Molecular Ecology (2009) doi: 10.1111/j.1365-294X.2009.04223.x

Relatedness and spatial proximity as determinants of host–parasite interactions in the brood parasitic Barrow's goldeneye (*Bucephala islandica*)

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Abstract

Recent studies, which have found evidence for kin-biased egg donation, have sparked interest in re-assessing the parasitic nature of conspecific brood parasitism (CBP). Since host–parasite kinship is essential for mutual benefits to arise from CBP, we explored the role of relatedness in determining the behaviour of conspecific nest parasites and their hosts in nesting female Barrow's goldeneyes (*Bucephala islandica*), a duck in which CBP is common. The results revealed that the amount of parasitism increased with host–parasite relatedness, the effect of which was independent of geographical proximity of host and parasite nests. Proximity per se was also positively associated with the amount of parasitism. Furthermore, while hosts appeared to reduce their clutch size as a response to the presence of parasitic eggs, the magnitude of host clutch reduction also tended to increase with increasing relatedness to the parasite. Hence, our results indicate that both relatedness and spatial proximity are important determinants of CBP, and that host clutch reduction may be an adaptation to nest parasitism, modulated by host–parasite relatedness. Taken together, the results provide a demonstration that relatedness influences host and parasite behaviour in Barrow's goldeneyes, resulting in kin-biased egg donation.

Keywords: Barrow's goldeneye, conspecific brood parasitism, kin-biased egg donation, natal philopatry, parasite behaviour, relatedness

Received 23 January 2009; revision received 30 March 2009; accepted 31 March 2009

Introduction

Conspecific brood parasitism (CBP) is a reproductive strategy found in a number of egg-laying animals (e.g. Field 1992; Brockmann 1993; Yom-Tov 2001; Loeb 2003). Recent empirical findings that related individuals lay eggs into each others' nests hint at the possibility of mutual benefits from kin-biased egg donation (Andersson & Åhlund 2000; Andersson & Waldeck 2007; Waldeck *et al*. 2008), through kin selection (Hamilton 1964), and recent theory has delineated the conditions under which such benefits can be achieved (Andersson 2001; Lopez-Sepulcre & Kokko 2002). These advances have put the nature of host–parasite interactions into new light (but see Andersson & Eriksson 1982), emphasizing the importance of kin selection and kin recognition as potential key elements dictating reproductive decisions among individuals (Andersson 2001; Lopez-Sepulcre & Kokko 2002).

Natal philopatry elevates local relatedness among individuals (Chesser 1991; Koprowski 1996; Ratnayeke *et al*. 2002), which may provide either the opportunity for cooperation (Queller 1994; van Baalen & Rand 1998; Griffin *et al*. 2004; Foster *et al*. 2006), or lead to competition between relatives (Mitteldorf & Wilson 2000; West *et al*. 2002; Griffin *et al*. 2004). If parasitism is selective rather than random, individuals have, at least in theory, a choice to parasitize kin or non-kin, depending on whether CBP is detrimental or not. The outcome of parasitizing hosts of close kin has been found to depend on the cost of parasitism, so that high costs of CBP, resulting in a net cost to hosts, will lead to the avoidance of related hosts, while lower costs coupled with inclusive fitness benefits may result in a net benefit to the host and thereby to the targeting of related

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hosts (Zink 2000; Andersson 2001; Lopez-Sepulcre & Kokko 2002).

The mechanisms leading to potential kin-biased egg donation are still unknown (for discussion about potential mechanisms, see Andersson 2001; Semel & Sherman 2001; Lopez-Sepulcre & Kokko 2002; Pöysä 2004; Andersson & Waldeck 2007). Natal philopatry has been viewed as a null hypothesis, because it results in elevated local relatedness which, coupled with parasite preference for nearby hosts, may lead to kin-biased egg donation. This null hypothesis has, however, been refuted by recent empirical evidence which suggests that some mechanism of kin recognition is involved in kin-biased egg donation (Andersson & Åhlund 2000; Andersson & Waldeck 2007; Waldeck *et al*. 2008). Seen from an inclusive fitness perspective, the overall effect of CBP on host fitness may not be negative and an alternative explanation based on cooperation has been proposed (Andersson & Åhlund 2000; Lopez-Sepulcre & Kokko 2002). Lopez-Sepulcre & Kokko (2002) underlined the need for some level of kin recognition, albeit imperfect, to be present if parasitizing relatives is to be beneficial.

