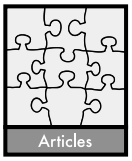

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Analysis of extra-pair paternity and conspecific brood parasitism in mallards *Anas platyrhynchos* using non-invasive techniques

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A method that was based on non-invasive sampling of genetic material was used to determine the rates of extra-pair paternity (EPP) and conspecific brood parasitism (CBP) in mallards. Maternal and offspring DNA were extracted from feathers in nest material and hatched eggshell membranes. Using 8 microsatelite loci, extra-pair offspring were detected in 48% of nests and accounted for 9.3% of all offspring. In addition, 10.1% of the offspring were confirmed to result from CBP, and 24% of all nests contained at least 1 offspring from CBP. Rates of conspecific nest parasitism were higher than those of related species, which might have been due to higher breeding densities at our study site. The incidence of EPP was distributed randomly (i.e. did not deviate from bionomial distribution) throughout the population, indicating that variations in pre-copulatory (e.g. female choice, mate guarding) or post-copulatory processes (e.g. sperm competition, cryptic female choice) do not affect the distribution of EPP among breeding pairs markedly. Yet, our data provide evidence of variation in the risk of being parasitized among breeding females. The occurrence of CBP and EPP was unaffected by the timing of the breeding attempt or breeding synchrony.

Extra-pair paternity (EPP) and conspecific brood parasitism (CBP) are alternative reproductive tactics that contribute to the variance in reproductive success in several animal taxa, including birds (Møller and Ninni 1998, Åhlund and Andersson 2001, Albrecht et al. 2007). Although EPP has been detected in most bird species (reviewed by Griffith et al. 2002), CBP occurs predominantly in precocial and semiprecocial bird taxa (Sorenson 1992, Yom-Tov 2001), where investments into post-hatching parental care are reduced (Sorenson 1992, Geffen and Yom-Tov 2001).

The fitness benefits from these strategies for the genetic father, in the case of EPP, or for the genetic mother, in CBP, are patent, because in these situations, genetic parents profit from siring additional offspring and reducing exertion during parental care, respectively. However, fitness pay-offs for the remaining players are still the subject of many controversies (López-Sepulcre and Kokko 2002, Pöysä 2004, Arnqvist and Kirkpatrick 2005, Albrecht et al. 2006b, Griffith 2007).

Considerable research over the past two decades has focused on testing evolutionary and ecological hypotheses with regard to the inter- and intraspecific variation in EPP and CBP. Nevertheless, the empirical data that support these hypotheses are incomplete in many aspects. In particular, CBP rates have been determined for few species using reliable genetic methods (Åhlund and Andersson 2001, Kraaijeveld et al. 2004, Nielsen et al. 2006). There is also an apparent taxon bias in EPP studies. Most attention has been paid to the investigation of EPP in passerine species, while only few studies have attempted to determine rates of EPP in precocial species such as waterfowl with intromittent copulation organ (Coker et al. 2002, but see Peters et al. 2003, Kraaijeveld et al. 2004).

This study was aimed at determining the rates of EPP and CBP in a ground-nesting dabbling duck – the mallard *Anas platyrhynchos*. The capture of hatchlings and incubating female ducks is methodically difficult (Hořák and Albrecht 2007), because the clutch hatches synchronously and the young typically leave the nest within several hours after hatching. In addition, the incubating females of certain species are sensitive to human disturbance, which can result in clutch abandonment after capture. These challenges may have impeded the progress of research on alternative reproductive strategies in waterfowl. Recently developed methods, based on the non-invasive collection of genetic material, might overcome these complications (Horváth et al. 2005, Schmaltz et al. 2006), although studies of reproductive tactics that are based solely on non-invasive sampling are rare (but see Rudnick et al. 2005).

