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Original Article

Thick eggshells of brood parasitic cowbirds protect their eggs and damage host eggs during laying

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Brood parasites lay thick-shelled eggs and numerous hypotheses have been proposed to explain the significance of this trait. We examined whether thick eggshells protect the parasite egg during laying events. We used eggs of the parasitic shiny cowbird (*Molothrus bonariensis*) and its hosts, the house wren (*Troglodytes aedon*) and chalk-browed mockingbird (*Mimus saturninus*) in South America, and the eggs of the parasitic brown-headed cowbird (*M. ater*) and its hosts, the house wren and red-winged blackbird (*Agelaius phoeniceus*) in North America. We experimentally dropped parasite eggs onto host eggs to simulate laying by the parasite, parasite eggs onto parasite eggs to simulate multiple parasitism, host eggs onto parasite eggs to simulate hosts laying from the height cowbirds lay, and stirred eggs to simulate jostling that may occur when cowbirds and hosts interact during laying events. We found that cowbird eggs were significantly less likely to be damaged than host eggs during jostling experiments, thereby failing to support the hypothesis that thick eggshells provide protection when eggs are jostled. These findings support the hypotheses that thick eggshells resist damage when laid from an elevated position, when additional cowbird eggs are laid onto them in multiply parasitized nests, and these eggs also damage host eggs when laid.

Key words: adaptation, brood parasitism, cowbird, eggshell, egg damage, Molothrus.

INTRODUCTION

Obligate avian brood parasites rely solely on other birds to care for their offspring and have evolved a series of specialized adaptations that allow them to exploit the parental investment of their hosts (Rothstein and Robinson 1998). One trait shared by many brood parasite lineages, including cowbirds (*Molothrus* spp.), cuckoos (*Clamator, Cuculus* spp.), and honeyguides (*Indicatoridae* spp.), is a thick eggshell (Gaston 1976; Picman 1989; Brooker and Brooker 1991; Spottiswoode 2013). Although it is widely accepted that thick eggshells are an adaptation for a brood parasitic lifestyle, the reason(s) why thick eggshells have evolved remains unclear and it is possible they provide multiple benefits. Numerous hypotheses have been proposed and we describe each below.

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Resists host puncture-ejection

One of the most often-cited hypotheses for the origin of a thick eggshell is that it resists host puncture-ejection (Swynnerton 1918; Spaw and Rohwer 1987). Hosts with small bills cannot grasp the parasitic egg between their mandibles and must puncture the egg and then remove it (Sealy 1996). However, Rothstein (1990) and Mermoz and Ornelas (2004) concluded there was scant evidence that increased eggshell thickness of the parasitic cowbirds was an adaptation to resist host puncture-ejection. Indeed, relatively few hosts of brown-headed (M. *ater*) and shiny (M. *bonariensis*) cowbirds eject parasitic eggs (n = 30 of 248 hosts and 9 of 267 hosts, respectively) and even fewer are puncture-ejecters (n = 8 and 2, respectively; Mason 1986; Peer and Sealy 2004; Reboreda et al. 2013; Lowther 2016). The majority of rejecters of cowbird eggs are large hosts that grasp-eject (Peer and Sealy 2004; Reboreda et al. 2013), similar to hosts of the parasitic great spotted cuckoo (*Clamator*)

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glandarius; Soler and Martinez 2000). Additionally, a thick eggshell does not appear to make puncture-ejection prohibitively costly (e.g. the host's bill bounces off of a parasite egg into the host egg damaging them) for small cowbird hosts (Sealy 1996), although it can in small cuckoo hosts (Antonov et al. 2006, 2009; Li et al. 2016).

Protection against damage during incubation by the host

This hypothesis suggests that a thick eggshell affords the parasitic egg greater protection during routine incubation by the host (Blankespoor et al. 1982). However, this possibility has been discounted because it is unlikely that the eggshells of any species would not be sufficiently strong to withstand normal incubation in a host nest (Spaw and Rohwer 1987; Brooker and Brooker 1991).