The roles of donors in studies of the role of relatedness in CBP have yet to be identified (e.g. Andersson & Waldeck 2007; Waldeck *et al*. 2008; but see Roy Nielsen *et al*. 2006). This may confuse any inferences about decision-making as the breeding status of the parasite is not known and these individuals may or may not have a nest site of their own. Three roles can be identified for breeding individuals within CBP systems: (i) nonparasites ('conventional' breeding), (ii) true parasites, and (iii) nesting parasites (Åhlund & Andersson 2001; Lyon & Eadie 2008). True parasites reproduce solely by donating eggs. Nesting parasites may prefer to donate eggs close to their own nest because they have better information about nest locations, or there may be constraints on time or travel distance. Thus, all other things being equal, nesting parasites are likely to prefer close nests over more distant ones even if the costs of travel will be greatly outweighed by the benefits of parasitizing. Such distance-constrained egg donation behaviour coupled with the presence of natal philopatry might lead to elevated host–parasite relatedness without requiring kin discrimination. However, if parasitizing is selective and relatedness plays a role in decision-making, or if both distance and relatedness are of importance, relatedness between interactants may not be driven by natal philopatry alone.

The aim of this study was to examine the role of relatedness in determining the behaviour of conspecific brood parasites and their hosts. We did this by genotyping 32 breeding females and 288 ducklings at 19 microsatellite loci from a Canadian population of the brood-parasitic Barrow's goldeneye (*Bucephala islandica*). Assignment of maternity to parasitic young in host nests allowed us to study the spatial genetic structure of brood parasitism, and most importantly, disentangle the effects of spatial proximity and relatedness on host–parasite interactions. We also investigated host responses to parasitism by analyzing the number of parasitic eggs in relation to the host's own clutch size and host–parasite relatedness, thereby shedding light on the possible presence of mechanisms leading to kin-biased egg donation in the CBP system of waterfowl, in which CBP is especially common (Yom-Tov 2001). By utilizing high-resolution genotypic data, we were able to study the behaviour of individuals of known roles in a CBP network, thereby gaining novel insights into the reproductive decision-making by both hosts and parasites.

Methods

Study area and population

The study was conducted at Riske Creek, close to the town of Williams Lake (51°52′N, 122°21′W), British Columbia, Canada, in 2006. The study area consists of a prairie plateau approximately 200 km^2 in size interspersed with a large number of ponds and lakes, with trembling aspen (*Populus tremuloides*) stands along the shores. The plateau is surrounded by mixed coniferous forest consisting mainly of lodgepole pine (*Pinus contorta*) and Douglas-fir (*Pseudotsuga menziezii*). Nest boxes were erected in the aspen and coniferous stands in 2004, as close to the shorelines of the wetlands as possible, resulting in a total of 127 nest boxes being placed around 36 ponds and lakes. The coordinates of all nest boxes were recorded using a global positioning system (GPS) unit. Seventy nests were initiated in 2006, half of which (35) hatched successfully.

Sampling and laboratory methods

During the 2006 breeding season, 32 females were caught by hand or by hand net from the nest aperture for banding, taking structural measurements (viz. weight, lengths of wing chord, culmen, radius-ulna and tarsus) and DNA sampling. To estimate the date of incubation onset, we used the egg flotation method of Kilpi & Lindström (1997), modified to Barrow's goldeneyes with an incubation period of approximately 32 days. The method provides a reliable estimate of the number of days the eggs have been incubated and hence allows the estimation of brood hatch date. DNA was sampled by using a syringe to extract *c*. 400 μL of blood from the carpal vein. Ducklings were sampled upon hatching by collecting *c*. 100 μL of blood from the medial metatarsal vein using small gauge lancets and capillary tubes. All blood samples were stored in tubes containing 70% ethanol and frozen at −20 °C until the DNA was extracted.

