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

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Abstract

The reliability of information that animals use to make decisions has fitness consequences. Accordingly, selection should favor the evolution of strategies that enhance the reliability of information used in learning and decision making. For example, hosts of avian brood parasites should be selected to increase the reliability of the information they use to learn to recognize their own eggs and chicks [1–3]. The American coot (*Fulica americana*), a conspecific brood parasite, uses cues learned from the first-hatched chicks of each brood to recognize and reject parasitic chicks [3]. However, if parasitic eggs are among the first to hatch, recognition cues are confounded and parents then fail to distinguish parasitic chicks from their own chicks. Therefore, hosts could ensure correct chick recognition by delaying parasitic eggs from hatching until after the first host eggs. Here we demonstrate that discriminatory incubation, whereby coots specifically delay the hatching of parasitic eggs, improves the reliability of parasitic chick recognition. In effect, coots gain fitness benefits by enhancing the reliability of information they later use for learning. Our study shows that a positive interaction between two host adaptations in coots—egg recognition and chick recognition—increases the overall effectiveness of host defense.

Results and Discussion

The ability to recognize offspring and other kin allows individuals to gain fitness benefits through investment in relatives [4–6]. Fitness benefits gained from such investment depend critically on the accuracy of recognition. Any investment directed toward unrelated individuals is wasted with respect to inclusive fitness, and thus selection should favor recognition cues that maximize reliability [7]. In a variety of taxa, kin recognition cues are learned from referent individuals and then applied to other individuals (e.g., insects [8, 9], fish [10], birds [11], and mammals [12]). Learning provides a simple and fairly reliable mechanism for acquiring recognition cues, but it also comes with the risk of errors when individuals learn cues from incorrect referents. For example, parents whose nests contain unrelated offspring (e.g., brood parasite hosts) could mistakenly learn unrelated offspring as their own [1, 3]. Thus, the reliability of information that an animal learns—that is, the likelihood that referent individuals are actually genetic offspring—affects the fitness gained from recognition and, in some cases, even influences whether learned recognition is adaptive at all [1]. An intriguing possibility is that selection could favor behaviors that manipulate the reliability of the information used during learning in a manner that benefits the learner. Of course, this does not imply that the animal is aware of the fitness consequences of its actions, but rather that selection favors behaviors that provide fitness benefits through more reliable recognition. Although theoretically expected, empirical examples of behaviors that alter the reliability of information are currently lacking. Convincing evidence for adaptive information manipulation in the context of learning would require two lines of evidence: first, that a behavior affects the reliability of information used during learning, and second, that altering the reliability of the information affects fitness.

Avian brood parasites and their hosts provide a model system for examining how the reliability of the learning process involved in recognition connects to fitness. Brood parasites lay eggs in the nests of other individuals (hosts) and leave these hosts to provide all care to the parasitic offspring. The high costs of brood parasitism have frequently led to the evolution of host defenses such as recognition and rejection of the parasitic eggs [13–16] and, more rarely, chick recognition and rejection [3, 17–19]. Host defenses such as egg and chick recognition often involve learned recognition cues [1–3, 20]. However, errors in this learning process can lead to acceptance of brood parasitic eggs or chicks, or even rejection of the host's own eggs or chicks. For example, hosts of brown-headed cowbirds (*Molothrus ater*) can be experimentally induced, through learning errors, to accept nonmimetic parasitic eggs [2, 20, 21]. With respect to chick recognition, Lotem [1] suggested that the risks and costs of misimprinting on parasitic chicks during the development of a recognition template could prevent the evolution of chick recognition in hosts of some brood parasites. This "cost of misimprinting" hypothesis suggests an important corollary: for chick recognition to be a beneficial strategy, hosts must have reliable learning mechanisms. We also expect natural selection to favor any host behaviors that improve the reliability of the learning mechanisms used; however, this hypothesis has received little empirical or theoretical attention.