Extra calcium for chick vigor

The eggshell is an important source of embryonic calcium for musculoskeletal development (Blom and Lilja 2004). Because some cuckoo nestlings require significant energy and strength to evict host eggs or nestlings, they may rely on extra calcium in their eggs to accomplish this task. This hypothesis has failed to receive support because the eggs of the non-evicting cuckoos are thickershelled than evicting cuckoo species (Antonov et al. 2012).

Resists bacterial infection

Female brood parasites visit numerous host nests and, as a result, are exposed to a variety of pathogens (Hahn and Reisen 2011). Antonov et al. (2012) suggested a thicker eggshell could help resist bacterial infection of the egg. This hypothesis has not been tested, however, the eggshell cuticle may be more critical in preventing infection of eggs (D'Alba et al. 2016). One would also predict that generalist parasites, which visit many species' nests and have greater exposure to pathogens, should have thicker eggshells than specialists that visit only one or a few host nests, but the opposite is true among the cowbirds (López 2013).

Protection in multiply parasitized nests

A thick eggshell may protect the parasitic egg by preventing other parasitic females from removing or damaging previously laid eggs in species that multiply parasitized host nests such as the cowbirds, *Clamator* cuckoos, and honeyguides (Brooker and Brooker 1991; Mermoz and Ornelas 2004; Spottiswoode 2013). Multiple parasitism and this type of damage is routine in nests parasitized by shiny and screaming (*M. rufoaxillaris*) cowbirds because these 2 cowbirds peck and puncture host and parasite eggs during the laying event (Hoy and Ottow 1964; Gloag et al. 2012; Fiorini et al. 2014). A thicker eggshell may also provide protection when additional parasite eggs are laid onto it or jostled into it when a nest is multiply parasitized (Soler and Martinez 2000).

Resists damage when parasitic eggs are laid from an elevated position

Brood parasites lay their eggs rapidly, usually within 30 s (Sealy et al. 1995; Peer and Sealy 1999; Gloag et al. 2013). Lack (1968) first suggested that a thick eggshell prevents damage to the parasite eggs during these laying events that frequently occur from an elevated position (see also Liversidge 1970; Wyllie 1981; Rothstein 1990; Soler and Martinez 2000; Antonov et al. 2012). *Clamator* cuckoos often "shoot" eggs from elevated positions (15 cm) frequently damaging host eggs (Gaston 1976; Antonov et al. 2012).

Soler and Martinez (2000) provided evidence that the frequent breakage of host eggs that occurred during laying was an adaptation to benefit the nestling cuckoo because these eggs did not typically hatch (see also Gaston 1976). Similarly, cowbird species have been observed laying from elevated positions (Rothstein 1990; Ellison et al. in review; Figure 1, Supplementary Video 1). They do not settle into the nest as hosts do (e.g. Neudorf and Sealy 1994), they lack a brood patch (Selander and Kuich 1963), and thus the tactile means of sensing how close they are to host eggs. They lay from above the rim of small hosts or they can be forced to raise up by large hosts during mobbing attacks (Gloag et al. 2013). They also tip their body forward while raising the tail during laying (Scott 1991; Supplementary Video 1). Despite this being the first hypothesis for the evolution of thick eggshells, proposed 50 years ago, Antonov et al. (2012) proclaimed that "the importance of the laying damage hypothesis is therefore still largely unexplored...".

Damages host eggs during laying

When thick-shelled parasitic eggs are laid from the nest rim or higher they may damage host eggs (Figure 2a–c). This type of damage has been recorded in hosts of the brown-headed cowbird (Blankespoor et al. 1982), shiny cowbird (Kattan 1998), bronzed cowbird (Peer and Sealy 1999), giant cowbird (M. oryzivorus; Fraga 2011), in addition to other brood parasites (Wyllie 1981; Soler et al. 1998). Studies quantifying eggs damaged by laying brood parasites are limited, but Soler et al. (1998) recorded damaged magpie (*Pica pica*) host eggs at 62.2% of nests (n = 360) parasitized by great spotted cuckoos. Nolan (1978) found that 6% of cowbird eggs laid (n = 79) damaged prairie warbler (*Setophaga discolor*) eggs and Blankespoor et al. (1982) found that significantly more red-winged blackbird (*Agelaius phoeniceus*) eggs (43% of 134 eggs) were cracked in parasitized nests compared to cowbird eggs (15% of 232 eggs).