Genomic DNA was extracted using the Chelex method of Walsch *et al*. (1991). Individuals were genotyped at 19 microsatellite loci described in Jaari *et al*. (2009) and listed in Table A1, Supporting information. Polymerase chain reaction (PCR) amplifications were carried out in a total volume of 10 μL (2 pmol of each primer, 1× QIAGEN Multiplex Master Mix, 0.5× Q-Solution, and *c*. 30 ng of DNA) using the same cycling profile (15 min at 95 °C, followed by 30 cycles of 30 s at 94 °C, 90 s at 56 °C and 60 s at 72 °C and a final extension for 10 min at 72 °C) for all markers, using a commercial multiplex PCR kit (QIAGEN). The PCR products were diluted 1:750 with MQ-water and mixed with Et-ROX 400 standard (GE Healthcare, Life Sciences) according to the manufacturer's instructions and were resolved in a MegaBACE 1000 capillary sequencer (GE Healthcare, Life Sciences). Genotypes were scored using Fragment Profiler 1.2 software (GE Healthcare, Life Sciences). More details about used primers, loci and their variability are given in the Table A1 and Jaari *et al*. (2009).

Detection of CBP

Loci were tested for deviations from Hardy–Weinberg equilibrium and linkage disequilibria using FSTAT 2.9.3.2 (Goudet 1995). Nonexclusion probabilities were calculated using Cervus 3.0 (Kalinowski *et al*. 2007). This statistic describes the average probability that the genotypic data will not exclude an unrelated candidate parent to be the parent — in this case mother — of an arbitrary offspring when the genotype of the other parent — in this case the father — is unknown.

Maternities were assigned to all the 288 sampled ducklings using the likelihood approach implemented in Cervus 3.0 (Kalinowski *et al*. 2007). Cervus estimates the likelihood ratios for each mother–offspring pair over all loci, given the genotypic data provided. Using the difference in likelihoods between the likeliest and second likeliest pair, Cervus can assign parentage and estimate confidence levels for the assignment through simulation. We use the default confidence levels of 80% and 95%, but only considered mother–offspring pairs assigned with the stricter confidence criterion (95%) in the further analysis. Our estimation was based on a 1% genotyping error rate and 60% of all breeding females in the population sampled, and was run for 10 000 iterations. Our results were not sensitive to this sampling proportion; using a 40% or a 90% sampling rate lead to the same conclusions as the chosen 60% sampling rate. Parasitism events were scored when offspring were assigned to other females than the one in the nest of which they were born, or when there were young in the nests that remained unassigned to any female. The mothers of the latter class of young remain unknown.

After assignment by Cervus, we counted the number of parasitic young in the nest of each female and recorded the known donors. The broods of three females were not sampled due to early hatching, and hence these three females were only included in the analyses of the behaviour of parasites and in calculating host–parasite relatedness estimates, as all of them parasitized other nests. One nesting female was not sampled, and so the brood in this nest (10 ducklings) was omitted as a host nest, and only used when analyzing the number of donated young. In total, 278 young hatching in host nests were included in our analyses.

Close relatedness between females may complicate motherhood assignment, because two related candidate mothers will have more similar likelihoods for motherhood than unrelated individuals, thus leading to a low difference in likelihoods and uncertainty in assignment. This could potentially overestimate the effect of relatedness on the degree of clutch reduction, as more young may remain unassigned to the host. To test whether such a bias exists, we conducted a correlation analysis between host–parasite relatedness and the number of unassigned young in the nest.

Patterns of relatedness

Host–parasite relatedness was estimated with Kinship 1.2 (Queller & Goodnight 1989) by calculating the pairwise relatedness coefficient r (*sensu* Queller & Goodnight 1989). This coefficient measures the genetic similarity of individuals taking into account the mean similarity in the reference population. The reference population in this case is represented by the sampled females, the relatedness values of which range from –0.65 to 0.71 with a mean (± SE) of –0.002 (± 0.008).

Natal philopatry is expected to elevate the local relatedness of individuals and relatedness should thus decline with increasing distance in a population where natal philopatry is present. Such trends may be hard to detect over short distances (Waldeck *et al.* 2008), so we used the entire span of the population to search for a possible spatial trend in relatedness. We used a generalized linear model (GLM) for relatedness over distance for all the 496 pairwise distances and r-values. The pairwise distances were calculated based on the coordinates of the nests of each incubating female. The distances ranged from 0.1 km to 18.5 km with a mean (± SD) of 7.45 km (± 0.22).