To analyze CBP and EPP rates, we gathered feathers from nest material as a source of maternal DNA and eggshell membranes as a source of young DNA (Pearce et al. 1997). We tested whether the prevalence of EPP and CBP differ significantly between nests in the mallard population (i.e. EPP and CBP events are distributed non-randomly). A non-random distribution of alternative

breeding tactics among individuals in a population suggests inter-individual variations in the susceptibility to EPP/CPB, governed by biologically relevant mechanisms. For EPP in particular, the ability of males to monopolize a social mate can vary (Neuhauser et al. 2001, Albrecht et al. 2007). Similarly, a parasitic female prefers safer nest sites, nests of high-quality hosts (Pöysä 2003, Pöysä and Pesonen 2007), or, simply, nests that are easier to detect (Semel et al. 1988), resulting in the skewed distribution of CBP events throughout nests.

We also tested whether the prevalence of CBP and EPP varied during the breeding season. For example, CBP has been proposed to be more prevalent at the end of the breeding season, because females that lose their first clutch due to predation might adopt this tactic as a 'best-of-a-bad-job' strategy (Sayler 1992). Concurrently, CBP might be associated positively with breeding synchrony, due the greater availability of clutches for parasitic females. It has been suggested that male searches for extra-pair copulations are constrained by mate guarding of social females during fertile periods. In such a situation, a negative correlation between breeding synchrony and EPP rates might be expected, due to the decline in number of unconstrained males (Dunn et al. 1999).

Methods

Study site and field work

The field work was performed in 2004 throughout the entire mallard breeding season (mid-April to mid-July) in the artificial fish pond Starý u Soběslavy in the Třeboň Biosphere Reserve (49°9′N, 14°43′E), Doudlebia, Czech Republic. Mallard nests were located on 3 artificial islands (3000–5000 m^2) in this fish pond. In extensive searches for nests in the grassland that surrounded the body of water that we studied, we observed that mallards bred here at much lower densities compared with artificial islands (2–3% of all found nests, Kreisinger unpubl.). Thus, we believe that we detected the majority of breeding attempts in this area, due to the limited area of the island, our high searching efforts, and the low breeding densities that existed off of the artificial island.

We found 55 nests during the entire breeding season. When a nest was located, we measured the eggs and vegetation parameters (Albrecht and Klvaňa 2004, Kreisinger and Albrecht 2008). A 'candler' (Weller 1956) was used to estimate the incubation stage. Previously identified nests were checked during each subsequent visit to the area, and their status was recorded (e.g. undisturbed, depredated, hatched).

Sampling of genetic material

Immediately upon finding a nest, we collected 10–15 feathers from the nest material. If the number of feathers was low (typically in nests with an incomplete clutch, Kreisinger unpubl.), we repeated the collection during subsequent visits.

Twenty-six of 55 nests survived until hatching (fatalities due to predation; Albrecht et al. 2006a, Kreisinger and Albrecht 2008). In all hatched nests, we searched the nest material and nest surroundings carefully for egg membranes. Membranes were dried at room temperature. Using this approach, we obtained material from most eggs that were present in the nests during the last visit before hatching (240 of 259 [93%] eggs).

Genotyping

Maternal DNA was extracted from an approximately 5–7 mm long terminal section of the calamus, containing the feather tip (umbiculus inferior) and superior umbilicus (Horváth et al. 2005), using the DNeasy Blood and Tissue Kit. Four to five feathers were used for the extraction. Offspring DNA was extracted from an approximately 0.25 cm^2 piece of egg membrane that contained chorionic blood vessels.

We used 8 microsatellite loci that were designed specifically for mallard (Table 1; Maak et al. 2003, Denk et al. 2004). Multiplex polymerase chain reaction (PCR) was performed for each DNA sample in a 10 µl reaction mixture that comprised 5 µl QIAGEN Multiplex PCR Kit, 4 µl of DNA solution (approx. 20–30 ng per reaction), and 1 µl of primer solution. Forward primers were fluorescently dyed. The PCR reaction consisted of a 15 min 95°C denaturation step, followed by 35 cycles of 1) denaturation at 94°C for 30 s, 2) annealing at 60°C for 90 s and 3) extension at 72°C for 60 s. The final extension step was 30 min at 60°C. PCR products were sized using the ABI 3100 automated capillary sequencer. Alleles were scored using GeneScan software.

