incubated boxes, and (3) between nests used by individual female wood ducks in different years.

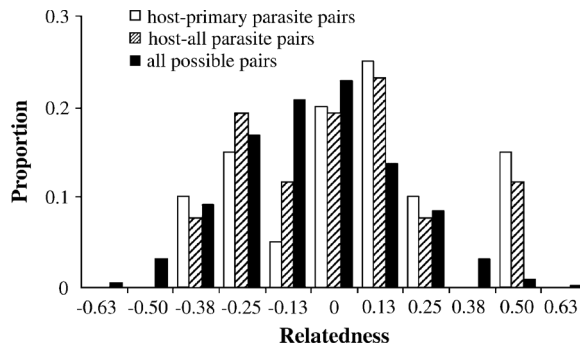


Figure 3
Distributions of relatedness between host and primary parasite, host and all parasites, and for all pairs of individuals in the sample (53 females).

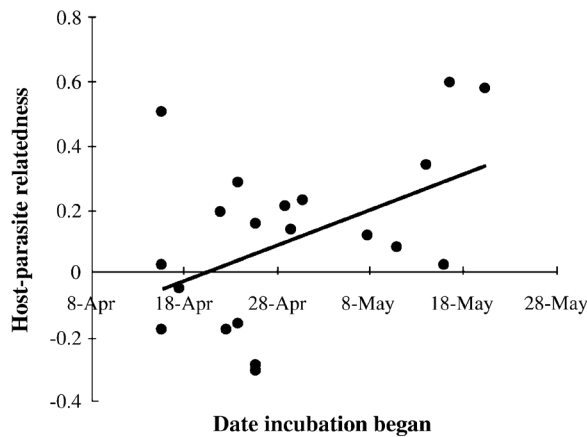


Figure 4
Relationship between host-primary parasite relatedness and timing of the start of incubation.

of the entire population ($n = 25$, $Z = 1.41$, $p = .16$). This suggests that females were not more likely to encounter relatives in their neighborhoods than in the population as a whole. Furthermore, similar estimates of relatedness from pairs drawn randomly from the neighborhood (0.01 ± 0.04) and from females in the population (0.02 ± 0.04 , $t = 0.20$, $df = 48$, $p = .85$), indicated that spatial clustering of relatives was minimal. Therefore, we used allele frequencies in the population of females to calculate subsequent relatedness values.

Focal nest observations allowed us to identify 27 host parasite pairs from marked females. Four of these 27 host-parasite pairs were identified as first-order relatives in Kinship (15%, Figure 3). The average relatedness of all parasites to their hosts was 0.04 ± 0.03 , whereas the average relatedness of a host female to her primary parasite was 0.11 ± 0.03 . From the perspective of parasites, hosts were not more related than expected by chance within the pool of females nesting in the neighborhood ($n = 25$, $p = .30$) or on the study area ($p = .12$). However, from the perspective of hosts, primary parasites were more related than would be expected by chance. This held true both for females in the neighborhood ($n = 20$, $p = .03$) and

for those on the entire study area ($p = .02$). In all four cases of a female parasitizing a first-order relative, she was the primary parasite.

We identified 58 of the 211 genotyped ducklings (27%) as hatching from eggs laid by a female other than the one incubating the nest. Twelve ducklings matched both the host and the primary parasite, and therefore, could not be assigned unambiguously to either. Ten of these 12 were among 53 ducklings from nests in which the primary parasite and host were related. If the 12 ducklings are assigned to the host, primary parasite production can be estimated as 2.0 ± 0.4 (range 0–6) ducklings per nest. If, however, they are assigned to the primary parasite, the estimate of primary parasite production per nest is 2.8 ± 0.6 (range 0–7) ducklings. Under either assumption, primary parasite production was greater than that of the second ranking sampled parasite in the same nest (0.3 ± 0.2 ducklings, $n = 7$ nests in which both parasites were sampled). For cases in which multiple parasites were known and sampled, of the parasite offspring, $85.8 \pm 5.0\%$ of the ducklings sampled were from the primary parasite.