Here we show that in the American coot, a species with frequent conspecific brood parasitism, hosts employ behaviors that alter the reliability of the information used to learn to recognize parasitic chicks. Within-species brood parasitism occurs at a high frequency in some coot populations, and successful parasitism is very costly to hosts [22, 23]. American coots exhibit multiple lines of defenses to reduce these costs, including both parasitic egg rejection [16, 24, 25] and parasitic chick rejection [3]. Egg recognition is based on visual characteristics, and over 40% of parasitized hosts reject parasitic eggs by burying them in the nest lining [16]. However, egg recognition does not always lead to direct rejection: some parasitic eggs remain in the nest but spend a disproportionate amount of time at the periphery of the clutch (Figure 1), which delays their hatching ("discriminatory incubation"; [16]; see below). Why

hosts do not reject these eggs outright is unclear; one possibility is that hosts are less certain about the identity of these eggs, and errors with discriminatory incubation are less costly than mistaken rejection of the hosts' own eggs. Host defense continues after hatching; parasitic chicks suffer significantly lower survival compared to host chicks as a result of chick recognition and active chick rejection by the host parents [3]. A series of cross-fostering experiments demonstrated that successful chick recognition is learned and depends critically on the identity of the chicks that hatch on the first day, the sensitive period when parents apparently learn cues for recognition [3]. When only host chicks hatch on the first day of hatching, parents learn correct recognition cues and are able to subsequently recognize and reject many parasitic chicks that appear later in the hatching sequence. However, if parasitic chicks hatch with host chicks on the first day, the hosts learn both the host and parasitic chicks as their own and accept all later-hatched chicks. Finally, when parents are exposed only to parasitic chicks on the first hatching day, they then mistakenly imprint on these chicks and pay a cost of misimprinting: they reject their own chicks that hatch later [1–3]. Therefore, the types of chicks that hosts encounter on the first day of hatching—a pure set of their own chicks, a mix of their own and parasitic chicks, or a pure set of parasitic chicks—determine whether hosts are able to reject parasitic chicks, accept both their own and parasitic chicks, or, worse, mistakenly reject their own chicks. Because first-hatched chicks are used as referents, hosts could improve the accuracy of chick recognition by positioning parasitic eggs in inferior incubation positions and thus delaying their hatching. We tested this hypothesis by measuring the effect of discriminatory incubation on the hatching patterns of host and parasitic chicks at naturally parasitized nests.

Most birds regularly shuffle the positions of the eggs in their clutch, and the incubation temperatures of eggs vary with location in the nest [26–28]. Some species take advantage of the temperature gradient in clutches and use preferential incubation positions to influence hatching patterns; to achieve these effects, parents actively control the amount of time that different eggs spend in the warmer central incubation positions [29, 30]. In coots, previous studies based on data collected from 1987 to 1990 showed that hosts use such positional effects to specifically target parasitic eggs [16, 25]. In both naturally parasitized nests and artificial parasitism experiments, parasitic eggs are actively pushed to cooler, outer positions in the clutch (Figure 1) [16, 25]. Parasitic eggs also take longer to hatch than host eggs in naturally parasitized nests [16]; we did not monitor incubation length in the experimental parasitism study [25]. However, parasitism rates and host responses to parasitic eggs vary among years and sites [31]. In the present study, we first confirmed that the same patterns of delayed hatching of parasitic eggs occurred during 2005–2008. Parasitic eggs had longer incubation periods than host eggs after controlling for laying order and egg size (mixed-effects model with nest as random factor: likelihood $X^2 = 12.8$, $p < 0.001$). As a result, parasitic eggs hatched significantly later than host eggs that were laid on the same day (Figure 2A; paired t test: mean difference = 1.0 days, $t_{21} = 3.5$, $p = 0.002$). The difference in the length of incubation period between host and parasitic eggs laid on the same day was predicted by two factors: (1) difference in amount of time spent in central incubation positions and (2) difference in egg size (linear regression: $F_{2,16} = 6.1$, adjusted $R^2 = 0.36$, $p = 0.01$). For a given matched pair of eggs,

Figure 1. Photo of a parasitized nest. The two parasitic eggs, indicated with p, are kept at inferior incubation positions at the outer edge of the clutch by the incubating bird.

the egg that was observed in central incubation positions more often (usually the host egg) hatched earlier, and this effect was independent of differences in egg size (Figure 2B; Wald $X^2 = 5.24$, $p = 0.02$; see Supplemental Experimental Procedures). We found no evidence that parasitic females lay eggs that inherently required longer incubation periods (Supplemental Experimental Procedures). In summary, parasitic eggs were found less often in the center of the clutch compared to host eggs, and this translated into a delay in hatching of the parasitic eggs, thus confirming the effects of discriminatory incubation in this population [16].

