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Supplemental Information

Hosts Improve the Reliability of Chick Recognition by Delaying the Hatching of Brood Parasitic Eggs

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Supplemental Experimental Procedures

Egg Size Difference Does Not Explain Delayed Hatching of Parasitic Chicks

We used linear regression to test for the combined effects of differences in egg size and relative egg incubation positions (e.g. time spent in central position; see Methods) on the difference in incubation lengths of host and parasitic eggs that were laid on the same day at the same nest (n = 19 pairs of eggs). Our initial full model showed no significant interaction between egg size and egg position, so the interaction term was dropped from further analyses. This two-factor regression model produced a significant fit ($F_{2,16}$ = 6.11, adjusted R^2 = 0.36, P = 0.01). We further tested for the effect of each predictor variable while controlling for the other covariate by using Wald tests. Both variables were significant: for a given pair of host and parasite egg, the egg that was more frequently in the center hatched relatively earlier (Wald X^2 = 5.24, P = 0.02), and the smaller egg hatched relatively earlier (Wald X^2 = 5.88, P = 0.02).

One remaining question is whether delayed hatching of parasitic eggs could be explained in part by the biased egg size—if parasitic eggs were larger relative to host eggs, this could delay their hatching. However, the pattern of relative egg size was opposite to this prediction: in 13 of 19 pairs of eggs, the host eggs were larger than the parasitic egg. Thus, the relative length of incubation period between pairs of host and parasitic eggs are better explained by relative egg position: host eggs are found in central positions of the clutch more often in 12 of 19 nests (no difference in 1 nest), and the degree of difference in egg position explains the degree of difference in incubation lengths (Figure 2b).

No Intrinsic Differences in Incubation Requirements

American coots are facultative brood parasites, and thus most parasitic females also lay eggs in their own nests. This allows us to further confirm that incubation length results from egg position effects rather than other intrinsic factors associated with the females laying the eggs. We asked whether females that use parasitism as a reproductive strategy tend to lay eggs that take longer to hatch than those of other females. One way to answer this question is to compare the lengths of incubation periods of eggs that known parasitic females lay in their own nest with the incubation period of other non-parasitic eggs in the population.

In 2005, we were able to identify 6 known parasitic females based on timing of egg-laying and visual matching of egg patterns by at least two people (refs for methods). We compared the incubation lengths of these females' eggs in their own nest (i.e., not parasitic eggs) with incubation lengths of eggs laid by 23 other females in their own nests. We only used eggs with exactly known laying date (N = 174 eggs). We built a general linear model with clutch size, position in the laying sequence, egg size, and female status (known parasite vs. non-parasite) as fixed-effect terms. Because the distribution of laying sequence was skewed towards earlier laying positions (fewer eggs in later laying sequence because few females lay very large clutches), we used a log-transformation. The response variable was the number of days from egg-laying to hatching. We then conducted Wald tests to determine the whether each fixed-effects term significantly affected incubation length.

Our analysis shows that clutch size, laying sequence and egg size all affected incubation length (Clutch Size: $X^2 = 32.6$, $P < 0.001$; Laying Sequence: $X^2 = 75.4$, $P < 0.001$; Egg Size: $X^2 = 4.8$, $P = 0.03$) but female status did not ($X^2 = 0.9$; $P = 0.34$). This shows that intrinsic differences in female quality between parasitic and non-parasitic females do not produce the difference in incubation lengths between host and parasitic eggs. Combined with the analysis showing egg position does affect incubation length, we feel confident that discriminatory incubation is a major factor delaying the hatching of parasitic eggs.

Our analysis does not address the question of whether parasitic females specifically lay parasitic eggs that require longer incubation periods. However, this scenario seems unlikely from an evolutionary perspective. If selection were to act on intrinsic incubation requirements of parasitic eggs, we would predict that parasitic eggs would be selected to hatch earlier, not later. We cannot test this idea with our current data set because all parasitic eggs are potentially subject to biased incubation. However, this factor could explain the tendency for parasitic eggs to be smaller.