

Egg puncturing by the brood parasitic Greater Honeyguide and potential host counteradaptations

Claire N. Spottiswoode^{a,b} and John F. R. Colebrook-Robjent^c

^aDepartment of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK,

^bDST/NRF Centre of Excellence at the Percy FitzPatrick Institute, University of Cape Town, Rondebosch 7701, South Africa, and ^cMusumanene Farm, PO Box 630303, Choma, Zambia

The brood parasitic Greater Honeyguide *Indicator indicator* frequently punctures one or more host eggs when laying its own. We investigated variation in egg puncturing to test the hypothesis that this behavior is adjusted adaptively and selects for host defenses in a coevolutionary manner. We first show that puncturing was indeed effective in reducing the number of host young that the honeyguide hatchling would have needed later to kill or to outpace in embryonic development. Within clutches, thicker shelled and rounder host eggs were more heavily punctured, implying that they were more difficult to damage effectively and that puncturing could exert selection on host egg properties. Moreover, host females laying clutches of relatively thick-shelled eggs were more likely to raise at least one of their own offspring successfully in spite of parasitism, implying selection in progress. We provide anecdotal evidence that heavily punctured clutches are sometimes deserted, suggesting a trade-off between the benefits of puncturing and this possible cost. Our data also support 2 consequent predictions: honeyguides should puncture clutches more intensely when laying late relative to the host and when parasitizing hosts with hatchlings likely to provide a more robust adversary to their own. Selection on host eggshell properties was also implied by honeyguide host species having thicker eggshells than congeneric nonhost species. Although correlational, these findings are consistent with the hypothesis that honeyguides modify their puncturing behavior in an adaptive manner and that their hosts are experiencing selection for laying thicker shelled and possibly more rounded eggs. *Key words*: brood parasitism, coevolution, egg shape, eggshell thickness, honeyguides, selection. [*Behav Ecol*]

Nestlings of the brood parasitic Greater Honeyguide *Indicator indicator* (Piciformes, Indicatoridae) are typically raised alone, thus monopolizing the parental care of their mainly Coraciiform and Upupiform hosts. Two behaviors contribute to this situation: first, the laying female honeyguide usually punctures some or all of the host eggs, presumably using either her bill or her claws (Friedmann 1955). So far as is definitely known within the Indicatoridae, egg puncturing is unique to the Greater Honeyguide, although similar behavior appears to have independently evolved at least twice in other brood parasitic lineages, in the Great Spotted Cuckoo *Clamator glandarius* (Soler 1990; Soler et al. 1997) and in the cowbirds *Molothrus* spp. (Icteridae) (e.g., Hoy and Ottow 1964; Carter 1986; Massoni and Reboreda 1999). Second, the honeyguide hatchling is equipped with a sharp hook on the tip of its bill, with which it kills any host hatchlings to have escaped or survived puncturing at the egg stage (Friedmann 1955; Ranger 1955); this behavior is common to all the members of the Indicatoridae for which hatchlings have been observed (Short and Horne 2001). This paper concerns the first of these 2 phenomena, which we hypothesize has selected for host defenses in a coevolutionary manner.

Egg-puncturing behavior brings clear potential benefits to a laying female brood parasite. Puncturing may reduce the number of host young with which the parasitic hatchling needs to compete (or in the case of honeyguides, to kill) (Soler et al. 1997). If the parasite lays her eggs late relative

to the host, puncturing may also ensure that host eggs do not hatch in advance of her own, thus avoiding cessation of incubation and consequent death of the parasitic embryo (Soler et al. 1997). Additionally, puncturing may directly reveal information about the state of development of the host clutch, thus allowing the parasite to refrain from parasitizing nests that are found too late in the incubation period (Massoni and Reboreda 1999). Yet, in Greater Honeyguides, there is considerable variation among parasitized clutches in the incidence and degree of egg puncturing (Friedmann 1955), and no other honeyguide species is definitely known to show this behavior. Unless an evolutionary lag is responsible, this suggests that egg puncturing, like other highly virulent behavior in avian brood parasites (Kilner 2005), is tempered by costs. One cost could be the risk of desertion, if the host female perceives that most of her clutch has been damaged and is no longer worth incubating to term. In Great Spotted Cuckoos, it has further been suggested that minimizing obvious damage to an egg has the advantage of reducing the chances that the host female will closely inspect her clutch and hence detect and reject the parasitic egg (Soler et al. 1997).

If egg puncturing can increase the parasite's reproductive success to the detriment of the host's, as suggested above, host counteradaptations might be expected to evolve. First, hosts should be expected to desert clutches that are conspicuously punctured, thus avoiding the costs of a doomed reproductive attempt and of raising the parasitic chick. Second, host females should lay thicker shelled or otherwise strengthened eggs, for 2 reasons: first, stronger eggs should require more puncturing attempts in order to be visibly damaged, thus providing a cue for the hosts to desert; second, females laying eggs that survive puncturing attempts may

Address correspondence to C.N. Spottiswoode. E-mail: cns26@cam.ac.uk.

Received 19 July 2006; revised 17 February 2007; accepted 5 March 2007.

raise surviving young if the honeyguide egg fails to hatch because it is infertile or was laid too late relative to the host eggs. In the latter instance, incubation may cease before the honeyguide egg hatches, or if it does hatch, it may be faced with adversaries much larger than itself. Host nestlings are hence sometimes found with a fertile but unhatched honeyguide egg (Colebrook-Robjent JFR, Spottiswoode CN, in preparation).

In this study, we explore these hypothetical adaptations and counteradaptations using correlative data from an extensive collection of parasitized clutches of different host species from Zambia. First, we test the assumption that egg puncturing leads to death of the host embryo concerned. Second, we test whether rate of egg puncturing is related to host egg morphology. If stronger eggs are more difficult to damage effectively, then a greater number of punctures is likely to be needed sufficiently to damage the embryo's environment because some attempts will fail fully to transpierce the shell. Within clutches, we should therefore expect a positive relationship between the number of punctures and 1) shell thickness and shell mass, because thicker eggshells are more resistant to puncturing and cracking (Ar et al. 1979; Blankespoor et al. 1982; Spaw and Rohwer 1987; Picman 1989), and 2) shell shape, because spherical eggs should theoretically have greater structural strength than ovate or tapered ones (Lack 1968; Vogel 2003), and have been empirically shown to be more puncture resistant in 4 Icterid species (Picman 1989). Third, we examine whether a high rate of puncturing of host clutches is associated with nest desertion, and hence whether its benefits are likely to trade off against the danger of hosts perceiving that they have been parasitized.