We determined whether discriminatory incubation would affect the reliability of information available for learned chick recognition by comparing observed hatching patterns with those predicted by egg-laying patterns. We used a simple assumption to predict when eggs should have hatched: that, all else equal, host and parasite eggs would have the same length of incubation period. This assumption is supported by the observation that parasitic females' eggs laid in their own nests do not differ in incubation time from nonparasitic females; the eggs are not intrinsically different (Supplemental Experimental Procedures). Given this, for each nest, we observed which host eggs hatched on the first day of hatching (i.e., the sensitive period when recognition cues are learned), and we predicted that all parasitic eggs laid along with these host eggs should also have hatched on the first hatching day, in the absence of effects of discriminatory incubation. We then compared this predicted hatching pattern of parasitic eggs with the observed hatching pattern. The longer incubation periods of parasitic eggs substantially increased the probability that only host chicks (i.e., correct referents) hatched on the first hatching day of the brood (Figure 3; G-test with Williams' correction: $G = 15.1$, df = 2, $p < 0.001$, n = 60 nests). More nests hatched only host chicks on the first hatching day than predicted based on egg-laying patterns (Figure 3; predicted: 39 of 60 nests; observed: 52 of 60 nests). This increase in nests with reliable referents was a result of 13 nests in which only host chicks hatched on the first day despite the expectation that a parasitic chick would hatch along with them. Only seven nests actually hatched both host and parasite chicks on the first day, whereas 20 nests were predicted to do so based on laying patterns (Figure 3). One nest hatched a single parasitic chick on the first day (Figure 3), and this was be-

Figure 2. Effect of discriminatory incubation on the incubation periods of host and parasitic eggs laid on the same day. (A) The difference between the length of the incubation period for host and parasitic eggs laid on the same day at the same nest (n = 22 pairs of eggs). Gray bars indicate the number of times the parasitic egg hatched ahead of the host egg, the white bar indicates the number of times the parasitic and host eggs hatched on the same day, and black bars indicate the number of times the host egg hatched first.(B) The differences in length of incubation period between matched pairs of host and parasitic eggs (n = 19 pairs of eggs for which we had egg position data) correspond with the relative frequency with which each egg was found in the center of the clutch. We plotted the raw data (not corrected for differences in egg size), and the line fit is based on a simple linear regression ($F_{1,17}$ = 4.9, adjusted R^2 = 0.18, p = 0.04). See Results and Discussion for full statistical analysis. Two overlapping points have been jittered along the vertical axis for clarity.

cause all of the host eggs that were laid before or with the parasitic egg failed to hatch at all.

Delaying the hatching of parasitic chicks with discriminatory incubation has at least two benefits. First, it causes parasitic chicks to hatch later in the hatching order than they would otherwise, which reduces their survival because later-hatched chicks generally suffer higher mortality [32]. Because each surviving parasitic chick costs the parents one of their own chicks [23], the reduced survival of parasitic chicks increases host chick survival. The second benefit of discriminatory incubation—enhanced reliability of chick recognition—results from the particular chick recognition mechanism of coots. Hatching spans several days (typically 3–8 days) at each nest, and hosts imprint on characteristics of the chicks that hatch on first day of hatching [3]. Delayed hatching eliminates many parasitic chicks from this pool of referents, and coots therefore gain an informational benefit by manipulating hatching patterns. A reliable pool of referents enables hosts to potentially reject all parasitic chicks in the brood, and because many parasitized host nests contain multiple parasitic eggs (50% of parasitized nests; mean number of parasitic eggs per nest = 2.1; maximum number of parasitic eggs = 15), the potential payoff for correct recognition should be large. We cannot determine which of the two benefits of discriminatory incubation, hatch order or enhanced information for learned chick recognition, was the primary factor in its evolution. However, it is clear that improved information for chick recognition yields fitness benefits and would contribute to the evolutionary maintenance of discriminatory incubation as a host defense.