Resists damage when eggs are jostled with eggs in a host nest

When a parasite quickly leaves a nest (Soler and Martinez 2000) or when it is violently attacked by hosts during laying (Neudorf and Sealy 1994; Gloag et al. 2013; Soler et al. 2014), parasite and host eggs can be jostled into one another and a thick eggshell may help to resist damage. Indeed, when Jacobin cuckoos (*Clamator jacobinus*) parasitize cape bulbuls (*Pycnonotus capensis*) roughly 50% of host eggs are damaged in such a manner (Krüger 2011).

In this study, we experimentally tested the hypotheses that the thick-shelled eggs of the parasitic shiny and brown-headed cowbirds resist damage when laid from an elevated position; the parasite eggs resist damage when jostled in host nests; and the parasite eggs damage host eggs during these events. We evaluated whether the cowbird eggs resist damage when: 1) they were laid from an elevated position onto another egg, 2) they were hit by another egg laid from an elevated position whether it be a host or another cowbird egg, and 3) when they were jostled with other eggs in the nest when hosts attack the parasites during laying. Because cowbird eggs are thicker and stronger than expected based on allometry (Picman 1989; Mermoz and Ornelas 2004), we predicted that, in each of these 3 types of events, cowbird eggs would suffer less damage than host eggs. Finally, we investigated the characteristics of the eggs of the shiny and brown-headed cowbirds and their respective large and small hosts.



Figure 1

(a, b) Two female shiny cowbirds laying in the nest of a rufous-collared sparrow (*Zonotrichia capensis*). Red asterisks indicate the host egg that was hit by a cowbird egg (a). Red arrows indicate the parasite eggs, one of which was hit by another cowbird egg (b). White arrows denote the egg leaving the cloaca and white lines show the relative height from which the parasitic egg is laid (~6.5 cm). Picture credit: Henrique R. Domingos, IPBio—Instituto de Pesquisas da Biodiversidade, Reserva Betary, Brazil. (c) Female brown-headed cowbirds at the moment of laying at the nest of a blue-gray gnatcatcher (*Polioptila caerulea*) and (d, e) Bell's vireos (*Vireo bellii*). White arrows denote the egg leaving the cloaca and white lines show the relative height between the parasitic egg and the bottom of the nest.

METHODS

Study sites

The fieldwork was conducted in the Reserva El Destino in Buenos Aires province, Argentina (35° 08′ S, 57° 23′ W) during the southern breeding season from October 2014 to January 2015, and in Scott County, Iowa, USA (41°49′ N, 90° 63′ W) and Rock Island County, IL (41° 28′ N, 90° 34′ W) during the northern breeding seasons in May–July of 2015 and 2016. To take into account the wide range of body sizes of hosts of both cowbird species, we used eggs of small and large hosts. In both locations, we collected freshly laid eggs of the shiny (adult female mass: 47 g; Reboreda et al. 1996) and brown-headed (adult female mass: 39 g; Dunning 1992) cowbirds and eggs of their respective hosts during the laying periods. In South America, the small host was the house wren (*Troglodytes aedon*, 11 g; Dunning 1992) and the large host was the chalk-browed mockingbird (*Mimus saturninus*, 73 g; Dunning 1992). In North America, the small host was also the house wren and the large host was the red-winged blackbird (42 g; Dunning 1992). The chalk-browed mockingbird nests in open cups and is parasitized at 89% of nests with an average of 3 cowbird eggs per nest (see Gloag et al. 2012 for further details). The house wren nests in cavities and is parasitized at 60% of its nests with an average of 1.7 eggs per nest (Tuero et al. 2007). The house wren is not parasitized as frequently in North America likely because they nest in boxes with entrances too small for cowbirds to enter (Pribil and Picman 1997) and the red-winged blackbird is parasitized at approximately 29% of nests at our study site, with an average of 1.3 cowbird eggs per nest (Peer 2017).