More ducklings left parasitized nests (11.8 ± 1.1) than unparasitized nests (9.1 ± 0.6 ducklings, $n = 113$, $U = 1026$, $p = .001$), despite lower hatching success in parasitized nests (0.62 ± 0.05 versus 0.75 ± 0.05 , respectively, $n = 113$, $U = 1143$, $p = .005$, also see Semel and Sherman, 2001), because parasitized nests contained more eggs. Few nests were abandoned ($n = 5$ of 22 genotyped hosts); however, a similar proportion of nests was abandoned when the primary parasite was a first-order relative of the host (one of five, 20%) as when parasites were not related (four of 17, 23.5%).

More parasitic females ($n = 17$ pairs, $Z = 2.10$, $p = .04$) and parasitic eggs ($n = 17$, $Z = 2.81$, $p = .01$) were detected per nest from genetic analysis than from behavioral observations. Simulations in the program Kinship revealed that among the 25 females banded as ducklings and returning to the study area, 37% of social mother-daughter pairs and 67% of nest mates actually were not related.

Seasonal patterns of host-parasite relatedness

Assumptions for linear regression were met (Shapiro-Wilk statistic = 0.96, $df = 20$, $p = .49$ and Levene statistic = 1.6, $df = 14,5$, $p = .33$). Host-primary parasite relatedness increased during the nesting season ($R^2 = .22$, $df = 19$, $p = .04$, Figure 4). Early in the season, host-parasite relatedness was similar to background levels. At the end of the season, host-parasite relatedness was similar to that expected for first-order relatives.

DISCUSSION

Our genetic analysis revealed that hosts were more closely related than expected to the parasite with the most ducklings leaving their nest and that this pattern could not be explained by strong natal philopatry. However, relatedness of all parasites matched that expected if parasites chose hosts randomly with respect to

relatedness, neither preferring nor avoiding relatives. This finding differs from conclusions in Semel and Sherman (2001) that females avoided parasitizing relatives, based on behavioral observations of social mother-daughter pairs and nest mates returning to this same population. This difference in results requires careful consideration.

The apparent discrepancy could have arisen for technical reasons. For example, related parasites might parasitize at a different time of day when observations would have missed them. Or, early parasitism by relatives may have been missed because observations of nests began after the first parasitic egg was laid. Another possibility is that capturing females at nests may have influenced the pattern of parasitism. Alternatively, biases might result from limitations of the genetic analysis; parasitic events by relatives were harder to detect due to genetic similarities among kin. However, this bias falsely reduces the number of ducklings resulting from parasitic relatives, and thus our finding is conservative and cannot be explained this way. Moreover, both data sets arise from field observations of parasites; genetics were used only to assess the level of parasitism and relatedness of observed host-parasite pairs.

We revisited analyses in Semel and Sherman (2001) to determine whether analytical differences might account for discrepancies. They examined whether the pattern of egg laying differed from random expectation within smaller, more localized areas (the five nearest boxes) to the parasitized box. In this analysis, they considered each egg an independent event. We did a similar analysis with genetic data, but used each female as the unit of comparison, rather than each egg. Parasites selected hosts randomly from among females nesting in these local areas ($p = .24$), consistent with findings reported here.

The genetic analysis may be detecting something the behavioral observations did not. Some of the parasites identified by Semel and Sherman (2001) may have come from parasitic eggs and so social relationships may have been misleading. However, this type of mistake might explain no effect of relatedness on parasitism—but not a positive effect.

Most primary parasites were not closely related to hosts (Figure 3). Higher average genetic relatedness of primary parasites arises from a small number of cases involving close relatives. How these rare events occur is not clear. Yearlings hatched from parasitic eggs could return to their natal box and lay parasitic eggs if their social mother has not returned. If their genetic mother or sister was the replacement at the natal box, they might then parasitize a relative. Or, if a parasite's natal box is occupied by her social mother, she could move on to nearby nest sites and parasitize her genetic mother or sister there. This occurred at least once; of 14 yearlings that returned to find their social mother in the natal box, five parasitized a different nest-box and one parasitized a genetic relative in this new box. However, returning yearlings generally laid eggs far from their natal box (.2 km, Figure 2). Few females nested in or parasitized their natal boxes even if their social mother was not there (four of 17, Semel and Sherman, 2001). In summary, our genetic results suggest that kinship has little influence on host choice, although we cannot eliminate the provocative possibility that behavioral mechanisms of avoiding kin produce a low frequency of parasitism by kin.