Table 1. Summary of statistics of microsatellite loci used to determine EPP and CBP in mallards. [a]name of locus described by *Peters et al. 2004 or [#]Maak et al. 2003), [b]number of individuals typed, [c]number of alleles, [d]observed heterozygosity, [e]expected heterozygosity, [f]polymorphic information content, [g]maternal non-exclusion probability, and [h]paternal non-exclusion probability.

Locus[a]	N[b]	K[c]	$H_{(Obs)}$[d]	$H_{(Exp)}$[e]	PIC[f]	$NonEx_{(1P)}$[g]	$NonEx_{(2P)}$[h]
Apl 12*	265	40	0.92	0.95	0.94	0.2	0.11
Apl 18*	268	29	0.94	0.92	0.91	0.27	0.16
Apl 36*	273	24	0.83	0.92	0.91	0.3	0.18
Apho 21[#]	259	16	0.73	0.85	0.84	0.45	0.29
Apho 2[#]	266	16	0.81	0.86	0.84	0.43	0.28
Apho 24[#]	270	17	0.63	0.76	0.74	0.59	0.41
Apho 24[#]	269	16	0.66	0.82	0.8	0.51	0.33
Apho 18[#]	275	4	0.34	0.3	0.28	0.96	0.85
					Combined	0.00089	0.00003

Fifty-five maternal and 240 offspring samples were gathered for genotyping. Genotyping of one maternal DNA sample failed. We were also unable to genotype 13 offspring samples from a nest that remained outdoors for a prolonged period after hatching. Consequently, 54 maternal and 227 samples were genotyped. There was no evidence of DNA contamination (i.e. more than 3 alleles per locus) of the maternal DNA samples. Although offspring DNA contamination occurred occasionally (<2% of samples), re-extraction of the contaminated sample resolved this problem.

The exclusionary probability of a random parent (computed by CERVUS 3, Kalinowski et al. 2007) of a given set of markers was high (0.9981 for the first parent and 0.9999 for the second, Table 1). A slight deficiency of heterozygotes was detected in our population (Table 1), which could have been due to the presence of null alleles (Pemberton et al. 1995; Table 1). Nevertheless, the observed deficiency of heterozyzgotes might also have been caused by the massive release of captivity-bred individuals by hunting organizations (Fišer et al. 1989).

Microsatellite data, based on non-invasively collected samples, can suffer from allelic drop out and allelic misprinting due to low DNA quality (Taberlet et al. 1999), which can bias EPP and CBP estimates. We examined this potential source of bias by evaluating the consistency of genotypes from identical DNA sources. We genotyped 64 pairs of DNA isolates from 32 egg membranes and 32 nest feathers. We did not find any evidence of allelic drop out or allelic misprinting. Amplification of a particular locus for one sample in the pair occasionally failed (less than 4% of samples); regardless, this source of inconsistency would not have resulted in false EPP or CBP identification.

Paternity/maternity analyses

We adopted conservative criteria to identify CBP and EPP young. A young offspring was considered to result from CBP or EPP if it did not match the putative mother or father at ≥ 2 loci and if at least one of these mismatches was not attributed to allelic drop out or null alleles (i.e. the putative female or father and CBP or EPP offspring were heterozygotes at this locus). EPP was analyzed in the sub-set of young in which CPB was not proved. We reconstructed the paternal genotype from the genotypes of the young in the nest and their genetic mother (Peters et al. 2003). If the father's genotype, which was compatible with the majority of non-CBP young in the nest, did not match a given young, the offspring was considered to have resulted from EPP.

In the next step, we searched for genetically compatible parents of EPP and CPB offspring using a procedure that is based on maximum likelihood, as implemented in Cervus 3.0 (Kalinowski et al. 2007). For EPP, we searched for the most likely genetic father, using the parental genotypes that were inferred from the genotypes of its genetic offspring and social female (above). Initially, we performed a simulation (10 000 cycles) using the known distribution of allele frequencies to estimate the critical 95% confidence interval for the differences in log-likelihood scores between the 'real' genetic and second-most likely father, based on the known maternal genotype. We estimated the proportion of sampled males to be 20%.

