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## Can intraspecific brood parasitism be detected using egg morphology only?

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Intraspecific brood parasites lay their eggs in the nests of conspecifics. There are a number of methods for detecting intraspecific brood parasitism (IBP) in birds based on egg morphology. Here we test Eadie's (1989) method, which calculates the Euclidean distances between eggs in a given clutch in a three-dimensional space (weight, length and width). A parasitised clutch is predicted to contain an egg (or eggs) that is significantly different from the clutch's other eggs. Data from three species were analysed. Our captive zebra finch *Taeniopygia guttata* clutches did not include any instances of IBP, the wild jackdaw *Corvus monedula* data were unlikely to contain any, and for the goldeneye *Bucephala clangula* data set we had an observational estimate of IBP. We simulated IBP in the zebra finch, jackdaw and goldeneye data to test whether the method reliably detects an experimentally 'parasitised' clutch. We show that the distributions of the test statistics greatly overlap in 'parasitised' and unmodified clutches, and are dependent on the clutch size. We therefore conclude that the method can only be used with caution, after calibrating it for a given population.

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Intraspecific brood parasitism (IBP) or egg dumping is an alternative reproductive strategy in which females lay their eggs in nests belonging to other individuals of the same species. Once thought to be relatively rare, molecular techniques have revealed that in a number of species IBP has the potential to heavily impact on individual reproductive success (reviewed in Arnold and Owens 2002). Molecular techniques can, however, be intrusive, since blood or tissue samples are needed, as well as expensive. If an alien egg in an avian clutch could be detected reliably with a fast, cheap and non-intrusive method, this method would be in great demand. An

example of such a method based on egg morphology has been developed by Eadie (1989) to detect parasitised clutches in goldeneyes, *Bucephala clangula* and *B. islandica*. Eadie's (1989) method, later used by Pöysä (1999, Pöysä et al. 2001), utilises egg measurements only. It is therefore quick, easy and cheap, but is it reliable and generally applicable to other bird species?

Here, we test Eadie's method for detecting IBP by using egg morphology data from three different species, the zebra finch *Taeniopygia guttata*, jackdaw *Corvus monedula*, and goldeneye *Bucephala clangula*. These three data sets should provide a good test of this method. The zebra finch data originate from a captive breeding experiment in which pairs were housed separately, so no IBP could occur. We know that egg dumping does occur in this species in the wild, with molecular analyses showing that 36% of nests contained chicks that were unrelated to both parents (Birkhead et al. 1990). This IBP will have large fitness costs for the hosts, thus it has been suggested that in response, females have evolved to lay eggs of a uniform size because this makes alien eggs easier to detect (Zann 1996). In wild jackdaws in the UK, the IBP rate in a nestbox population was shown to be zero using molecular techniques (Henderson et al. 2000). The absence of IBP in the jackdaw and zebra finch datasets provided a good test of the performance of the method as we could then randomly move eggs among clutches to simulate brood parasitism. Then, we analysed a data set from a Scottish population of common goldeneyes, in which IBP was known to have occurred. These data were compared to those found in common goldeneye populations studied by Eadie (1989) in North-America and by Pöysä (1999) and Pöysä et al. (2001) in Finland. Finally, the IBP simulation was repeated using our goldeneye data.

## Material and methods

### Eadie's method for detecting parasitised clutches

The method Eadie (1989), see also Pöysä 1999) developed to detect parasitised clutches is based on egg morphometrics (length, width and weight). To test whether a clutch contained parasitic eggs, the measurements of all the eggs were converted to  $z$ -scores by standardising each measurement to zero mean and unit variance across the population (e.g. Sokal and Rohlf 1995). Using these  $z$ -scores, the similarity between any two eggs in the clutch were calculated as a regular m-space Euclidean distance,  $d_{ij}$  (e.g. Davis 1986):

$$d_{ij} = \sqrt{\sum_{k=1}^m (X_{ik} - X_{jk})^2} \quad (1)$$

where  $X_{ik}$  was the  $k$ th variable on egg  $i$ , and  $X_{jk}$  denoted the  $k$ th variable measured on egg  $j$ , and  $m=3$  as three measurements were taken on each egg.

For a clutch of  $N$  eggs the number of these distances ( $d_{ij}$ ) equalled  $N(N-1)/2$ . The greatest of the distances, i.e. the one between the two most dissimilar eggs in the clutch, was the maximum Euclidean distance (MED). Eadie (1989) used the MEDs to distinguish between parasitised clutches (revealed by large MEDs) and non-parasitised clutches (small MEDs) in a population of Barrow's goldeneye *Bucephala islandica* and goldeneyes in British Columbia, Canada. He concluded that a cut-off point of 2.5 in MED of a given clutch was an objective criterion to identify parasitised clutches in goldeneyes. Specifically, MEDs above 2.5 were a sufficient signature of parasitism. Pöysä (1999) and Pöysä et al. (2001) used the same method to identify parasitised clutches in three goldeneye populations in Finland. They suggested, however, that a higher cut-off point of 3 should be used to identify parasitised clutches.