Parasites who are relatives may have higher success and this could occur in several ways. First, parasites might attain higher success in the nests of relatives through more synchronous egg laying. Synchrony with the host may enable primary parasites to lay more eggs before incubation begins and result in more eggs incubated through term, whereas eggs in poor synchrony would still be developing when the rest of the clutch hatched and departed the nest. Better synchrony between relatives could arise because of genetic effects on the timing of breeding (e.g., Blondel et al., 1990; Sheldon et al., 2003). Indeed, synchrony of laying increases success of parasitic eggs in other ducks (Sorenson, 1993).

In our study, parasites were observed during laying and the first few days of incubation, after which, we did not disturb nests. As a consequence, all observed parasites were in synchrony with hosts. Therefore, the greater success of primary parasites over other parasites cannot be explained by synchrony alone but might be explained by the addition of more eggs during the period of synchrony. Laying chronology for multiple parasites at a nest ($n = 5$) provided limited support for this; primary parasites either laid more eggs than other parasites ($n = 2$) or laid the same number as other parasites, but the eggs laid by the other parasites did not hatch.

Related females might also lay parasitic eggs more successfully if hosts are less aggressive to related parasites. Semel and Sherman (2001) observed interactions at the box between hosts and parasites that ranged from the box shaking and feathers being torn to prolonged co-occupancy. The genetic relatedness of females in these encounters was known in eight instances; in all cases, females were not related. Therefore, we could not determine whether hosts reacted less aggressively toward relatives. Åhlund (2005) also reported that goldeneye hosts and parasites occasionally encountered each other at the nest and that the nature of encounters varied, but relatedness of the females involved was not reported.

Random host selection accompanied by reduced host aggression toward kin could produce a variety of distributions of host-parasite genetic relatedness, depending on how effective hosts are at deterring unrelated parasites. If hosts are able to exclude unrelated parasites only rarely, the distribution of host-parasite pairs should reflect random host selection by parasites and resemble the distribution of relatedness for all pairs in the population (Figure 3). However, if hosts are occasionally able to deter unrelated parasites, then related parasites should be able to lay more eggs. If so, the distribution of host relatedness to primary parasites should be skewed toward relatives, as we observed.

Another alternative is that hosts may assist related parasites in some way but may be unable to deter unrelated parasites when away from the nest. During early clutch development, wood duck hosts are at the nest for less than an hour daily (Clawson, 1975; Semel and Sherman, 2001). Andersson and Åhlund (2000) suggested such a mixture of cooperation between relatives and parasitism by unrelated individuals in goldeneyes. In that species, hosts were more related than expected to the parasite that laid the most eggs in nests, but average relatedness of hosts to all parasites was lower. Furthermore, mean host-primary parasite relatedness was 0.13, which is similar to our data for wood ducks. Goldeneye nest mates associated in contexts other than egg laying, and Andersson and Åhlund (2000) suggested that relatives recognized each other and jointly visited potential nest sites. Young wood duck

females also prospected for nest sites together (Semel and Sherman, 2001), but relatedness between these females was unknown.

Semel and Sherman (2001) interpreted the behavior of older parasites as competition for prime nest sites rather than “pure” parasitism and the behavior of younger parasites as the reverse, based on differences in parasite behavior between age classes. Yearling parasites initiated laying later in the season, laid eggs quickly, vocalized loudly at the nest, and often visited boxes in pairs. In contrast, older parasites initiated laying earlier, spent more time at the nest as laying progressed, and did not vocalize or visit nest-boxes in pairs. We examined our sample of genotyped birds of known age (13 adult and four yearling parasites). All four yearlings were primary parasites, whereas 69% of adult parasites were primary parasites. Host-parasite relatedness was 0.22 ± 0.16 for yearlings and 0.05 ± 0.07 for adults ($t = 1.1$, $df = 15$, $p = .29$, power < 0.33). Interestingly, relatedness between hosts and parasites increased as the season progressed (Figure 4), consistent with younger, more related parasites laying later in the season, and suggestive that relatedness affects the success of yearling parasites the most.