Chick recognition and rejection is a rare host defense strategy [33, 34], and a key question is whether the enhanced reliability of referent chicks due to discriminatory incubation would have been necessary for the initial evolution of chick rejection. Such a stepping stone scenario is feasible because discriminatory incubation enhances fitness independently of chick recognition by handicapping survival of parasitic chicks even without recognition, and it thus could have preceded the evolution of chick recognition. However, fitness estimates suggest that discriminatory incubation would not have been a necessary precondition for the evolution of chick

Figure 3. Increase in Reliability of a Chick Recognition Template as a Result of Discriminatory Incubation. Black bars show the predicted proportion of parasitized nests (n = 60) predicted to hatch only host eggs, a mix of host and parasitic eggs, or only parasitic eggs on the first hatching day, based on the assumption that host and parasitic eggs laid on the same day hatch on the same day. White bars show the observed proportions of parasitized nests with host-only, mixed, or parasite-only templates, based on the types of chicks (host or parasite) hatching on the first day. More nests hatched only host chicks than predicted, based on laying sequence.

recognition; chick recognition would have been a favorable host strategy even in the absence of discriminatory incubation. For example, the effects of discriminatory incubation on hatching patterns can be removed by examining the hatching patterns predicted from egg-laying patterns alone, as we have done (Figure 3). This analysis indicates that without discriminatory incubation, 65% of all hosts that hatch parasitic chicks would hatch only their own chicks on the first day of hatching, compared to a mere 2% that would hatch only parasitic chicks on the first day. The remaining nests, in which parasites are predicted to hatch along with the first-hatched

host chicks (33%), would be neither better nor worse off than hosts that indiscriminately accept all chicks. Thus, even without the benefits of discriminatory incubation, the majority of hosts would enjoy the benefits of correct chick recognition while very few would suffer the costs of misimprinting solely on parasitic chicks [1].

The unique combination of two different major host defenses in American coots—egg recognition and chick recognition—contrasts with host defenses observed in other brood parasite hosts. Many hosts are able to recognize and reject parasitic eggs, but few hosts can recognize and reject parasitic chicks [33, 34]. Notably, the three cuckoo hosts now known to recognize parasitic chicks, superb fairy-wrens (*Malurus cyaneus*), large-billed gerygones (*Gerygone magnirostris*), and mangrove gerygones (*Gerygone laevigaster*), all lack the ability to recognize and reject parasitic eggs [17–19]. One influential hypothesis to explain the rarity of hosts that use both egg and chick recognition is that once one effective host strategy evolves, it reduces the potential benefits that can be gained through other lines of defense, and hence selection for secondary defenses is diminished [34–36]. However, our study shows that egg recognition and chick recognition can coexist as complimentary host defenses. Whether separate lines of defense interact negatively or positively is likely to depend on the specific natural history context (e.g., relative hatching patterns) and the mechanisms of recognition that hosts employ.

Our analysis is an extension of the idea that a key factor in the evolution of host strategies is access to reliable information that allows hosts to reduce costly errors associated with host defenses [1]. Brood parasite hosts often make adaptive use of information to reduce error rates of defensive strategies such as egg rejection. For example, a host can collect information about the risk of parasitism through direct experience during early stages of nesting [37–39] or through social learning [40] and then adaptively adjust rejection rates to reduce the risk of costly rejection errors [38, 41]. Our study provides the first evidence that host behavior in one context (e.g., incubation) can directly improve the reliability of information that they use at a later stage (e.g., chick recognition). More generally, we show that the fitness benefits of learning can be enhanced as a consequence of the actions of the learner itself. Future models of host parasite coevolution may need to consider mechanisms of information acquisition and information manipulation as additional components of host strategies.

Experimental Procedures

Nest Monitoring and Detection of Parasitic Eggs

We monitored nests on several wetlands near Williams Lake, British Columbia from 1987 to 1990 (417 nests) and from 2005 to 2008 (284 nests). We monitored each nest every 1–4 days during the egg-laying period, depending on site and year. On each visit, all new eggs were given a unique number with a permanent marker, and their lengths and widths were measured using calipers. We then calculated egg size (volume) using the equation egg size = $0.51 \times \text{length} \times \text{width}^2$ [42]. We employed widely used demographic techniques to detect parasitism [22, 43, 44]. We detected most parasitism by the appearance of more than one new egg per day (coots lay daily) and then visually compared features such as egg color and spotting patterns to determine which of the new eggs were laid by brood parasites. New eggs laid after a skip in laying of 2 or more days were also considered to be instances of brood parasitism, and these late-laid eggs also differed in appearance from the rest of the eggs in the nest. The accuracy of these methods has been independently verified both by statistical tests using egg features [32] and by DNA fingerprinting [23]. In 2005–2008, all eggs were taken from the nest once pipping commenced (i.e., the shell first showed signs of cracks as the chick in-

side began the hatching process) and were hatched in incubators. Nests were checked daily during the hatching period, and for all eggs (i.e., those that hatched in a nest and those that hatched in an incubator), the first day the chick was observed completely out of the egg was considered the hatching day when calculating the length of the incubation period.