### Egg morphology data

The zebra finch data were from a captive population held at the University of Glasgow and were collected in January–February 2001. Each breeding pair was housed in a separate cage so that no IBP could occur. The number of clutches used was 56 (clutch size  $\bar{x}=4.11$ ,  $SD=1.53$ ). Zann (1996) concluded that the variance in egg size within females was consistent and that between females was significantly different in both wild and captive zebra finches. So, we were confident that the absence of egg dumping in this population did not increase within-female egg variance, or that the relatively high quality diets in captivity did not decrease the within-clutch variance in egg size.

The jackdaw data were from a nestbox population in Wytham Woods, Oxfordshire, UK. The data were

collected during one breeding season (1986) and each egg was measured on the morning that it was laid. For further details on data collection, see Arnold and Griffiths (2003). We analysed 69 clutches (clutch size,  $\bar{x}=3.91$ ,  $SD=0.82$ ). Molecular parentage analyses have shown that the IBP rate in jackdaws is zero (Henderson et al. 2000), so we assumed that each clutch was laid by a single female.

The goldeneye population was breeding in Strathspey in Inverness-shire, Scotland. Between 10 March and 21 June 2002 a total of 25 occupied nest boxes were visited daily and the eggs were measured on the day of laying. In 14 of the 25 nest boxes (clutch size,  $\bar{x}=15.93$ ,  $SD=3.50$ ), IBP was observed, based on the egg laying interval. A nest was considered to be parasitised if more than one egg was laid in 24 hours (mean laying interval is 1.2 days for goldeneye), or if an egg was laid more than 6 days after the rest of the clutch. Eadie (1989) used the same criteria for goldeneye, and these criteria have also been used for other species (e.g. Arnold 1987, McRae 1997, 1998). By using these egg-laying interval criteria, seven of the clutches were considered to be non-parasitised (clutch size,  $\bar{x}=9.86$ ,  $SD=1.57$ ). In a further four of the nest boxes it was uncertain whether IBP had occurred or not, as daily checks of these nests were not possible (clutch size,  $\bar{x}=8.25$ ,  $SD=4.19$ ).

For all three species, clutches consisting of one or two eggs were excluded from the analyses as at least two eggs are needed to calculate the MED, and for any meaningful interpretation of the results. One person measured all the eggs within each species to ensure consistency.

### Simulation of IBP

To test whether Eadie's (1989) method could successfully detect parasitised clutches when it is not obvious that IBP has occurred, we simulated this form of IBP by modifying the original data. For each species we first calculated the MEDs of the original clutches according to equation 1. Then, we simulated IBP by moving one randomly chosen egg from each original clutch to another randomly chosen clutch within the data set. The MEDs of the parasitised clutches were then calculated. This was repeated 1,000 times for each data set. It should be noted that all the original goldeneye clutches were included in this simulation, regardless of their classification of being parasitised or not. This was done for three reasons: First, we were not 100% certain that we had detected all cases of IBP in the field. Second, the sample size would have been small otherwise, because only seven clutches were classified as non-parasitised. Finally, we wanted to demonstrate that in previously parasitised clutches further, simulated, parasitism increased the average MED value, albeit less than

when the original clutch was unparasitised (e.g. in zebra finches and jackdaws).

## Data analyses

For each species, we compared the MED distributions of the original ('non-parasitised') and the modified ('parasitised') clutches. For Eadie's (1989) method to work, the MEDs of the parasitised clutches should have been on average higher than those of the original clutches. Thus, we would have expected this change to be largest in zebra finch, followed by jackdaw, and to be smallest in goldeneye, based on the increasing probability that IBP has occurred. This comparison should also indicate where the possible cut-off point for each species should be. This comparison was done by bootstrapping. So, for each of the three species 1,000 random samples were drawn from the distributions of MEDs calculated for the simulated data sets. We kept the sample size of these bootstrapped samples the same as in the original data set (56, 69 and 25 for zebra finch, jackdaw and goldeneye, respectively). Calculating the lower 95% percentile of these bootstrapped distributions gave a 5% risk level with which to compare the median MED value calculated using the original data sets. If the median of the original data set was lower than the lower 95% percentile of the bootstrapped distribution, we could conclude that simulated IBP increased the median MED value significantly.

The clutch size of parasitised nests was predicted to increase in species that do not remove host eggs, so next we tested whether the MEDs increased with the clutch size as shown earlier by Eadie (1989), Pöysä (1999) and Pöysä et al. (2001). Finally, the MEDs of the goldeneye clutches known to have been naturally parasitised were compared to the MEDs of those clutches that were unlikely to have been parasitised.