In order to assess whether host–parasite pairs were more related than expected by natal philopatry, we compared pairwise host–parasite relatedness with that of close neighbours (e.g. Waldeck *et al.* 2008). This was carried out by comparing the mean r-values of host–parasite pairs with neighbours nesting within a kilometre radius of each other ($n = 29$) using an ANOVA. The 1-km distance interval was chosen based on the structure of the open and patchy landscape in the area, and so that the interval would contain a sufficient sample size for the analyses. We also compared the pairwise relatedness of the remaining pairs of females with the two above-mentioned groups, to test whether close neighbours and/or host–parasite pairs were on average more closely related than the rest of the population.

Parasitism behaviour

To examine the relative importance of relatedness and distance in determining the degree of parasitism by nesting parasites, we built a multinomial logistic regression model (McCullough & Nelder 1989), with relatedness and distance between nests being used to explain the probability that female i laid each egg in the nest of female j. The model is conditional on the total number of eggs laid by female i in a nest other than her own, and also on the nest of female j being available. Nests initiated after the onset of the focal individual's incubation period were defined as unavailable. The model was fitted using a Bayesian approach (e.g. Gelman *et al*. 2004), with vague priors for the two regression coefficients (i.e. normal distributions with mean 0 and variance 10). The model was fitted in OpenBUGS through the Brugs package (Thomas *et al*. 2006). Five chains were run, and after a burn-in of 100 iterations, the next 10 000 iterations from each chain were used. Full details of the model are given in Table A1.

Host response to parasitism

We analysed host responses to parasitism by examining how the total number of offspring produced by a female was affected by the total number of parasitic young received, and the relatedness of the host to the parasites. This was carried out by using a GLM with the number of own young in the host nest as the dependent variable, and the number of parasitic young in the same nest and mean relatedness to parasites as explanatory variables. Relatedness was not calculated in some nests as the parasite had not been sampled, so we used multiple imputation to estimate the distribution of the missing values (e.g. Little & Rubin 2002). This essentially equals treating them as extra variables to be estimated, and our inferences are thus being averaged over the likely values of the missing data. The nature of missing data is commonly nonrandom, and multiple imputation is currently the most efficient method of removing such bias (Nakagawa & Freckleton 2008). Using this method meant that we could use all of the data in the analysis, giving us extra precision for our inference. However, the results remain qualitatively the same even if the imputation procedure is omitted (see Discussion).

The model was fitted using a Bayesian approach, with vague priors (normal distributions with mean 0, variance 1 for the regression coefficients, mean 0, variance 10 for the intercept). For the missing data, the relatedness was given a normal distribution, with the mean and standard deviation being estimated from the data: the mean had a normal prior with mean 0, variance 2, and the standard deviation had a uniform prior between 0 and 1. The analyses described above allowed us to simultaneously assess the independent effects of the number of eggs donated and mutual host–parasite relatedness on the number of host eggs produced.

Detection of a reduction in host clutch size may not indicate that the host actually laid fewer eggs, but that it was induced to donate eggs to avoid having too many eggs in its own nest, thus diminishing the costs of incubation or even avoiding nest desertion (cf. McRae 1995; Jaatinen *et al*. in press). In order to assess the role of this alternative process when considering host responses to parasitism, we calculated the correlation between the number of received young and the number of young donated. A positive correlation would indicate that apparent host clutch reduction was in fact an artefact of parasitism-induced egg donation by the host. In order to examine the effects of host–parasite relatedness on the total size of host clutches, we calculated the correlation between the total number of hatched young in host nests and the host's mean relatedness to parasites. Since we use hatched ducklings to infer the numbers of host and parasite young, it is important to control for the effect of total clutch size on the hatching success of eggs. To test whether the total number of incubated eggs affected the fraction of hatching young, we did a correlation analysis between these two variables.

Results

The loci used were in Hardy–Weinberg equilibrium and unlinked. The nonexclusion probability per locus ranged from 0.34 to 0.99, and the combined probability over all loci was 0.0004. Thus, the probability by which a false candidate parent was not excluded as a parent was very low.

Assignment and relatedness trends

The assignment of maternity for the 288 ducklings sampled revealed 43 young assigned to other females than the owner of the nest, and 42 young remained unassigned. Thus, 50.1% of the parasitic young came from identified females. There was no correlation between mean host–parasite relatedness and the number of unassigned young in the nest ($r_p = 0.35$, $P > 0.15$, $n = 18$), indicating that relatedness between hosts and parasites did not affect the efficiency by which Cervus assigned maternity.