Figure 2

Damage to host eggs during natural cowbird laying and simulated laying events. (a) Chalk-browed mockingbird (*Minus saturninus*) eggs were damaged by shiny cowbird (*M. bonariensis*) punctures. (b) A red-winged blackbird (*Agelaius phoeniceus*) egg and (c) a chestnut-capped blackbird (*Chrysomus ruficapillus*) egg dented during cowbird laying events. (d) Chalk-browed mockingbird and house wren eggs damaged during simulated laying events.

Experimental design

We conducted 2 experiments, an egg-dropping and an egg-jostling experiment similar to those by Soler and Martinez (2000) on the great spotted cuckoos and its magpie host. To standardize the experimental conditions between sites, we used a plastic artificial nest with a nest cloth lining that was 12-cm wide and 6-cm deep. House wren nests have an average diameter of 5.1 cm and depth of 6.7 cm; mockingbirds 9.4 cm and 9.4 cm, respectively, and red-winged blackbirds 12 cm and 7 cm, respectively (Yasukawa and Searcy 1995; Fiorini V, unpublished data). A single egg was collected from host nests and was used once in a test assigned randomly to an experiment. It is possible that we used eggs from the same female cowbird because we did not track their individual laying. To simulate laying, an egg (Egg1) was placed on a ruler secured to the nest rim and then pushed off onto the egg (Egg2) in the nest below. We dropped a parasite egg onto a host egg, a host egg onto a parasite egg, or a conspecific egg onto another. The distance from the nest rim to the bottom of the nest was standardized at 6 cm, and an egg (Egg1) was dropped onto the second egg (Egg2) in each trial. We used this distance based on our video analysis of shiny and brown-headed cowbirds laying (n = 39)laying visits; Ellison et al. in review; Figure 1). It should be noted that the distance from which the host eggs were being dropped is not typical for hosts, but this served as a control treatment to simulate laying of a thinly shelled host egg from the cowbird laying height. Egg2 was on the floor of the nest along with 3 plaster similar-sized eggs to simulate a host clutch. We did not use natural eggs as companion eggs to minimize the number of eggs collected. We filmed the majority of the tests to confirm that Egg1 had been dropped onto Egg2 and not onto a plaster egg. We recorded whether the eggs were cracked or dented (Figure 2d). In the egg-jostling experiments, we placed 2 eggs into the nest and "stirred" them with a wooden dowel in a figure eight motion for an average of 8 ± 1 s and averaged 9 ± 2 figure 8 movements per trial (Supplementary Video 2). This movement caused the eggs to ricochet off one another. In each trial, the nest contained either a parasite egg and a host egg or 2 conspecific eggs.

Egg and eggshell measurements

The length (L) and width (W) of a subsample of eggs were measured with calipers (Mitutoyo, accuracy: ± 0.02 mm), and mass using a digital scale (Precise 200A; accuracy: ± 0.002 g). We calculated the aspect ratio (W/L) as an index of egg shape (Picman 1989) and used egg volume (V) as an index of egg size and it was calculated from the equation: $V = 0.498 \times L \times W^2$ [mL] (Spaw and Rohwer 1987). After conducting the egg-dropping and egg-jostling tests, we measured the eggshell thickness (T) of a subsample of eggs using a micrometer (Mitutoyo 103–129; graduation: 1 µm; accuracy: ± 2 µm) with the shell membrane removed. We measured the thickness of 3–4 shell fragments (2–3 mm²) from the equatorial region of the egg because this is the area where most damage occurs during these events (Figure 2).