## Results

The MED medians of zebra finches, jackdaws and goldeneyes increased with simulated brood parasitism (see Fig. 1 for distributions). As expected, the median MED of zebra finches increased the most, from 1.89 to 2.95 (56.1%), followed by the jackdaws, from 2.02 to 2.81 (39.1%). In goldeneyes, however, this increase was very small, from 4.41 to 4.55 (3.2%). The lower 95% values for the bootstrapped median MED values of the simulated data sets of each species were: 2.66 (zebra finch), 2.63 (jackdaw) and 4.01 (goldeneye). Thus, we can conclude that at the 5% risk level, both zebra finch and jackdaw medians were higher in the simulated data sets than in the original ones, but that there was no significant difference in the goldeneye.

It was possible that the median MED of the original clutches was not smaller than that of the artificially parasitised clutches simply because of smaller, and perhaps atypical samples of natural egg-size variation. However, bootstrapped medians of the original clutches,

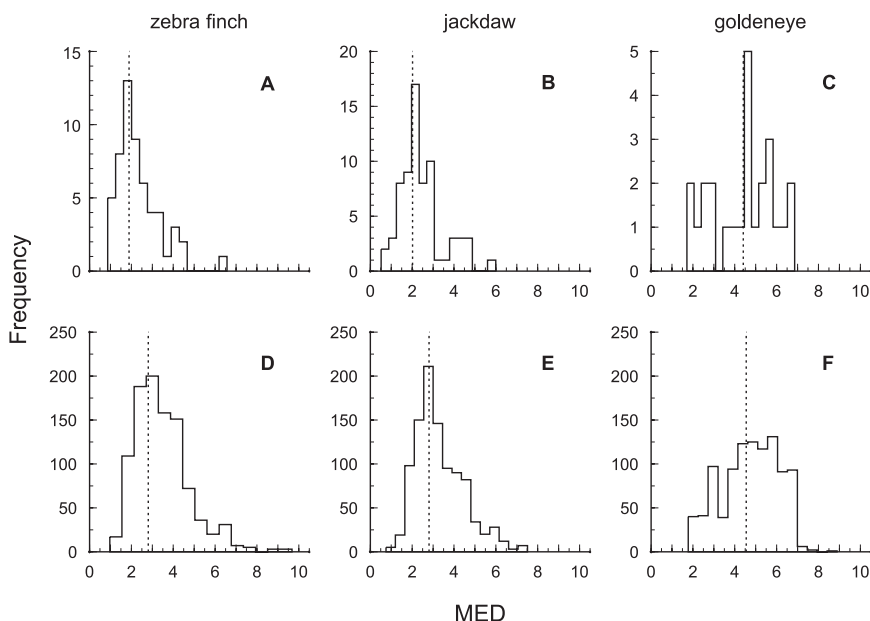


Fig. 1. The frequency distributions of the maximum Euclidean distances (MEDs) of the original clutches (A–C) and the simulated parasitised clutches (D–F) for zebra finches, jackdaws and goldeneyes, respectively. The superimposed dotted lines indicate the MED medians of each frequency distribution. Note the different scale of y-axis in different panels.

using 1,000 re-sampling rounds, were practically identical to the original estimates: zebra finch 1.91, jackdaw 2.03, and goldeneye 4.40 (original estimates, respectively, 1.89, 2.02 and 4.41).

The MEDs in all these species also increased with clutch size (Fig. 2; zebra finch  $r=0.38$ ,  $N=56$ ,  $P=0.004$ , jackdaw  $r=0.56$ ,  $N=69$ ,  $P<0.001$ , and goldeneye  $r=0.45$ ,  $N=21$ ,  $P=0.04$ , all these values were based on the original unmanipulated data). In the goldeneye, an analysis of covariance showed that brood parasitism, clutch size and their interaction all contributed to the clutch MED when they were included in the model (Table 1). These clutches, known to have been parasitised, had a slightly higher mean MED ( $\bar{x}=4.77$ ,  $SD=1.50$ ) than those clutches that were not believed to have been parasitised ( $\bar{x}=3.69$ ,  $SD=1.38$ ).