The finding that hosts and primary parasites are more closely related on average than expected, even after considering female philopatry, supports a role of relatedness in intraspecific nest parasitism of wood ducks. Without kin recognition, parasitism of relatives is favored only if hosts benefit directly (López-Sepulcre and Kokko, 2002). Recognition is crucial if parasitism is costly; females should only parasitize relatives if costs are low and success is higher in nests of related hosts (López-Sepulcre and Kokko, 2002). We did not examine costs and benefits of parasitism, but host survival did not seem to differ for parasitized and unparasitized females; 72% of parasitized and 67% of unparasitized females returned the next year. Furthermore, parasitized females that returned had received a similar number of parasitic eggs (5.2 ± 1.4) the previous year as those that did not return (3.7 ± 1.5 , $t = 0.70$, $df = 15$, $p = .50$). Studying costs and benefits of parasitism and mechanisms of kin recognition will clarify the role of relatedness in parasitism.

Proximity of kin may lead to interactions favored by kin selection, but also can lead to competition among relatives for limited resources (Murray and Gerrard, 1984; Taylor, 1992; Wilson et al., 1992), which reduces the benefits of cooperation (West et al., 2002). The potential for competition among relatives is rarely considered (Griffin and West, 2002; West et al., 2002) and can lead to overestimation of the importance of kin selection (West et al., 2001). We found no evidence of spatial clustering of relatives in this population of wood ducks. Returning females tended to nest farther from their natal boxes than their mother (Figure 2), which may have served to separate related females. Therefore, if kinship confers benefits in some cases of parasitism, these are unlikely to be opposed by costs due to competition.

In summary, kinship may influence the reproductive tactics of female wood ducks. However, this effect is subtle, influencing some females in some circumstances. Some females appear to produce more ducklings when they parasitize a relative, but the evidence for kinship affecting whether a female parasitizes and where is weak. Thus, brood parasitism, like many alternative reproductive behaviors, is influenced by a number of ecological and social factors, including kinship, making its ecology and evolution even more complex than previously supposed.

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REFERENCES

- Åhlund M, 2005. Behavioural tactics at nest visits differ between parasites and hosts in a brood-parasitic duck. *Anim Behav* 70:433–440.
- Alcock J, 2005. *Animal behavior*, 8th ed. Sunderland: Sinauer Associates.
- Anderson MG, Rhymer JM, Rohwer FC, 1992. Philopatry, dispersal, and the genetic structure of waterfowl populations. In: *Ecology and management of breeding waterfowl* (Batt BDJ, Afton AD, Anderson MG, Ankney CD, Johnson DH, Kadlec JA, Krapu GL, eds). Minneapolis: Minnesota University Press; 365–395.
- Andersson M, 1984. Brood parasitism within species. In: *Producers and scroungers* (Barnard CJ, ed). London: Croom Helm; 195–228.
- Andersson M, 2001. Relatedness and the evolution of conspecific brood parasitism. *Am Nat* 158:599–614.
- Andersson M, Åhlund M, 2000. Host-parasite relatedness shown by protein fingerprinting in a brood parasitic bird. *Proc Natl Acad Sci U S A* 97:13188–13193.
- Blondel J, Perret P, Maistre M, 1990. On the genetical basis of the laying date in an island population of blue tit. *J Evol Biol* 3:469–475.
- Brockmann HJ, Grafen A, Dawkins R, 1979. Evolutionary stable nesting strategy in a digger wasp. *J Theor Biol* 77:473–496.
- Buchholz WG, Pearce JM, Pierson BJ, Scribner KT, 1998. Dinucleotide repeat polymorphisms in waterfowl (family Anatidae): characterization of a sex-linked (Z-specific) and 14 autosomal loci (Ph.D. dissertation). *Anim Genet* 29:323–325.
- Clawson RL, 1975. *The ecology of dump nesting in wood ducks*. Columbia: University of Missouri-Columbia.
- Dominey WJ, 1984. Alternative mating tactics and evolutionary stable strategies. *Am Zool* 24:385–396.
- Eadie JMCA, Sherman PW, Semel B, 1998. Conspecific brood parasitism, population dynamics and the conservation of cavity-nesting birds. In: *Behavioral ecology and conservation biology* (Caro TM, ed). Oxford: Oxford University Press; 306–340.
- Fields RL, Scribner KT, 1997. Isolation and characterization of novel waterfowl microsatellite loci: cross-species comparisons and research applications. *Mol Ecol* 6:199–202.
- Goodnight KF, Queller DC, 1999. Computer software for performing likelihood tests of pedigree relationship using genetic markers. *Mol Ecol* 8:1231–1234.
- Goudet J, 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). <http://www.unil.ch/izea/software/fstat.html>.