We then test for an evolutionary response in hosts and parasite. First, we test for evidence of selection currently operating in the host species with the largest sample of parasitized clutches (the Little Bee-eater *Merops pusillus*) by relating a parasitized female's egg morphology to whether or not any of her clutch was likely to survive honeyguide parasitism. Specifically, selection on eggshell strength predicts that hosts laying thicker and rounder eggs should have a higher probability of being able to raise surviving young of their own, despite being parasitized by a honeyguide. Second, if excessive puncturing is costly because of host desertion, we expect puncture rate to increase with its potential benefits. Among clutches within host species, puncture rate should be greatest when the female honeyguide has laid relatively late, as in such cases incubation might otherwise cease when the host young hatch, and moreover the honeyguide hatchling would benefit most from not needing to kill any host hatchlings. Among host species, egg puncturing should be most beneficial when the host hatchling is most difficult for the honeyguide hatchling to kill, which should be the case in the largest bodied hosts. Third, if parasitism by Greater Honeyguides selects for eggshell thickening in their hosts, then we should expect that host species ought to have thicker eggshells relative to their egg sizes (given that shell thickness increases with egg size in birds, e.g., Ar et al. 1979; Spaw and Rohwer 1987; Brooker MG and Brooker LC 1991) than closely related nonhost species with similar nesting biology.

Our null hypothesis is that variation in puncture rate among eggs and nests might result from varying time constraints among laying events or varying degrees of clumsiness among honeyguide females, rather than any adaptive adjustment according to the expected payoff. The prediction that most clearly contrasts with that of the foregoing hypothesis is the second mentioned above, because an indiscriminately puncturing female should produce more damage to thinner and weaker eggshells.

METHODS

Study site and species

All data were drawn from eggs and records held in the Musumanene Museum, Choma, Zambia, collected by J.F.R.C.R. during 1969–2002. Permission for preceding and subsequent fieldwork was in 1988 explicitly granted by the Choma Magistracy. Parasitized clutches came mainly from the Choma District of Zambia's Southern Province, within a 28-km radius of the town of Choma (16°49'S, 26°59'E), with additional clutches from the Lundazi, Mpika, and Lusaka Districts of Zambia. The habitat in the Choma District is largely broad-leaved woodland dominated by *Brachystegia*, interspersed with agricultural fields and seasonally flooded grassy depressions. Honeyguide eggs were identified by a combination of shell color, roundness and gloss, and thicker shells (depending on host species), and honeyguide identity (Greater vs. Lesser Honeyguide *Indicator minor*) was confirmed by the presence of parasite young (details in Colebrook-Robjent JFR, Spottiswoode CN, in preparation). The Greater Honeyguide is the only egg-puncturing brood parasite to breed at our study site.

In the Choma District, as probably elsewhere in Zambia, Greater Honeyguides parasitize a range of generalist woodland and savannah species; in common with hosts of other *Indicator* spp., these are all hole nesters. Consistent efforts were made to find nests of all bird species in the Choma region, usually by observing host behavior (typically by flushing incubating birds), and no evidence suggested that any host species were disproportionately conspicuous. All host species bred synoptically in tree cavities, except Little Bee-eater (terrestrial burrows) and African Hoopoe *Upupa africana* (cavities in termite mounds, as well as tree holes). Main hosts were the Little Bee-eater (39% of host nests parasitized, representing 64% of all Greater Honeyguide-parasitized nests), Striped Kingfisher *Halcyon chelicuti* (29% and 5%, respectively), Greater Scimitarbill *Rhinopomastus cyanomelas* (20% and 6%), Green Woodhoopoe *Phoeniculus purpureus* (13% and 9%), and African Hoopoe (46% and 13%); additionally, single parasitized clutches of Capped Wheatear *Oenanthe pileata* (13% and 1%) and Black Tit *Parus niger* (4% and 1%) were recorded (Colebrook-Robjent JFR, Spottiswoode CN, in preparation). The duration of host incubation required to hatch Greater Honeyguide eggs is unknown, but honeyguide embryos are thought to develop more rapidly than those of their hosts (Short and Horne 2001).

Egg characteristics

At the time of collection, the following information was recorded for each egg (host and parasite) by J.F.R.C.R.: 1) Egg length and breadth, measured using Vernier calipers accurate to 0.1 mm. We used these to estimate egg shape (as the ratio of egg breadth to egg length, which gives a value that increases with increasing egg roundness) and egg volume (as $0.51 \times \text{length} \times \text{breadth}^2$; Hoyt 1979). 2) Whether any embryo present was living and still viable, or dead and rotting, as assessed from egg contents during blowing (dead embryos were distinguished by their opaque and solid quality, discolored blood, and, if present, signs of decomposition and rotting). 3) The state of incubation: 0 = unincubated or fresh; 1 = slight or trace (developed for an estimated 0–20% of the incubation period); 2 = minute to very small embryo (20–40%); 3 = small medium to medium-sized embryo (40–65%); 4 = large embryo (65–80%); 5 = very large embryo, near hatching (>80%). These subjective categories were scored consistently and blindly to the hypotheses under test. 4) Any observations (typically absence of host parents in attendance) that

revealed whether the clutch was under active incubation or deserted.

Subsequently, 5) the number of puncture holes in each egg was counted by J.F.R.C.R. under a $\times 10$ magnifying glass, blindly to the hypotheses under test. Eggs that were completely broken open as a presumed consequence of puncturing had to be excluded because puncture holes could not be counted accurately (see below). To assess repeatability, J.F.R.C.R. recounted (blindly to previous counts) puncture holes in a random sample of 93 host eggs. Although the size as well as the number of puncture holes may also be important, it is difficult to quantify and we have no information on the relative damage caused by holes of different sizes. 6) The mass of cleaned, empty eggshells was measured by J.F.R.C.R. using an Acculab PP2060D electronic balance accurate to 0.001 g. 7) Eggshell thickness was measured by C.N.S. to the nearest 0.001 mm using a Mitutoyo micrometer (model number 293-723-30) specially modified for the purpose (see also Green 1998; Gosler et al. 2005). Shell thickness measurements were taken at the shell surface opposite the blowhole, in the region of the equator, thus minimizing any confounding effect of variation in shell thickness in different regions of the egg (see Gosler et al. 2005). To calculate measurement repeatability, C.N.S. remeasured (blindly to previous measurements) a random sample of 44 honeyguide and host eggs.