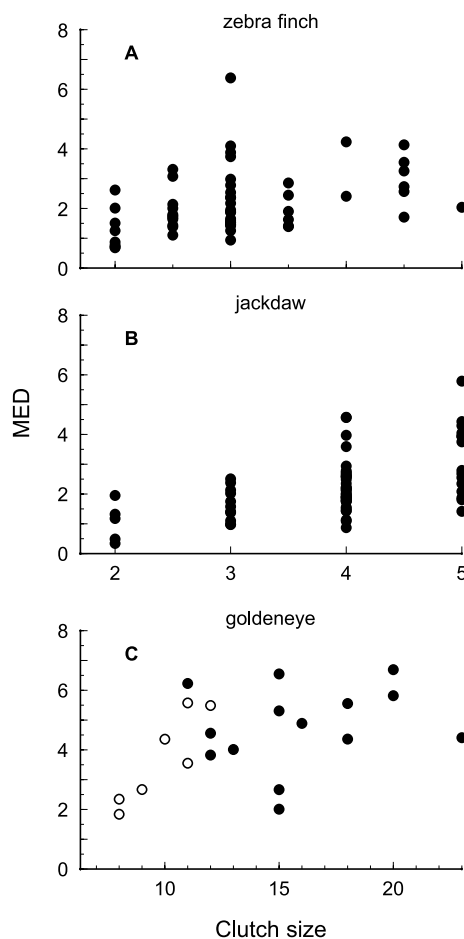


Fig. 2. The maximum Euclidean distance (MED) of the original clutches plotted against the clutch size in (A) zebra finches, (B) jackdaws and (C) goldeneyes. In goldeneyes, the filled dots indicate broods which were likely to have been naturally parasitised, and the empty dots show non-parasitised clutches.

## Discussion

Based on three different species, we have shown that Eadie's (1989) method for detecting intra-specific brood parasitism does not seem universally applicable. While the MEDs increased as expected when broods were artificially parasitised (Fig. 1), the resulting MED distributions of parasitised and non-parasitised clutches show considerable overlap. This means that it was difficult to set a reliable cut-off point for assigning an egg as parasitic. In practice, the within- and between-female variation in egg morphology in non-parasitised nests may not be known, and therefore cannot be easily estimated without using a molecular method to detect parasitism. In addition, if the brood parasite does not expel one of the host's eggs, then the clutch size will increase. However, a large MED can indicate a clutch with a large number of eggs, but not necessarily a parasitised clutch (see also Pöysä 1999), thus the MED cut-off point will be even more difficult to define.

Brown and Sherman (1989) pointed out that a method for detecting intra-specific brood parasitism based on differences between various egg traits necessarily relies on the assumption that between-female variation exceeds within-female variation. This seems to be common in birds, with about 70% of the variation in egg mass being due to between-female variation (Christians 2002). Importantly, our study species did not show atypical proportions of between-female variation (i.e. repeatability values, see Lessells and Boag 1987) compared to other bird species (Christians 2002; Table 1) for egg size, egg length, width and weight in zebra finch: 0.67, 0.60, 0.44; jackdaw: 0.50, 0.57, 0.49; goldeneye (only clutches classified as non-parasitised included): 0.04, 0.18, 0.15, respectively. It is interesting that the between-female variation in egg size estimated for goldeneyes was the lowest among the species examined, even though they probably had the highest rate of IBP. Thus, it is unlikely that our results were affected by abnormally low between-clutch variation in our data.

A comparison of our goldeneye results with those of earlier studies (Eadie 1989, Pöysä 1999, Pöysä et al. 2001) showed that the MED values reported here were rather similar. However, after considering the difference between the MED values of parasitised and non-

Table 1. Summary statistics of the analysis of covariance for the relationship between MED and clutch size in the naturally parasitised and non-parasitised goldeneye nests (see text for details).

Source	SS	MS	df	F	P
Clutch size	3.98	3.98	1	7.68	0.013
Parasitism (0 or 1)	2.77	2.77	1	5.35	0.034
Clutch size $\times$ parasitism	2.78	2.78	1	5.36	0.033
Error	8.81	0.52	17		



parasitised clutches, our results indicated greater overlap (Fig. 2C). It is possible that the methods used to identify parasitised nests in our study were not reliable enough. Andersson and Åhlund (2001) have shown, using protein fingerprinting, that brood parasitism could occur in two thirds of goldeneye nests – a proportion clearly higher than indicated by the methods used here. Another possibility is that the Scottish goldeneye population is atypical. It is rather small and marginal, consisting of only about 120 breeding pairs (Dennis and Pöysä 1997). This gives rise to the possibility that small population size, together with relatedness of hosts and parasites, and heritability in egg size (e.g. Christians 2002) creates less between-female variation than in other, larger populations. Indeed, as indicated by the relatively low repeatability values in our goldeneye data (cf. above), this could have been the case.

Taken together, our results support Eadie's (1989) conclusion that this method for detecting IBP in birds can only be applied with considerable caution. Calibrating the cut-off point for the MED measurement is demanding, and it is possible that at least in some cases clutch size has to be taken into account. It may be possible, that to make the initial calibration reliable, molecular techniques are needed, in which case the need for the MED based method is greatly reduced. Thus, we echo the cautionary tone of McRae (1997) on using egg morphology for detecting intraspecific brood parasitism.

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