For CBP offspring, we performed a similar simulation to estimate the 95% confidence interval for log-likelihood differences between the genetic and second-most likely mothers, assuming that 70% of breeding females were sampled. Maternity and paternity were assigned when the most likely mother and father matched the parasitic young at all loci and when the 95% confidence criterion was fulfilled.

Kingroup ver. 2 (Konovalov et al. 2004) was used to estimate the number of females or males that sired CBP and EPP offspring in a particular nest. Kingroup computes pairwise relatedness between individuals using an algorithm, specified in Queller and Goodnight (1989). It is possible to compare the likelihood of 2 alternative relatedness hypotheses using this software. To determine the presence of CBP offspring that were laid by more than one female in a particular nest, we compared the likelihood of the null hypothesis that a given pair of parasitic offspring in a nest consisted of full or half-siblings; the alternative hypothesis was that these individuals were unrelated.

Similarly, to determine whether the presence of multiple EPP offspring in a particular nest resulted from fertilization by more than one extra-pair male, the likelihood of the null hypothesis, i.e. that a given pair of EPP offspring in a particular nest were full siblings, was compared with the alternative hypothesis that they were half-siblings. Finally, we examined the parasitism of more than one clutch by a single female by comparing the likelihood that a given pair of CBP offspring from two different clutches consisted of unrelated individuals with the alternative hypothesis that these individuals were half- or full siblings.

Statistical analyses

Using a generalized linear model (GLM), we test for the association between CBP, EPP, and breeding synchrony, and nest initiation date. The ratio of CPB (or EPP) versus non-CPB (or non-EPP) young in a nest was included as a binomial response variable. Alternatively, the presence or absence of CBP and EPP young was coded as a binary response (i.e. detected vs undetected). Because the results of both approaches were identical, we have presented the results of the former only.

Nest initiation date was considered the Julian date of the laying of the first egg (estimates based on back dating; Kreisinger and Albrecht 2008). We used a modified version of the synchrony index (Kempenaers 1993) to estimate breeding synchrony for a particular female. Breeding synchrony for a female corresponds to the proportion of females in the population that are simultaneously fertile (Yezerinac and Weatherhead 1997). Here, we defined the fertile period as the egg-laying stage to avoid an a priori assumption concerning sperm competition (Peters et al. 2003). This criterion is also plausible with respect to CBP, because most CBP events in mallards occur during egg laying (Kreisinger unpubl.).

We calculated the significance of explanatory variables according to F statistic using a stepwise deletion process – i.e. by comparing the change in deviance between models

that contained the variable in question with the model in which this variable is deleted (Crawley 2002).

The observed proportions of EPP young in nests were compared with the expected proportions, implied by binomial distribution, to test for the nonrandom distribution of EPP in the population (Bouwman et al. 2006). This approach, however, might not provide sufficiently robust results for CBP, due to its dependence on random processes that have low biological relevance (e.g. laying ability of a particular parasitic female). Thus, we compared the observed numbers of females that contributed to CBP in a particular nest with the expected value from the Poisson distribution. The Poisson distribution can be derived, based on single parameter, the mean, which was calculated by dividing the total number of individuals that were involved in CBP by the number of genotyped clutches. On reanalysis of the randomness of CBP and EPP distribution, assuming a binomial or Poisson distribution, respectively, identical results were obtained. All statistical analyses were performed in R 8.1. (R Development Core Team 2008).

Results

Conspecific brood parasitism

We identified 23 parasitic young (10.1%) in 6 of 25 nests (24%). The level of support for this result was high, because a mismatch with the maternal genotype occurred at ≥ 3 loci, with the exception of one individual. Three nests contained 6 parasitic eggs, one nest had 2, and 2 nests had one such egg. A parasitic offspring was assigned to its genetic mother in only one case. By relatedness analysis, we did not reject the null hypothesis that a given pair of offspring consisted of unrelated individuals for all pairwise comparisons for CBP offspring from different broods, suggesting that none of the females laid parasitic eggs in more than one nest.