Analyses of egg viability were confined to those eggs where this could be determined with certainty. All unincubated or infertile host eggs, and all clutches where the honeyguide egg was unincubated (indicating recent laying and puncturing), were excluded, even if host egg viability could still be determined (e.g., if the egg contents had leaked). Analyses of puncture rate in relation to eggshell properties were carried out on a more conservative subset as well as on the full data set because some nests may have contained an incomplete complement of host eggs if badly damaged eggs (with potentially nonrandom shell properties) were removed by hosts. Therefore, we repeated the analyses including only nests for which we could be very confident that no host eggs were missing, defined as those containing the maximum or one less than the maximum recorded unparasitized clutch size for each host species (see Colebrook-Robjent JFR, Spottiswoode CN, in preparation).

Analyses of a clutch's shell properties were examined in relation to whether the honeyguide, host, or neither party had the potential to gain at least one surviving offspring. The host was scored (independently by both authors, who concurred in all cases) to have no reproductive success if 1) the clutch was deserted, 2) a honeyguide nestling was present, or 3) at least one honeyguide embryo was viable and its state of incubation was not more than 2 incubation scores less advanced than that of the host. The host was scored to have accrued or be very likely to accrue some reproductive success if 1) a surviving host nestling (as well as at least one unhatched egg to provide information on shell morphology) was present or 2) if at least one viable embryo was present that was more than 2 incubation scores ahead of the honeyguide, suggesting that it would hatch well in advance of the honeyguide. If there was any uncertainty over these criteria, the clutch was excluded. Three nests met criterion 1), and 2 nests met criterion 2): one in which the honeyguide embryo was dead and rotten and the host embryos about to hatch, and one in which the honeyguide egg had only just begun to develop, whereas a host egg had a medium-sized embryo. The final sample size was 47 Little Bee-eater clutches where outcome could be scored (compared with 9 or less for the other hosts). We assigned scores under the assumption that honeyguide eggs that were unincubated were viable and again under the more conservative assumption that only honeyguide eggs for which incuba-

tion had begun were viable (hence excluding clutches where the honeyguide egg was wholly unincubated and thus yielding a total of 32 clutches).

Analyses of puncture rate in relation to timing of laying concerned the behavior of a female honeyguide in response to the properties of the whole host nest, rather than the properties of individual eggs; hence, clutches were the unit of analysis. We analyzed the maximum number of puncture holes as well as the mean, as even a single perceptibly damaged egg could be a sufficient cue to prompt host desertion. The independent variable was a binary score of whether the honeyguide egg had been laid significantly late relative to the Little Bee-eater eggs. This was defined as late enough relative to the host to prevent the honeyguide from catching up and hatching before or soon after a viable host embryo (irrespective of whether any was present). We took this to be when the host eggs' incubation score was 3 or greater (i.e., a medium-sized or larger embryo) if host embryos were killed when the honeyguide laid, or if they were not, then when the host eggs' degree of embryonic development was more than 2 states of incubation (according to the categories defined above) ahead of honeyguide's. Clutches for which this was impossible to determine (e.g., if eggs were infertile or broken) were excluded.

The comparative analysis of shell properties of hosts and their close relatives was confined to the Halcyonidae and Meropidae, because the 3 Upupiform host species have no close phylogenetic relatives in Zambia. Within each family, we measured the eggshell thicknesses of all confamilial (in all cases also congeneric) species, which are not regular host species in Zambia. Although there are records from elsewhere in Africa of parasitism of White-fronted Bee-eater *Merops bullockoides*, Chestnut-bellied Kingfisher *Halcyon leucocephala* and Brown-hooded Kingfisher *Halcyon albiventris* (Short and Horne 2001), we are confident that these species are not regular hosts in Zambia owing to an absence of any parasitism records among substantial numbers of nests found by J.F.R.C.R. ($N = 12, 57, \text{ and } 24$ nests, respectively). There is one exception, the Swallow-tailed Bee-eater *Merops hirundineus*, for which there is one confirmed Zambian parasitism record (Colebrook-Robjent JFR, Spottiswoode CN, in preparation).

Statistical analyses

Repeatability of eggshell thickness measurements was calculated according to the formulae in Lessells and Boag (1987). Owing to non-normality of residuals from the count data, even after transformation, we could not apply these to repeated counts of puncture holes. Therefore, we instead calculated statistical significance of repeatability using a general linear model with a quasipoisson error distribution, with repeated counts as the dependent variable and egg identity as the independent variable.

Predictions about egg viability and eggshell traits in relation to puncture rate concerned variation among eggs within a clutch. Analyzing within-clutch variation rather than pooling all eggs is appropriate because 1) eggs within a clutch are not statistically independent, having been laid by the same female, and 2) there may be individual differences in the behavior of female honeyguides that could affect puncture rate independently of the properties of the clutch. Therefore, in order to examine within-clutch patterns (confined to clutches with more than one surviving egg per clutch), we used mixed-effects models, with puncture rate or egg viability as the dependent variable, respectively, egg traits or puncture rate as fixed effects, and clutch identity nested within host species (for the 5 host species with more than one parasitized nest per species) as random effects. This approach allowed different

intercepts to be modeled per clutch and per host, thus examining within-clutch variation while allowing mean differences among clutches and among hosts to be taken into account. The significance of random effects was assessed by comparing alternative models using likelihood ratio tests. When the dependent variable was egg viability (live vs. dead), we used binomially distributed errors with a logit link function.

Predictions about selection on the egg traits of individual host females, and about the laying behavior of individual parasitic females, concern variation among parasitized host clutches within host species. Hence, for these analyses, the unit of analysis was the mean of each Little Bee-eater clutch, as this was the only host with adequately large samples of parasitized clutches ($N = 41\text{--}48$ clutches) to permit a clutch-level rather than egg-level analysis. Lastly, predictions concerning differences in honeyguide laying behavior among host species (including all 7 recorded host species) were tested using host species means (means of clutch means because eggs within a clutch are not statistically independent) as the unit of analysis, after ascertaining that interspecific differences existed using a linear mixed model with host identity was as a fixed factor and clutch identity as a random factor.