Statistical Analyses

To compare the hatching patterns of host and parasitic eggs, we collected egg-laying and hatching sequence data from the same population in 2005–2008 (n = 15 nests, 148 eggs) as was studied previously (1987–1990: [16, 25]). We constructed a mixed-effects model with nest as random factor and with position in the laying sequence, egg size, and egg type (host versus parasite) as fixed effects. The response variable was the length of incubation period, defined as the number of days between the laying and hatching of an egg. Because incubation begins partway through the laying period, eggs laid early in the laying sequence will have longer incubation periods, as defined here. Because there was no interaction between the fixed-effects terms, we eliminated these interaction terms in further comparisons. We then conducted likelihood ratio tests to determine the relative effects of each fixed term on the length of incubation period. We also conducted a paired t test to more directly compare the effect of discriminatory incubation on the relative incubation lengths of 22 pairs of host and parasitic eggs from the 2005–2008 sample that were matched pairs—i.e., laid on the same day in the same nest.

To confirm that the observed differences in incubation lengths between host and parasitic eggs laid on the same day were due to egg positioning effects, we conducted a separate analysis examining the relationship between the differences in egg positions at a nest for a given matched pair of host and parasitic eggs and the difference in hatching times for those same eggs. A previous experimental study using foreign eggs (i.e., eggs that were not laid parasitically but obtained from a second nest) showed that egg recognition by hosts, and not intrinsic properties of parasitic eggs, determines relative frequencies with which host or parasitic eggs occupy central positions in the clutch [25]. During 2005–2007, we revisited active nests several times during egg incubation (range 6–23 times) and visually assessed which eggs were in the center of the clutch of eggs (defined as approximately 3/4 of the egg surrounded by other eggs). We did not make enough revisits to nests in 2008 for this analysis. For each matched pair of parasitic and host eggs laid on the same day (n = 19 pairs of eggs at 14 nests), we compared the number of times each egg was found in the center of the clutch throughout the incubation period (eggs were either in the center or on the outside). We calculated the difference between the number of times the host egg was found in a central position and the number of times the parasitic egg was found in a central position and divided this value by the number of nest visits, which creates a relative index of egg position independent of the total number of visits. Positive values of this index occurred when the host egg was in the center of the clutch more often than the parasitic egg, whereas a negative value indicates that the parasitic egg was in the center more often. A value of zero indicated that both host and parasitic egg were found in central positions equally often at a given nest. In addition to this relative egg position index, we also calculated the difference in egg size and used both of these as covariates in a linear regression model to test how they affected the relative incubation period (host egg incubation length minus parasite incubation length). This allowed us to conduct a Wald test to determine the effects of egg position on incubation length while controlling for any egg size effects. The effect of egg size on incubation length is presented in the Supplemental Experimental Procedures.

We quantified the effect of discriminatory incubation on reliability of chick recognition using 60 nests in which at least one parasitic egg survived to hatching and for which we had accurate information for both the egg-laying sequence and the hatching sequence (n = 33 nests for 1987–1990; n = 27 nests for 2005–2008). At one nest, we inferred hatching patterns based on extensive pipping of the first three eggs (the nest was not rechecked for 2 days, so the actual hatching pattern was not observed). In all cases, we predicted that parasitic eggs should have hatched on the same day as the host egg laid on the same day. We used a G-test (or log-likelihood ratio test) to compare the predicted and observed frequency of nests that hatched host chicks only, a mix of host and parasitic chicks, or only parasitic chicks on the first hatching day. We used a Williams' correction because of small sample size [45].

All statistical analyses were conducted using R version 2.9.2.

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Supplemental Information

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of Chick Recognition by Delaying

the Hatching of Brood Parasitic Eggs

Daizaburo Shizuka and Bruce E. Lyon

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.