Although there was some evidence for a negative relationship between pairwise relatedness and distance, the relationship was not statistically significant ($F_{1,495} = 3.37$, $P = 0.07$; Fig. 1), and explained very little of the variation in the data ($R^2 = 0.7\%$). There were significant differences in mean relatedness between host–parasite pairs, close neighbours, and the remaining population (ANOVA: $F_{2,493} = 10.13$, $P < 0.001$). The mean relatedness (± SE) of host–parasite pairs was 0.08 (± 0.04) and that of close neighbours 0.11

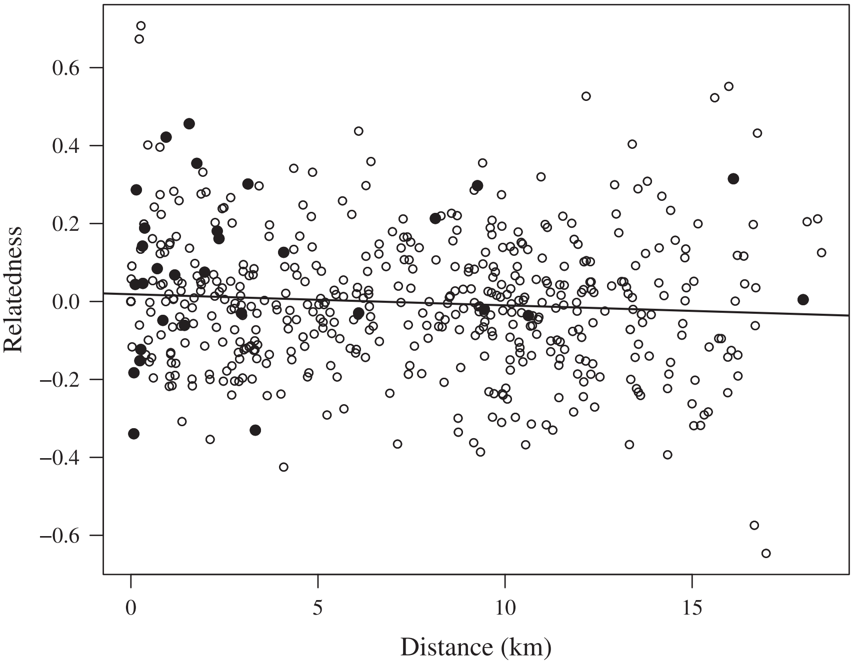

Fig. 1 Distance and relatedness between goldeneye females. Pairs indicated by open circles did not exchange eggs, whereas those indicated by filled circles exchanged eggs.

(± 0.041); the two did not significantly differ from each other ($P = 0.84$, Tukey's test). However, both relatedness coefficients were significantly higher than that of the remaining population ($P = 0.011$ and 0.001 respectively, Tukey's test), the mean (± SE) of which was −0.015 (± 0.0081).

Parasitism behaviour

The number of parasitic eggs laid into host nests increased with decreasing distance {$b = -0.17$, 95% highest posterior density interval [−0.28, −0.08, Pr(coefficient > 0) < 0.001]; Fig. 2}. The number of parasitic eggs also increased with relatedness between host and parasite {$b = 2.4$, 95% highest posterior density interval [0.70, 4.43, Pr(coefficient < 0) = 0.004]; Fig. 2}.

Clutch reduction and relatedness

There was evidence that the number of host offspring decreased with increasing relatedness to the parasite and increasing numbers of parasitic offspring in the nest: $\log(R) = 2.1 - 0.06\times$ parasitic offspring $-0.77\times$ relatedness (Table 1, Fig. 3, R = expected number of offspring). However, although the confidence intervals were wide (−0.13 to 0.015 for number of parasitic offspring, −1.54 to −0.003 for relatedness), the posterior probabilities that the estimates are not negative were all around 0.05 (0.06 and 0.025 for number of parasitic offspring and relatedness, respectively), meaning that there is a very small probability that the effect