Statistical analyses were carried out using the software R (R Development Core Team 2006). Unless otherwise stated, we modeled the log-transformed number of puncture holes ($\log[\text{holes} + 1]$) as a continuous variable, as residuals were normally distributed. There were 2 exceptions where log transformation failed to normalize residuals: the late-laying analysis, for which the number of holes was instead square-root transformed, and the analysis of among-host differences in puncture rate, for which puncture rate was instead modeled as count data with a quasipoisson error distribution, in a generalized linear mixed model (GLMM). Additionally, for all analyses, we checked whether similar results were found when puncture rate was instead modeled as count data (using GLMMs where appropriate), and in all cases this was so.

RESULTS

Measurement accuracy and incidence of puncturing

Eggshell thickness measurements (repeatability = 0.953, $F_{43,44} = 41.90$, $P < 0.001$) and counts of puncture holes ($F_{92,93} = 10.60$, $P < 0.001$) were both highly significantly repeatable. In total, 180 of 229 host eggs (79%) were punctured, at least one egg in 69 of the 74 (93%) host clutches. Considering only punctured clutches, on average 85% (± 2.7) of eggs in a clutch were punctured. Among those eggs that were punctured and where the holes could be counted ($N = 164$ eggs), the mean number of puncture holes was 7.3 ± 0.5 , range 1–37. Host eggs were distributed among different host species as follows: Little Bee-eater (number of parasitized clutches: 46; range of host eggs present per clutch: 1–5), Striped Kingfisher (4; 1–4), Greater Scimitarbill (5; 2–3), Green Woodhoopoe (4; 1–4), African Hoopoe (11; 1–6), Capped Wheater (1; 2), and Black Tit (1; 1).

Is egg puncturing associated with host embryo death?

Egg viability at the time of blowing could be determined with certainty for 132 eggs, in 41 clutches drawn from 5 host species. Figure 1 illustrates mean levels of puncturing for viable ($N = 62$) and dead ($N = 80$) embryos, for all clutches and host species pooled, and shows that eggs with more than 12 puncture holes always had dead embryos. The mean difference between viable and dead embryos was 7.8 puncture holes. When we analyzed this relationship in a GLMM with viability as the response variable, rates of embryo death were

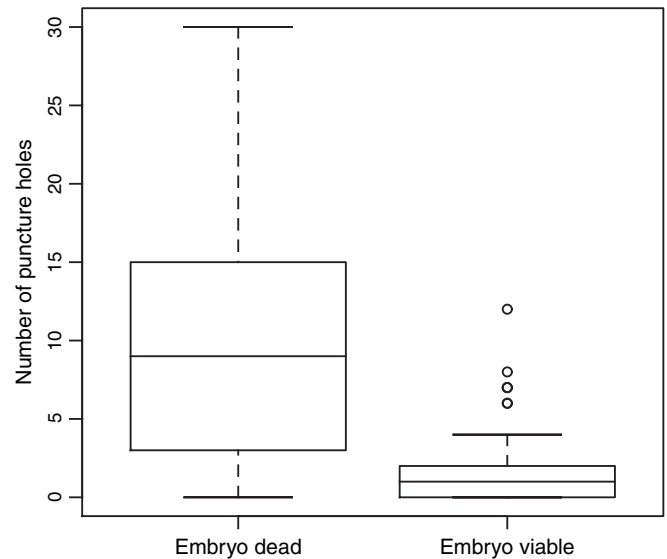


Figure 1

Number of puncture holes per incubated egg in clutches parasitized by the Greater Honeyguide in relation to whether the embryo was living or dead at the time of collection. See main text for statistical tests taking into account clutch and host species membership.

indeed significantly higher in eggs with more puncture holes (slope \pm standard error [SE] = 2.31 ± 0.32 , $t_{90} = 7.20$, $P < 0.001$), independent of mean differences between clutches and host species. This is likely to represent a minimum difference because additional punctured embryos may have been destined to die subsequently.

Is rate of egg puncturing related to host egg morphology?

Data were available for more than one egg per clutch in 194 eggs, comprising 59 clutches from 5 host species. Within clutches, the number of puncture holes was significantly greater in eggs that were thicker shelled (shell thickness effect: slope \pm SE = 19.01 ± 6.03 , $t_{133} = 3.15$, $P = 0.002$) and, independently in the same model, more rounded in shape (shape effect, where a value of shape closer to one indicates a more rounded shell: slope \pm SE = 4.01 ± 1.46 , $t_{133} = 2.75$, $P = 0.007$). The significance of the random factors suggested that there were differences in intercept among nests/females within host species (likelihood ratio = 29.03, $P < 0.001$) but not among host species (likelihood ratio = 1.68, $P = 0.20$). This analysis was not confounded by any collinearity between egg properties because shell thickness and shape were unrelated to each other ($t_{134} = -0.08$, $P = 0.94$). Results were very similar if shell mass (which was also unrelated to shape, $t_{132} = 0.02$, $P = 0.59$) was used as a surrogate for shell thickness ($N = 192$ eggs; shell mass effect: slope \pm SE = 4.63 ± 1.51 , $t_{131} = 3.07$, $P = 0.003$; shape effect: slope \pm SE = 2.68 ± 1.20 , $t_{131} = 2.23$, $P = 0.027$).

When we repeated these analyses for the subset of nests for which we could be very confident that no broken eggs had been previously removed by hosts (see Methods), the sample size was reduced to 121 eggs, in 29 clutches of 4 host species. Nonetheless, results were extremely similar, suggesting that any removal by hosts of heavily damaged eggs did not generate any bias in the analyses. This was so when shell thickness was measured directly (shell thickness effect: slope \pm SE = 21.44 ± 7.57 , $t_{90} = 2.83$, $P = 0.006$; shape effect: slope \pm SE = 6.49 ± 1.76 , $t_{90} = 3.69$, $P < 0.001$), as well as when shell mass was used as a surrogate ($N = 119$ eggs; shell mass

effect: slope \pm SE = 4.47 ± 1.92 , $t_{88} = 2.32$, $P = 0.023$; shape effect: slope \pm SE = 4.75 ± 1.52 , $t_{88} = 3.13$, $P = 0.002$).

Do hosts desert excessively punctured clutches?