Statistical analysis

Experiments

To predict the probability of damage for each egg type in the eggdropping and jostling experiments, 2 logistic regression models were fitted using generalized linear models (GLM) assuming a binomial error structure, and we used the *glm* function and logit as the link function in the base package in R. Damage suffered by the focal egg was a binary response (yes/no), and egg type (parasite, large host, small host), treatment, and the interaction between egg type and treatment were predictor variables. We also performed a similar analysis based on individual species comparisons (Supplementary Data). In the egg-dropping experiment, the predictor treatment had 6 levels: egg dropped onto a parasite egg, egg dropped onto a large host egg, egg dropped onto a small host egg, egg hit by a parasite egg, egg hit by a small host egg, and egg hit by a large host egg. In the egg-jostling experiment, the predictor treatment had 3 levels: egg jostled with a parasite egg, egg jostled with a small host egg, and egg jostled with a large host egg. We checked the model assumptions through diagnostic statistics. We determined the significance of the models using the *anova* function. When significant differences were found, multiple comparisons (Tukey's post hoc tests) via the *glht* function in the "multcomp" package (Bretz et al. 2010) with adjusted *P* values were performed.

Egg and eggshell measurements

To evaluate if the egg parameters (aspect ratio, volume, mass, shell thickness) differed among species, the data were analyzed using generalized least squares (GLS) fitted by a restricted maximum likelihood (REML) via gls function from the "nlme" package in R (Pinheiro et al. 2014). The heteroscedastic variance in the model residuals was controlled by modeling the variance structure using the appropriate function (among varIdent, varPower, and varExp) according to the Akaike's information criterion (AIC) (Zuur et al. 2007, 2009). The anova function was used on the GLS models to determine significance and multiple comparisons (Tukey's post hoc tests) were performed via the glht function in the "multcomp" package with adjusted P values (Bretz et al. 2010). Egg size is a confounding variable that must be controlled for because it is positively correlated with shell thickness and egg strength (Picman 1997). Therefore, we also performed linear regression models (LM) with shell thickness as a dependent variable, and egg volume and mass as independent variables. To validate these models, we confirmed the assumptions of homogeneity of variance with the Breusch-Pagan test, the normally distributed residuals of the Shapiro-Wilk test, and the presence of autocorrelation in the residuals by Durbin-Watson test (Zuur et al. 2009, 2010). Finally, we evaluated if the residuals of the regression between shell thickness versus egg volume differed among species using generalized least squares (GLS).

All analyses were conducted using R statistical software version 3.4.1 (R development Core Team 2016). The α level was set at P < 0.05 and the values are expressed as mean \pm standard deviation. Plots were created using the "ggplot2" package in R.

Ethical note

All procedures performed in studies involving animals were in accordance with the ethical standards of the institutions at which

the studies were conducted. All work complied with the Argentinean Law for the Conservation of Wild Fauna (Ley Nacional de Fauna 22421/81) and was conducted with the authorization from the Organismo Provincial de Desarrollo Sostenible, Argentina (Permit number 202/12-O.P.D.S.). Experiments were likewise approved by the Western Illinois University IACUC (#15–17) and permit numbers SC863, NH16.5062, and MB122718-1. Our study followed the ASAB/ABS Guidelines for the Use of Animals in Research and all eggs were collected fresh, prior to incubation.

RESULTS

Egg-dropping and egg jostling experiments

The anova results confirmed that the interaction term between egg type and treatment was nonsignificant in both the egg-dropping and egg-jostling experiments. The predictor egg type was significant in these 2 experiments, whereas predictor treatment was only significant in the egg-dropping experiment (Table 1). In the egg-dropping experiment, the parasite egg had a low probability of being damaged (3.6%) when it collided with another egg, that is, when it was dropped onto an egg or when it was hit by an egg, regardless of whether the egg was a host or another parasitic egg (Figure 3a). The probability of damage to the large (45.9%) and small (32.8%) host eggs were significantly greater than that for the parasitic egg (Tukey's test: P < 0.001 for both pairwise comparisons), and there were no significant differences between the 2 host egg types (Tukey's test: P = 0.153; Figure 3a). An egg was significantly more likely to be damaged when it was hit by a parasitic egg (52.2%) and when it was dropped onto a parasitic egg (32.3%) (Tukey's test: P = 0.002; Figure 3b), but had a low probability of being damaged (overall 8.0 %) when it was hit by a small or large host egg or when it was dropped onto small or large host egg (Tukey's test: P > 0.06 for all pairwise comparisons; Figure 3b). The results were similar when analyzed based on individual species comparisons (Supplementary Figure 1).