In 3 nests, the relatedness analysis supported the hypothesis that CBP offspring in the nests are less related than full or half-siblings, suggesting parasitism of these three clutches by more than one female. The distribution of the counts of females that parasitized individual nests deviated significantly from the Poisson distribution ($DF = 1$, $\chi^2 = 7.298$, $p = 0.0069$), which would be expected under a random distribution of parasitic events in the studied population.

There were no differences in the number of eggs that were laid by parasitized versus non-parasitized females (Mann–Whitney U test, $Z = 0.181$, $p = 0.8559$). In addition, the number of parasitic eggs in a nest and the number of eggs that were laid and incubated by females did not correlate (Spearman correlation, $R = 0.023$, $p = 0.9119$). Consequently, the clutches in nests that were parasitized were significantly larger compared with those in non-parasitized nests (Mann–Whitney U test, $Z = 2{,}209$, $p = 0.0272$).

We found no relationship between the proportion of parasitic eggs and breeding synchrony index (GLM, slope $= 0.0024$, $F_{(1,23)} = 0.9205$, $p = 0.3478$) or nest initiation day (GLM, slope $= 0.5303$, $F_{(1,23)} = 2.7969$, $p = 0.108$).

Extra-pair paternity

After excluding CBP young, we detected 19 (9.3%) extra-pair offspring. Twelve of 25 nests (48%) contained at least one extra-pair young. Six nests contained one extra-pair young, 5 nests had 2, and one nest contained no such young. The distribution of observed proportions of extra-pair young in individual nests did not deviate from the expected binomial distribution ($DF = 2$, $\chi^2 = 1.8713$, $p = 0.3923$). We assigned EPP offspring to the 95% confidence inferred paternal genotype in 4 cases. The proportion of assigned extra-pair offspring was significantly higher than expected, based on the proportion of CBP offspring that were assigned (binomial test, $p = 0.0019$). This test is based on the proportion of successfully assigned parents that sired all detected CBP or EPP young. At the same time, we accounted for the loss of information on parental genotypes, because parental information was available for only 44% of nests that were sampled due to nest predation.

In 3 cases, the relatedness of EPP offspring in one nest likely corresponded to the half-sibling hypothesis rather than the full sibling null expectation, suggesting that fertilization of a particular female by more than one extra-pair male occurred. But, fertilization of more than one EPP offspring in a particular clutch by a single male could not be excluded in 3 cases.

There was a positive, but non-significant relationship between the proportion of extra-pair young (without taking CBP eggs into account) and breeding synchrony (GLM, slope $= 0.0004$, $F_{(1,23)} = 2.9694$, $p = 0.0849$) and nest initiation day (GLM, slope $= 0.5110$, $F_{(1,23)} = 2.6515$, $p = 0.1177$).

Discussion

Extra-pair paternity

Extra-pair paternity was confirmed in 19 of 204 (9.3%) non-parasitic young. One or more extra-pair young was detected in 12 of 25 analyzed nests (48%); these values are slightly higher compared with a study that was based on allozyme markers (Evarts and Williams 1987). Nevertheless, our estimates are consistent with those of Denk (2005).

Although the EPP rates that we observed in mallards do not exceed EPP rates in other waterfowl species, they are some of the highest rates that have been recorded in this group. EPP is supposedly absent in many waterfowl species, such as Barrow's goldeneye *Bucephala islandica* (Eadie et al. 2000) and blue duck *Hymenolaimus malacorhyncos* (Triggs et al. 1991), and estimates that are based on genetic methods have rarely exceeded 5%; 2–5% in Ross's geese *Chen rossi* and lesser snow geese *Chen caerulescens* (Dunn et al. 1999), and 4% in gadwalls *Anas strepera* (Peters et al. 2003). The black swan *Cygnus atratus* is an exception, 15% of extra-pair young (Kraaijeveld et al. 2004).