Three of the total of 84 parasitized clutches of all host species (52 of them Little Bee-eaters) were judged, by the absence of attending adults and by cold eggs, to be deserted at the time of finding. It is likely that the actual rate of nest desertion was higher because active nests are generally easier to find. The small number of deserted clutches (all of Little Bee-eaters) found means that any conclusions must remain qualitative. Two deserted parasitized nests contained punctured host eggs, with a respective maximum of 10 and 37 (the highest number ever found) puncture holes per host egg, whereas a third contained no host eggs. A further single deserted clutch was found that did not contain a honeyguide egg, but had been heavily punctured (maximum of 13 holes), suggesting that a honeyguide egg had been present and had been ejected by the hosts. For comparison, none of a total of 92 unparasitized clutches of Little Bee-eaters found at the egg stage was judged to be deserted. These anecdotal observations suggest that Little Bee-eaters sometimes deserted heavily punctured clutches but that heavily punctured clutches were frequently not deserted.

Is host egg morphology related to the probability of surviving honeyguide parasitism?

Hosts were occasionally able to raise nestlings in spite of being parasitized: at least one host nestling was found alongside an unhatched but clearly fertile honeyguide egg in 2 of 8 cases of Green Woodhoopoe parasitism, 1 of 12 cases of African Hoopoe parasitism, and 3 of 60 cases of Little Bee-eater parasitism. In further cases, the honeyguide egg was at the time of collection considerably outpaced in development by at least one viable host egg. This analysis is confined to the Little Bee-eater because only in this species did sufficient unhatched host eggs remain to provide information on host shell properties. Little Bee-eater clutches that were scored as very likely to accrue some reproductive success in spite of being parasitized ($N = 5$ clutches) had significantly thicker shelled eggs than those where the host was scored as very likely to fail, irrespective of whether clutches with unincubated honeyguide eggs were assumed to be viable (all data: slope \pm SE = 0.0065 ± 0.0027 , $R^2 = 0.126$, $F_{1,41} = 5.92$, $P = 0.019$) or were excluded (incubated subset: slope \pm SE = 0.0069 ± 0.0031 , $R^2 = 0.160$, $F_{1,26} = 4.97$, $P = 0.035$; see also Figure 2). However, there was no difference in shell shape between Little Bee-eater clutches with and without any likely reproductive success (all data: slope \pm SE = 0.0003 ± 0.0152 , $F_{1,45} = 0.0003$, $P = 0.99$; incubated subset: slope \pm SE = 0.0040 ± 0.0161 , $F_{1,30} = 0.062$, $P = 0.81$). Although we had no a priori reason to predict this relationship, Little Bee-eater clutches that were judged likely to gain some reproductive success had eggs of significantly smaller volume eggs than those judged likely to gain none (all data: slope \pm SE = -0.177 ± 0.084 , $R^2 = 0.091$, $F_{1,45} = 4.48$, $P = 0.040$; incubated subset: slope \pm SE = -0.195 ± 0.083 , $R^2 = 0.157$, $F_{1,30} = 5.57$, $P = 0.025$).

Do honeyguides puncture more when laying late?

Late incidences of honeyguide laying were recorded from all but one host species (Greater Scimitarbill). Honeyguides laid eggs throughout the host developmental period, even when host eggs were close to hatching, as evidenced for example by a barely developed honeyguide egg alongside host eggs containing very large embryos. In the only species with sufficiently

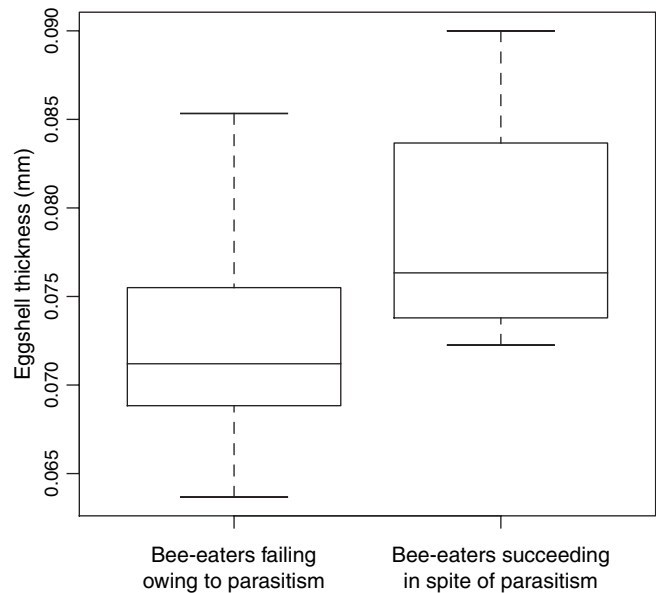


Figure 2

Thickness of Little Bee-eater eggshells in relation to whether or not they were able to raise at least one surviving nestling in spite of Greater Honeyguide parasitism. See main text for statistical tests taking into account clutch membership.

large samples to allow analysis, the Little Bee-eater, clutches parasitized by a late-laying honeyguide female ($N = 6$) had both a higher mean (Figure 3; slope \pm SE = 1.36 ± 0.46 , $R^2 = 0.185$, $F_{1,39} = 8.83$, $P = 0.005$) and a higher maximum (slope \pm SE = 1.17 ± 0.53 , $R^2 = 0.112$, $F_{1,39} = 4.92$, $P = 0.033$) number of puncture holes per surviving egg than clutches where the honeyguide laid soon after the host ($N = 35$). The mean difference in number of puncture holes between late-laying and timely laying honeyguides (7.7 holes, Figure 3) was greater than the mean difference in number of puncture

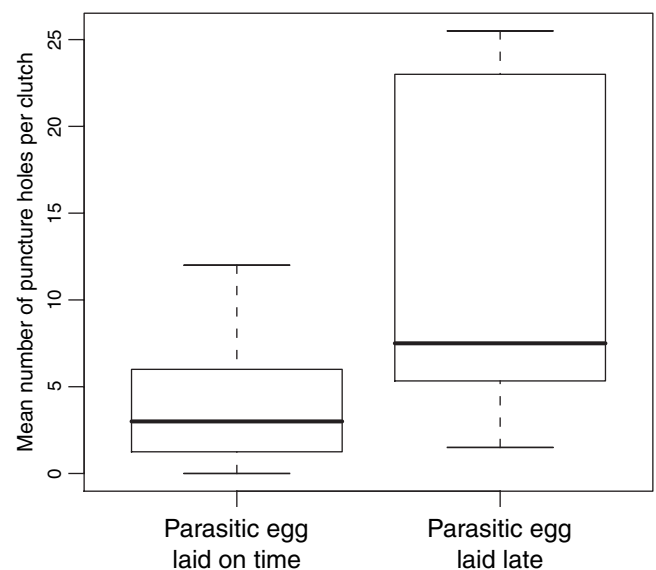


Figure 3

Mean number of puncture holes per egg in parasitized clutches of Little Bee-eaters in relation to whether or not the host eggs' state of incubation was significantly in advance of the parasite's, indicating late laying by the latter.

holes between viable and dead Little Bee-eater embryos (6.2 holes; subset of data in Figure 1, which refers to all host species), suggesting that such a difference would probably have been sufficient to cause embryo death. Considering all possible causes of honeyguide breeding failure (including nonindependent data on late laying, as well as nest desertion), for those Little Bee-eater clutches where both timing of honeyguide laying and probability of honeyguide success could be assessed, one of 6 (17%) late-laid parasitism attempts were judged likely to succeed, compared with 18 of 21 (86%) correctly timed parasitism attempts.