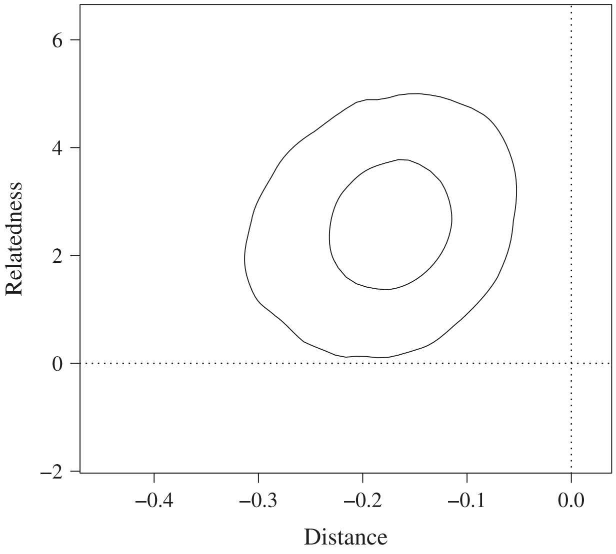

Fig. 2 50% and 95% Bayesian confidence regions for the regression coefficients from a multinomial generalized linear model for the effects of host–parasite relatedness and distance on the number of eggs donated by parasites. The confidence region is above the zero line for relatedness (horizontal dashed line), showing that the regression coefficient for the effect of relatedness on the number of parasitic eggs donated is positive, that is, the number of parasitic eggs donated increases with relatedness. The confidence region is below the zero line for distance, indicating a negative relationship between distance and the number of parasitic eggs donated, that is, the number of eggs donated decreases with distance.

Table 1 Mean (± SE) host clutch size in parasitized and unparasitized nests, the average number of donated eggs and the mean total clutch size (sum of host and parasitic eggs) in all nests. The number of donated eggs represents all nests including ones in which the female donated zero eggs (*n*, sample size)

	Value	*n*
Average own clutch size:		
Parasitized	6.27 ± 0.55	22
Unparasitized	7.71 ± 0.68	7
Total	6.62 ± 0.49	29
Average number donated	110 ± 0.29	29
Average total clutch size:		
Parasitized	10.18 ± 0.57	22
Unparasitized	7.71 ± 0.68	7
Total	9.59 ± 0.50	29

of both relatedness and the number of parasitic eggs on the number of host eggs would be the opposite (positive) or indeed absent. Hosts did not donate eggs in response to parasitism, as there was no correlation between the number of parasitic eggs received and the number of eggs donated by the host ($r_p = -0.19$, $P > 0.3$, $n = 18$). The total number of young hatched in host nests was also uncorrelated with host–parasite relatedness ($r_p = -0.34$, $P > 0.16$, $n = 18$), and total clutch size was unrelated to the proportion of eggs hatching ($r_p = -0.17$, $P > 0.3$, $n = 29$). Details about clutch sizes in relation to parasitism status and the numbers of eggs donated are presented in Table 1.

Discussion

We found that host–parasite relatedness followed the pattern of spatial genetic structuring expected under natal philopatry (Waldeck *et al.* 2008), with both close neighbours and host–parasite pairs being more closely related to each other than to random females drawn from the population. However, we found that both distance and relatedness to potential hosts were associated with the number of eggs donated by nesting parasites, and that hosts appeared to reduce their clutch size as a response to parasitism. While host–parasite relatedness might be explained through more synchronous egg laying of related females, a condition facilitating parasitism (Sorenson 1993), this explanation is made less likely by our finding that the degree of host clutch reduction was positively related to host–parasite relatedness; a novel insight in the context of CBP research. Both the latter result and the finding of relatedness affecting the degree of parasitism suggest that hosts and parasites modify their behaviour in response not only to the behaviour of one another but also to their kinship to one another.