Breeding densities influence the opportunity for extra-pair copulation, and hence, intra-specific EPP rates in birds (Westneat and Sherman 1997). Nevertheless, it is unclear whether high breeding densities elevate EPP rates in waterfowl. Intra-specific data on the relationship between

breeding density and EPP rate are based primarily on species for which feeding and breeding sites overlap (Westneat and Sherman 1997). Waterfowl feeding sites, however, are often separated from nesting sites, and areas that are preferred as feeding sites are not necessarily suitable for nesting (Owen and Black 1990, Grand et al. 1997). Thus, even if the relation between EPP and density is maintained, it is possible that EPP rates are determined primarily by the densities of conspecifics that lie outside of breeding areas. Yet, the rates of assignment of individual extra-pair offspring to potential genetic fathers were relatively high in our study, indicating that locally breeding paired males are primary involved in EPP.

Mate guarding and frequent within-pair copulation are the predominant strategies for preventing paternity loss in waterfowl (Sorenson 1994, Cunningham 2003). Simultaneously, however, mate guarding can prevent males from seeking extra-pair copulation (Birkhead and Møller 1992). If such a phenomenon exists, we would expect a negative correlation between the occurrence of EPP and breeding synchrony (Dunn et al. 1999), due to within-season variations in the proportions of males that are constrained by the guarding of fertile females. Yet, the empirical support for this hypothesis in waterfowl is mixed (Dunn et al. 1999, Peters et al. 2003). Our data do not indicate robust constraint against the male search for EPP as a result of mate guarding, because the correlation between the proportion of extra-pair young in the nest and breeding synchrony or nest initiation date was non-significant and the direction of this relationship is opposite than those predicted by the 'male constraint hypothesis'.

The distribution of extra-pair fertilization in our population did not deviate from the bionomial distribution (i.e. null expectation). A non-random distribution of EPP suggests that systematic processes govern its occurrence, and thus, individual variation in the probability of achieving extra-pair copulation or losing paternity in one's own nest (Bateman 1948, Møller and Ninni 1998).

Although this issue has evolutionary implications, only a few studies have tested it in waterfowl. In particular, EPP in the black swan *Cygnus atratus* is frequent; yet, it does not contribute markedly to variations in the reproductive success of males (Kraaijeveld et al. 2004). Similarly, extra-pair mating success (measured as extra-pair copulation frequency) does not correlate with social rank or phenotype in mallard males – characteristics that affect pairing success with social females (Cunningham 2003). Consistently with these studies, our data, despite the limited sample size, suggest that there is relatively low variation in the male's ability to achieve extra-pair copulation and lose paternity in his own nest and/or that there is low variation in the female's ability to resist extra-pair copulations.

Conspecific brood parasitism

The genotypes of 10.1% of the young that we analyzed (23 of 237) did not match the genotype of their putative mother, i.e. CBP was confirmed. At least one CBP young was found in 6 of 25 nests that were analyzed (24%).

CBP has been observed in more than 40% of waterfowl species (Geffen and Yom-Tov 2001), although direct estimates that are based on genetic data are rare (Åhlund and Andersson 2001, Kraaijeveld et al. 2004, Nielsen et al. 2006). Several hypotheses, such as competition for nest sites and the detectability of nests by potential hosts, have been proposed to explain inter-specific differences in the prevalence of CBP in waterfowl (Sayler 1992, Geffen and Yom-Tov 2001, Lyon and Eadie 2008). CBP rates are generally high in cavity-nesting species, among which competition for nest sites is expected to be high, and in colonial and semi-colonial species, for whom the opportunity to find a suitable host nest is also high (Beauchamp 1997).