Do honeyguides puncture more when hosts are large bodied?

There was a significant difference in puncture rate among hosts ($F_{6,63} = 3.81$, $P = 0.003$) (as anticipated by Friedmann 1955); hence, using species means ($N = 7$, based on a total of 70 clutches) as the unit of analysis appears justified. Clutches of host species with larger egg volume (and hence larger hatching size) had a significantly greater number of puncture holes in them than clutches of smaller egged host species (Figure 4; slope \pm SE = 1.67 ± 0.048 , $R^2 = 0.707$, $F_{1,5} = 12.06$, $P = 0.018$). However, the confidence with which different species means were calculated varies greatly owing to different numbers of parasitized clutches found, as shown by the sizes of the symbols in Figure 4. If this was taken into account by weighting each data point by the number of clutches from which it was calculated (log-transformed), the relationship was qualitatively similar, but lost statistical significance (slope \pm SE = 1.89 ± 0.50 , $R^2 = 0.533$, $F_{1,5} = 5.70$, $P = 0.063$). These patterns were not confounded by hosts with larger eggs also having thicker eggshells because there was only a weak interspecific relationship between these traits (slope \pm SE = 186 ± 193 , $R^2 = 0.156$, $F_{1,5} = 0.92$, $P = 0.38$; weighted analysis similar), and eggshell thickness was unrelated to puncture rate among species ($R^2 = 0.047$, $F_{1,5} = 0.25$, $P = 0.64$; weighted analysis similar).

Have host species evolved thicker eggshells than nonhosts?

Eggshell thickness varied significantly among species (*Merops*: $R^2 = 0.803$, $F_{4,63} = 64.01$, $P < 0.001$; *Halcyon*: $R^2 = 0.916$, $F_{4,36} = 98.23$, $P < 0.001$; Table 1), justifying the use of species means (means of clutch means) as data points. Figure 5 plots for each genus the interspecific relationship between eggshell thickness and egg volume. In general, shell thickness increased with egg size, but within each genus, the current host species had substantially thicker eggshells than expected for its egg size. Within each genus, the mean log-transformed

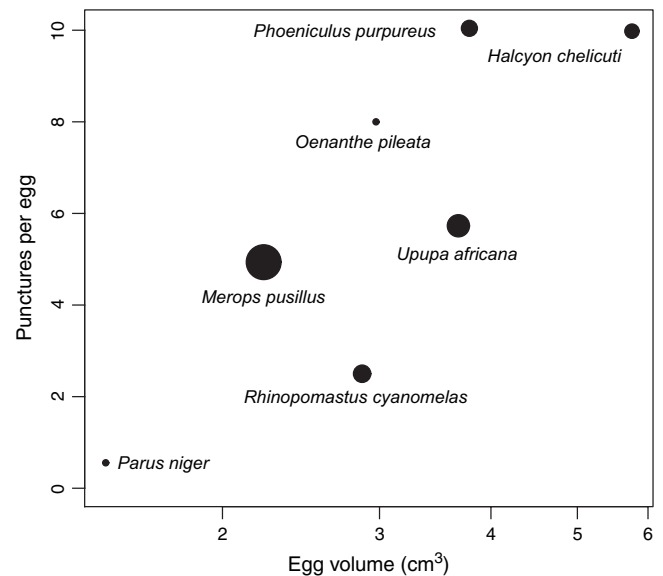


Figure 4

Mean number of punctures per host egg across host species, in relation to host egg volume. See main text for statistical tests. The diameter of each data point is proportional to the logarithm of the number of clutches from which the species mean was calculated.

ratio of eggshell thickness to egg volume was significantly greater in the host species compared with the 4 nonhost species (one-sample *t*-tests, *Merops*: $t_3 = -3.28$, $P = 0.023$; *Halcyon*: $t_3 = -4.26$, $P = 0.012$). Moreover, when the mean thickness of individual clutches of hosts was compared only with the non-host species with the most similar egg size, both host species had significantly greater absolute eggshell thickness (2-sample *t*-tests, Little vs. Blue-breasted Bee-eaters: $t_{52} = 2.03$, $P = 0.048$; Striped vs. Chestnut-bellied Kingfishers: $t_{26} = 13.03$, $P < 0.001$). The relationship between eggshell thickness and egg volume in kingfishers in Figure 5 remained very similar if Cerylid (Pied Kingfisher *Ceryle rudis*) and Alcedinid (Malachite *Alcedo cristata* and Pygmy Kingfishers *Ispidina picta*) species were also plotted (data not shown). These results might be biased if within parasitized host nests thinner shelled eggs were completely broken because this would generate a spuriously high mean thickness for hosts. We therefore repeated all the above analyses including only unparasitized host clutches and parasitized clutches with the maximum clutch size still present ($N = 5$ eggs for Little Bee-eaters; Colebrook-Robjent JFR, Spottiswoode CN, in preparation). In all cases results (not

Table 1

Eggshell thickness and volume (means \pm SE for clutch means, ranges for individual eggs) of Striped Kingfishers and Little Bee-eaters, hosts of the Greater Honeyguide, and of congeneric *Halcyon* and *Merops* species that are not currently regular hosts in Zambia