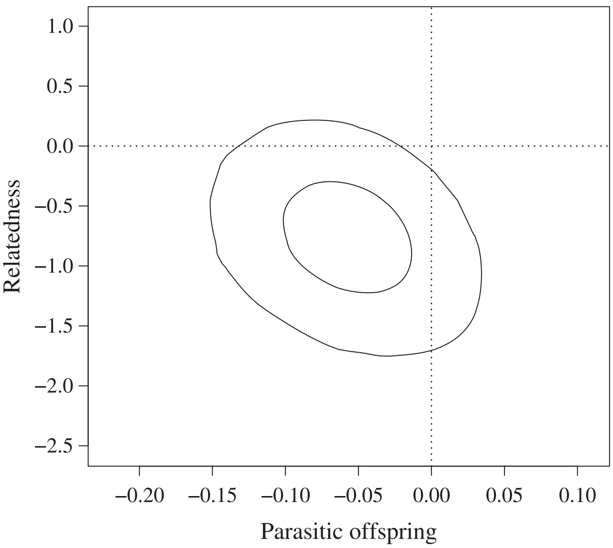

Fig. 3 50% and 95% Bayesian confidence regions for the regression coefficients from a generalized linear model of the effects of host–parasite relatedness and the number of parasitic offspring received on the number of own eggs laid by hosts (assumed to be Poisson distributed). The confidence region is mainly below the zero line for relatedness, indicating that host clutch size has a negative relationship with relatedness to parasites, that is, the number of eggs hosts lay in their own nests decrease with relatedness to parasites. The confidence region is also mainly below the zero line for the number of parasitic offspring, indicating that the number of eggs hosts lay in their own nests decrease with the number of parasitic eggs deposited in their nests. Although the 95% Bayesian confidence regions cross the zero lines for relatedness and the number of parasitic offspring, the posterior probabilities that the estimates are not negative were small (0.025 and 0.06 for relatedness and the number of parasitic offspring, respectively).

Spatial relatedness and parasite behaviour

Several studies have documented natal philopatry in female waterfowl (Anderson *et al.* 1992; Pöysä *et al.* 1997; Andersson & Åhlund 2000; Ruusila *et al.* 2000; Scribner *et al.* 2001; Andersson & Waldeck 2007; Waldeck *et al.* 2008), some of which have also found elevated relatedness between closely breeding females (McKinnon *et al.* 2006; Andersson & Waldeck 2007; Waldeck *et al.* 2008). It is thus important to assess whether the observed pattern of relatedness is the result of natal philopatry alone.

Our results show that both distance and relatedness *independently* affected the extent of parasitism by nesting parasites, so that the number of eggs donated increased with both decreasing distance and increasing relatedness. Hence, natal philopatry — although possibly present in our study population — is not the sole factor elevating host–parasite relatedness. In fact, the positive effect of decreasing distance on the amount of parasitism may be the result of two, not mutually exclusive, processes. First, natal philopatry may be facilitating the proximity of relatives. Second, a closely related species, the common goldeneye (*Bucephala clangula*), has been documented to prioritize safe nest sites when parasitizing (Pöysä 1999). A closer examination of the fate of host nests included in our study revealed that they had not been subject to predation once over the past three years. Neighbouring common goldeneye nests have been found to have a high probability of sharing the same fate (predated vs. successful; Pöysä 1999), and the probability of sharing the same fate also decreases with distance between nests (H. Pöysä, Finnish Game and Fisheries Research Institute, Joensuu, personal communication). Thus, nesting parasite females may target nests close to their own, safe nests in order to elevate the survival probability of their parasitic offspring.

Our finding that the number of parasitic eggs donated by nesting parasites increased with relatedness is in accordance with previous studies (Andersson & Åhlund 2000; Roy Nielsen *et al*. 2006; Andersson & Waldeck 2007; Waldeck *et al*. 2008). These studies found the relatedness between the host and the parasite laying most eggs into the host nest to be higher than expected by natal philopatry alone in wood ducks (*Aix sponsa*; Roy Nielsen *et al*. 2006), common goldeneyes (Andersson & Åhlund 2000) and eider ducks (*Somateria mollissima*; Andersson & Waldeck 2007; Waldeck *et al*. 2008). Our results thus add to the evidence that relatedness may at least partially determine the outcome of host–parasite interactions. With the high-resolution genotypic data at hand, we can thus confirm that the opportunity for kin selection to arise in this system exists.

If CBP is detrimental to the host, why do parasites preferentially target relatives? We argue that the net outcome of CBP need not be negative to hosts (see 'Host responses' below), as it would then make no sense for relatives to target one another.