- Greenwood PJ, 1980. Mating systems, philopatry and dispersal in birds and mammals. *Anim Behav* 28:1140–1162.
- Griffin AS, West SA, 2002. Kin selection: fact and fiction. *Trends Ecol Evol* 17:15–21.
- Griffith S, Owens IPF, Thuman KA, 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. *Mol Ecol* 11:2195–2212.
- Gross MR, 1996. Alternative reproductive strategies and tactics: diversity within the sexes. *Trends Ecol Evol* 11:A92–A98.
- Hamilton WD, 1964a. The genetical evolution of social behavior. I. *J Theor Biol* 7:1–16.
- Hamilton WD, 1964b. The genetical evolution of social behavior. II. *J Theor Biol* 7:17–52.
- Hasselquist D, Sherman PW, 2001. Social mating systems and extrapair fertilizations in passerine birds. *Behav Ecol* 12:457–466.
- Lo´pez-Sepulcre A, Kokko H, 2002. The role of kin recognition in the evolution of conspecific brood parasitism. *Anim Behav* 64: 215–222.
- Maniatis T, Sambrook J, Fritsch EF, 1982. *Molecular cloning—a laboratory manual*. Cold Spring Harbor: Cold Springs Harbor Press.
- Manly BFJ, 1997. *Randomization, bootstrap and Monte Carlo methods in biology*. New York: Chapman and Hall.
- Murray MG, Gerrard RJ, 1984. Conflict in the neighborhood: models where close relatives are indirect competition. *J Theor Biol* 111: 237–246.
- Queller DC, 1994. Genetic relatedness in viscous populations. *Evol Ecol* 8:70–73.
- Queller DC, Goodnight KF, 1989. Estimating relatedness using genetic markers. *Evolution* 43:258–275.
- Semel B, Sherman PW, 2001. Intraspecific nest parasitism and nest-site competition in wood ducks. *Anim Behav* 61:787–803.
- Seutin G, White BN, Boag PT, 1991. Preservation of avian blood and tissue samples for DNA analysis. *Can J Zool* 69:82–90.
- Sheldon BC, Kruuk LEB, Merila J, 2003. Natural selection and inheritance of breeding time and clutch size in the collared flycatcher. *Evolution* 57:406–420.
- Sorenson MD, 1993. Parasitic egg laying in canvasbacks: frequency, success, and individual behavior. *Auk* 110:57–69.
- Stai SM, Hughes CR, 2003. Characterization of microsatellite loci in wild and domestic Muscovy ducks (*Cairina moschata*). *Anim Genet* 34:387–389.
- Taylor PD, 1992. Altruism in viscous populations—an inclusive fitness model. *Evol Ecol* 6:352–356.
- West SA, Murray MG, Machado CA, Griffin AS, Herre EA, 2001. Testing Hamilton’s rule with competition between relatives. *Nature* 409:510–513.
- West SA, Pen I, Griffin AS, 2002. Cooperation and competition between relatives. *Science* 296:72–75.
- Westneat DF, Sherman PW, 1997. Density and extra-pair fertilizations in birds: a comparative analysis. *Behav Ecol Sociobiol* 41:205–215.
- Westneat DF, Stewart IRK, 2003. Extra-pair paternity in birds: causes, correlates, and conflict. *Annu Rev Ecol Evol Syst* 34:365–396.
- Wilson DS, Pollock GB, Dugatkin LA, 1992. Can altruism evolve in purely viscous populations. *Evol Ecol* 6:331–341.
- Zink A, 2000. The evolution of intraspecific brood parasitism in birds and insects. *Am Nat* 155:395–405.