	N	Thickness	Volume
Striped Kingfisher <i>Halcyon chelicuti</i> (host)	9 (24)	0.106 \pm 0.002, 0.094–0.118	5.77 \pm 0.13, 4.99–6.44
Chestnut-bellied Kingfisher <i>Halcyon leucocephala</i>	19 (67)	0.079 \pm 0.001, 0.069–0.093	5.60 \pm 0.09, 4.39–7.07
Brown-hooded Kingfisher <i>Halcyon albiventris</i>	10 (36)	0.092 \pm 0.002, 0.078–0.106	7.31 \pm 0.13, 5.97–9.07
Woodland Kingfisher <i>H. senegalensis</i>	2 (3)	0.133 \pm 0.004, 0.130–0.136	8.89 \pm 0.28, 8.33–9.33
Blue-breasted Kingfisher <i>H. malimbica</i>	1 (3)	0.143 \pm 0.005, 0.140–0.144	13.88 \pm 0.40, 13.46–14.15
Little Bee-eater <i>Merops pusillus</i> (host)	52 (155)	0.072 \pm 0.001, 0.059–0.090	2.25 \pm 0.06, 1.26–3.02
Blue-breasted Bee-eater <i>M. variiegatus</i>	2 (4)	0.064 \pm 0.004, 0.061–0.070	2.76 \pm 0.28, 2.47–2.99
Swallow-tailed Bee-eater <i>Merops hirundineus</i>	2 (7)	0.082 \pm 0.004, 0.073–0.089	3.30 \pm 0.28, 2.65–4.16
White-fronted Bee-eater <i>Merops bullockoides</i>	8 (24)	0.095 \pm 0.002, 0.084–0.106	3.84 \pm 0.14, 3.54–4.11
Southern Carmine Bee-eater <i>M. nubicoides</i>	4 (5)	0.107 \pm 0.003, 0.095–0.118	7.08 \pm 0.21, 6.14–7.55

Sample sizes refer to number of clutches, followed by number of eggs in parentheses.

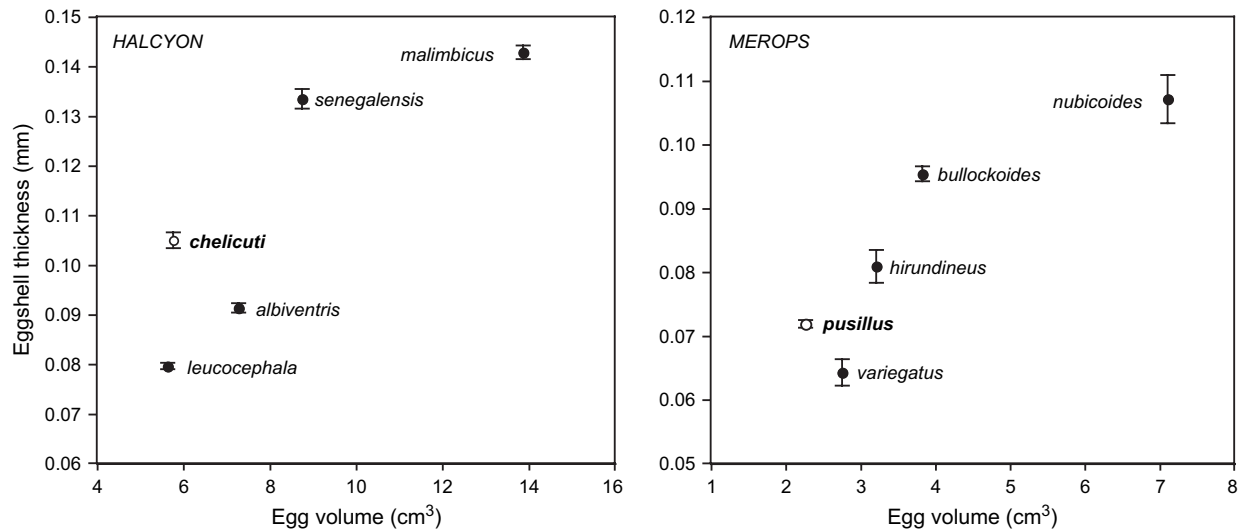


Figure 5

Interspecific relationship between eggshell thickness and egg volume among Zambian representatives of 2 genera of Coraciiform birds, each including one species regularly parasitized by the Greater Honeyguide (open symbol) and 4 species that are unparasitized or rarely parasitized (closed symbols). Data points are species means \pm SE.

shown) were extremely similar to those reported above, suggesting that surviving eggs did not represent a biased sample.

DISCUSSION

The patterns we have found are consistent with a coevolutionary scenario that embryo killing by adult Greater Honeyguides is an effective adaptation to increase the parasitic hatchling's chances of success and exerts selection for host counteradaptations. A higher rate of puncturing was associated with a greater probability of host embryo death and was increased in situations where it was likely to confer the greatest advantage to the parasitic offspring, namely 1) when the honeyguide egg was laid late relative to the host clutch and 2) when host hatchlings were likely to be more difficult adversaries for the honeyguide hatchling to overcome, owing to their larger body size. Two lines of evidence suggested that this behavior exerted selection on host egg properties: first, stronger eggs (as reflected by shell mass, thickness, and roundness) were more extensively punctured. That this relationship was positive and not negative supports the hypothesis that puncturing is adjusted in an adaptive fashion, rather than resulting from variation in degree of constraint or clumsiness of indiscriminately puncturing honeyguides. Second, host females laying thicker shelled (but not rounder) eggs were more likely to be able raise at least one surviving offspring in spite of honeyguide parasitism (albeit involving a small sample of nests) than those that lost all reproductive success when parasitized.

Because this evidence is entirely correlative, causality cannot be demonstrated with certainty, and alternative explanations for these patterns need to be considered. Massoni and Reboreda (1999) provide evidence that egg puncturing is used by Shiny Cowbirds *Molothrus bonariensis* principally to assess the host's state of incubation, rather than to reduce competition or ensure adequate incubation for the host embryo. This seems unlikely to explain puncturing by Greater Honeyguides, however, because unparasitized clutches were very rarely punctured (1 of 169 clutches of the 5 common host species analyzed here; the one unparasitized nest that was punctured had shell fragments outside the nest entrance, suggesting that a honeyguide egg may have been previously ejected).

We predicted and found that thicker shelled and rounder shelled eggs should contain more puncture holes because they should be more difficult to damage and hence should require more attempts to damage effectively. However, other mechanisms could also generate this association. For example, honeyguide eggs are generally more rounded than those of their hosts (Colebrook-Robjent JFR, Spottiswoode CN, in preparation; Friedmann 1955; Short and Horne 2001) and are substantially thicker shelled than those of either Little Bee-eater or Striped Kingfisher (but not of the Upupiform hosts; Colebrook-Robjent JFR, Spottiswoode CN, in preparation). A laying female Greater Honeyguide may benefit from ensuring that the thickest shelled and roundest eggs in a host clutch are adequately damaged, because the presence of a prior honeyguide egg in the clutch would be likely to cause her own to fail, owing to its head start in rapid embryonic development. What the observed relationship clearly can exclude is that honeyguides puncture eggs indiscriminately and without adaptive adjustment because such a scenario should result in the weakest, not the strongest, eggs in a clutch being most heavily punctured.