Host responses

Hosts responded to parasitism by reducing their clutch size and relatedness modulated this reduction. In fact, the effect of relatedness appeared stronger than that of the number of parasitic eggs, underlining the importance of host–parasite relatedness. These results are robust with respect to the statistical methods applied, since our conclusions remained unchanged also without the imputated data (see Methods section and Fig. 3 above), using a GLM (model: $P = 0.002$; relatedness: $P = 0.018$; parasitic offspring $P = 0.046$, $n = 18$). The observed clutch size reduction was real and not an artefact of parasitism-induced egg donation: the amount of parasitism in the host's nest did not induce egg donation by the host. Theoretically, conspecific brood parasitism may favour an increase (Ruxton and Broom 2002) or a reduction in the host's own clutch size (Lyon 1998); however, parasitism-induced increases in clutch size have to our knowledge never been empirically confirmed. In addition to the results presented in our current study, reduced host clutch size as a response to parasitism has also been confirmed by some other empirical work (Andersson & Eriksson 1982; Erikstad & Bustnes 1994). Nest parasitism is frequent in our study population and clutch reduction may be a way of mitigating the risk of parasitism-induced nest desertion by hosts (Jaatinen *et al*. 2009) as a consequence of overly large clutches which may potentially jeopardize hatchability.

On the other hand, our finding that relatedness to the parasite as well as the number of parasitic eggs received reduced host clutch sizes is slightly puzzling if only seen from the evasion of adversity point of view. Relatives indirectly harm themselves by afflicting adversity upon kin and should avoid doing so if possible. While incubating enlarged clutches is costly (Erikstad & Tveraa 1995; Monaghan & Nager 1997; Hanssen *et al*. 2003; Hanssen *et al*. 2005; de Heij *et al*. 2006), hosts accepting eggs may accrue benefits that are realized during the subsequent brood-rearing stage (Eadie *et al*. 1988; Lepage *et al*. 1998; Smith *et al*. 2005; Öst *et al*. 2008). In fact, potentially positive effects of brood size on offspring survival has been reported in Barrow's goldeneyes (Smith *et al*. 2005), as well as in other waterfowl (Lepage *et al*. 1998; Öst *et al*. 2008). However, the empirical evidence for positive effects of larger brood size is so far inconclusive, as an experimental study on Barrow's goldeneyes found no relationship between brood size and offspring survival (Eadie & Lyon 1998).

As the behaviour of both parasites and hosts are affected by relatedness, it may be difficult to deduce whether the actions of the parasites or the hosts are primarily responsible for the kin-biased egg donation observed (but see Andersson & Åhlund 2000). However, the outcome is of importance: while the total number of ducklings hatched in hosts' nests remained unaffected by relatedness to parasites, host–parasite relatedness significantly elevated the proportion of hatching parasitic young. This increase in the proportion of parasitic young resulting from host–parasite relatedness is in line with predictions based on kin selection theory and indicates the presence of a mechanism to kin-biased egg donation controlled by the parasites, by the hosts, or by both.

In conclusion, Barrow's goldeneyes are more likely to donate eggs to their relatives than predicted by the spatial pattern of relatedness alone, as well as to reduce their clutches in the face of parasitism, particularly so when the parasite is a relative. Some mechanism other than natal philopatry alone is likely to be acting for such patterns to arise, and experiments are needed to identify the exact mechanisms involved in kin-biased egg donation. Other important follow up steps include determination of the fitness achieved by egg recipients, donors and receiving donors, as well as elucidating whether negotiation between hosts and parasites occurs.

Acknowledgements

We wish to thank Perttu Seppä and Lotta Sundström for encouragement and discussion at the early stage of the project. We also thank Kaisa Välimäki for commenting the molecular methods and Jed Scharf for field assistance. Sveinn Are Hanssen and two anonymous reviewers provided valuable comments to the manuscript. The study was financially supported by the Academy of Finland (project numbers 104582 to M.Ö., 106143 to R.B.O'H, and 213663 to J.M.), The Finnish Cultural Foundation (to K.J.), Delta Waterfowl Foundation (to K.J.), and Oskar Öflunds stiftelse (to K.J. and M.Ö.).

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Kim Jaatinen shows interest in breeding ecology and behaviour, Sonia Jaari in molecular ecology and genetics of wild populations. Robert O'Hara expresses inordinate interest in Bayesian confidence intervals and Markus Öst in Eiders. Juha Merilä is interested in the biology of wild animal populations.

Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1 Characteristics of the microsatellite loci in the study population of Barrow's Goldeneyes, including repeat motif, size range (base pair), primer sequences (F- forward; R-reverse), GenBank accession numbers, number of alleles (A), expected heterozygosity (He), observed heterozygosity (Ho), and polymorphic information content (PIC)

Statistical Anlayses