In contrast, the occurrence of CBP is lower among ground-nesting dabbling ducks of tribe *Anatinae* (Sayler 1992), because they typically breed at low densities in the grasslands that surround bodies of water. Particularly in mallard, CBP has been estimated to occur in 0–10% of nests. (Bengtson 1972, Duebbert et al. 1983). Similarly, low CBP rates have been reported for other ground-nesting dabbling duck species (Peters et al. 2003, reviewed by Sayler 1992). CBP rates in our mallard population, in which nests aggregated at high densities on artificial islands (55 nests across 12 000 m^2 of artificial island during the breeding season) exceeded these estimates, however, indicating that the tendency to adopt the CBP tactic is highly flexible within species and that it depends on environmental factors, such as the arrangement of the nesting habitats (Semel et al. 1988). But, we can not exclude the possibility that our sampling strategy biased our estimates of CBP rates compared with the true population mean, due to nest predation. Specifically, CBP rates can be over-estimated if parasitic females prefer nests that are less likely to be detected by predators (Pöysä 2003, Pöysä and Pesonen 2007). Conversely, if the detection of clutches by parasitic females relies on similar clues that are used by nest predators, CBP rates will be under-estimated.

We succeeded in assigning a CBP young to a parasitic female in only one case. There was an apparent discrepancy between the low and relatively high assignment of CBP vs EPP young, respectively, to genetic parents. This finding suggests that non-breeding females contribute primary to nest parasitism ('best of a bad job' hypothesis, sensu Sorenson 1991) and/or that our study location was visited by non-resident breeding females that were searching for available host nests.

Although the negative consequences of CBP appear to be low in waterfowl (reviewed by Lyon and Eadie 2008), CBP might be associated with decreased hatching success and prolonged incubation periods, which often affect host breeding performance (Dugger and Blums 2001, Nielsen et al. 2006). In mallards, the adverse effects of brood parasitism seem to be low or moderate. Extremely enlarged clutches due to CBP (>25 eggs in one nest) did not occur frequently in our population, and moderately enlarged clutches (15–21 eggs in a clutch, possibly due to CBP) have successful hatch rates that are comparable with non-CBP clutches (Kreisinger unpubl.). Further, our data did not indicate a clutch size reduction that was laid by the host female in response to CBP.

Our data suggest that there is individual variation in the probability of being parasitized, because parasitic events were not distributed randomly throughout the population.

Several non-exclusive processes might have contributed to this result. First, parasitic females might have chosen nests that were easily detectable or accessible. Notably, the choice of host might have been affected by knowledge of the host's quality or the quality of its nest site (Pöysä and Pesonen 2007). Finally, potential hosts might have varied in their ability to defend against parasitic intruders. Unfortunately, our relatively limited sample size did not allow us to evaluate these possibilities quantitatively.

Conclusions and methodical considerations

This study shows that a non-invasive sampling overcomes some of the challenges that are associated with sampling live specimens and is a good alternative to more time-consuming approaches (Peters et al. 2003, Denk 2005). This non-invasive approach is suitable for obtaining data on CBP and EPP, as well as capture–recapture data, which are useful for estimating re-nesting rates, degree of breeding site fidelity, and inter-annual dispersal (Kreisinger et al. unpubl.).

Conversely, potential drawbacks of this methodology are worth noting. In particular, this method does not allow obtaining offspring DNA (i.e. eggshell membrane) from depretated clutches. Hence, in any association between the probability of nest predation and the occurrence of CBP (Pöysä 2003, Pöysä and Pesonen 2007), non-invasive methodologies might provide biased estimates of the mean CBP in a population.

Nevertheless, despite this limitation, non-invasive sampling is suitable for estimating individual reproductive success. The combination of non-invasive sampling and other methods, such as protein fingerprinting (Andersson and Åhlund 2001) and estimation of CBP based on egg morphology (Pöysä et al. 2009), might in part overcome these drawbacks.

Although our data do not appear to be influenced by problems that might have arisen in non-invasively collected samples, such as allelic drop out, allele misprinting, and DNA contamination. But, this approach should be carefully applied to other waterfowl species, because the conditions that promote DNA degradation, such as nest humidity, vary interspecifically.

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