If, as supported by our data, Greater Honeyguides do make strategic decisions about how extensively to puncture host eggs in relation to their state of incubation, how do they assess the latter? Visual cues (e.g., shell darkening) seem unlikely to be used in the poor light environment of the tree holes or burrows used by its hosts. Host behavior (e.g., incubation attentiveness or an absence of direct observations of egg laying) is perhaps a more likely cue. Massoni and Reboreda (1999) also suggest various possible mechanisms by which puncturing by cowbirds might itself provide tactile information on state of incubation, which may well also operate in Greater Honeyguides. Although we cannot be certain that puncturing necessarily always kills embryos rather than only slowing down their development, at worst the latter would result in a smaller, not greater, discrepancy in states of embryonic development between honeyguide and host. Therefore, it ought only to add noise to our data, rather than generating any bias in favor of our predictions.

The interspecific analysis of puncturing rate in relation to hatchling size (approximated by egg volume) is perhaps the most prone to confounding factors because of the allometric

relationship between egg size and a suite of life history traits (e.g., Martin et al. 2006). Most notably, incubation periods may be shorter in smaller host species laying smaller eggs, which may increase a honeyguide's benefit from halting their development, because they have a higher probability of hatching in advance of its own egg. Although difficult to test directly because of limited information on incubation periods of host species (known for only 5 of the 7 in this study), it seems unlikely to explain the interspecific pattern reported in Figure 4, because it would predict a negative relationship between puncture rate and egg size, and this was not observed. If female Greater Honeyguides do indeed adjust their puncture rate according to the host species involved, this would be consistent with the suggestion that host-specific genotypes may have evolved in the honeyguides (Spottiswoode CN, Quader S, Colebrook-Robjent JFR, in preparation).

Given that avian eggshells are composed of 97% calcium carbonate (Rahn and Paganelli 1989), eggshell thickening by hosts appears likely to involve increased investment of calcium, which in other environments can be a limiting resource to egg-laying birds (e.g., Graveland and Drent 1997; Gosler et al. 2005). If calcium is also limiting in honeyguide hosts, this may impose an additional cost of honeyguide parasitism at the population level. It may be worth considering whether a laying host female's optimal strategy is then to spread her available calcium equally among eggs in a clutch, or rather to vary it such that an indiscriminate honeyguide female could inadvertently cause perceptible damage to at least one egg. Such a form of mineshaft canary might more readily reveal whether parasitism has taken place. If calcium is alternatively not limiting in these groups of hosts, an interspecific implication may be that there is only weak selection against the retention of any eggshell-related adaptations to brood parasitism in species which, although not currently parasitized in Zambia, might be former hosts that have "beaten" the honeyguide. However, even were this the case, it would only render the observed findings that hosts lay thicker shelled eggs than nonhosts conservative because it should result in currently unparasitized species retaining thicker eggshells.

If puncturing host eggs can improve a parasite's reproductive success, why is the Greater Honeyguide the only member of the Indicatoridae to do so? Put another way, why have other honeyguide species settled at a different level of parasitic virulence (Kilner 2005)? The trade-off between the costs and benefits of egg puncturing may be influenced not only by the responses of hosts but also by conspecific competitors. An important consequence of egg puncturing is that in cases of multiple parasitism, the first-laying parasite is likely to lose all reproductive success because her eggs will probably be punctured by a subsequently laying female. Hence, egg puncturing should select against conspicuous behavior around host nests, in order to avoid revealing their location to conspecifics. It is striking that Greater Honeyguides (rate of multiple parasitism 5%; Colebrook-Robjent JFR, Spottiswoode CN, in preparation) are markedly more elusive around host nests (e.g., Friedmann 1955) than Lesser Honeyguides *I. minor* (rate of multiple parasitism 28%). Lesser Honeyguides do not puncture eggs (Friedmann 1955) and are very conspicuous and pugnacious around the nests of their barbet hosts (e.g., Payne 1992; Hanmer 1997), which they appear closely to monitor (Short and Horne 1992). The latter, in turn, might by reducing the chance of late laying, reduce the possible benefits of puncturing. Thus, secretive and frequently late laying by the Greater Honeyguide may together with egg puncturing form a suite of interrelated behaviors, the direction of causality among which is difficult to determine.

Given that the results reported here are purely correlative, how can they be tested by experimental manipulation? The

most sketchily supported prediction investigated here, that hosts do retaliate against Greater Honeyguide parasitism by deserting conspicuously damaged clutches, could clearly be tested by observing host reactions to punctured and unpunctured experimental eggs, introduced early in the incubation period. This is an important mechanism to test, because it is the most obvious cost that could temper indiscriminate puncturing by honeyguides, and thus provides the first line of host defense in the coevolutionary scenario suggested by this study.

We are very grateful to the Bruce-Miller family, Bruce Danckwerts, and Peter Leonard for their help and hospitality in Zambia and to them and many others for essential assistance with fieldwork: Dylan R. Aspinwall, Kazekani, Charles and Geoffrey Banda, Lazaro Hamusikili, Angelo Mudukula, Domonic and Refi Munkombwe, Tom L. Savory, Giles Siabulanda, Kenneth Siangonya, Fred Sinoya, Dunne Siyapolo, and J. C. Sweetman. We thank Judge Kalima of the Choma Magistracy for permission to conduct fieldwork, Douglas Russell and Robert Prys-Jones at the Natural History Museum (Tring, UK) for the kind loan of the micrometer used for eggshell thickness measurements, and Jörn Schärlemann for designing the instrument. We are also very grateful to Nick Davies, Rebecca Kilner, Suhel Quader, and 2 anonymous referees for their extremely helpful advice and comments and to A. Lindsay Greer and Daniel Kuan Li Oi for their advice about the strength of thin-shelled objects. C.N.S. was supported by Sidney Sussex College, Cambridge and the DST/NRF Centre of Excellence at the Percy FitzPatrick Institute, University of Cape Town.

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