During the egg-jostling experiment, the parasitic egg had a low probability of being damaged (0%) when it was jostled with conspecific or host eggs (Figure 3c). The probability of damage for the large host eggs was 8.3% and 6.6% for the small host eggs, but these rates did not differ significantly from that of the parasitic egg (Tukey's test: P > 0.9 for all pair-wise comparisons; Figure 3b). When we analyzed the jostling experiments based on individual species comparisons there were also no significant differences (Supplementary Figure 2).

Egg and eggshell measurements

The eggs of all 6 species differed significantly in their estimated values of mass (GLS: $F_{5, 52} = 381.00$, P < 0.001), volume (GLS:

Table 1

Anova results of the Generalized Linear Models for egg-dropping and egg-jostling experiments

	Predictor	df	Deviance Residual	df	Residual Deviance	P value
Egg-dropping experiment	NULL			697	761.09	
	treatment	5	144.66	692	616.43	<0.001
	egg type	2	125.67	690	490.76	<0.001
	treatment: egg type	6	7.12	684	483.64	0.310
Egg-jostling experiment	NULL			330	115.97	
	Treatment	2	1.70	328	114.26	0.427
	egg type	2	18.30	326	95.96	<0.001
	treatment: egg type	2	0.00	324	95.96	1.000

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Figure 3

(a) *Egg-dropping experiment:* damage (%) to the three egg types (large host: gray bar, parasite: black bar, and small host: white bar) when they collided with another egg; that is, when they were hit by or dropped onto an egg (striped); (b) eggs damaged (%; striped bar) when they were hit by or dropped onto an egg of a large host (gray egg), a parasite (black egg), or a small host (white egg). (c) *Egg-jostling experiment:* damage (%) to the three egg types (large host: gray bar, parasite: black bar, and small host: white bar) when jostled with another egg (striped egg). The asterisks (*) indicate the eggs in which the damage was measured. Tukey tests with different letters indicate statistically significant differences (P < 0.05). Sample sizes are indicated in parentheses.

 $F_{5, 52} = 570.43, P < 0.001$, aspect ratio (GLS: $F_{5, 52} = 49.97$, P < 0.001), and eggshell thickness (GLS: $F_{5, 52} = 397.22$, P < 0.001; Table 2). Shiny cowbird eggs were 43% larger, 54% heavier, and more rounded than brown-headed cowbird eggs (Tukey's test: P < 0.001 for pairwise comparisons of volume, mass, and egg shape; Table 2). Mockingbird and blackbird eggs were 45% and 36% larger, 42% and 39% heavier, and less rounded than the eggs of their respective parasites (Tukey's test: P < 0.001 for pairwise comparisons of volume, mass, and egg shape; Table 2). Southern and northern house wren eggs were 60% and 53% smaller, and 60% and 50% lighter than eggs of their respective parasites. Shiny cowbird eggs were more rounded than wren eggs (Tukey's test: P = 0.011), whereas brown-headed cowbird and wren eggs had similar egg shape (Tukey's test: P >0.080 for pair-wise comparisons; Table 2). Southern wren eggs were 23% larger and 20% heavier than northern wren eggs (Tukey's test: P < 0.001 for egg volume and mass), however, the northern wren's eggshells were 22% thicker than those of the southern wren (Table 2).

Finally, both parasitic species had thicker eggshells than their respective hosts without controlling for egg size (Tukey's test: P < 0.001 for pairwise comparisons; Table 2). When we controlled for egg size, the residuals of the regression between shell thickness and egg volume showed differences between parasitic and host species (GLS: $F_{5, 52} = 153.93$, P < 0.001). Both parasitic species had higher residual values than their respective hosts (Tukey's test: P < 0.001 for pair-wise comparisons; Table 2). Similar results were obtained using the independent variable egg mass. Approximately 98% of the mass variability was explained by the fitting linear regression obtained from volume (LM: $F_{1,56} = 2289.7$, P < 0.001, Adj. \mathbb{R}^2 : 0.975).

DISCUSSION

We found that cowbird eggs were damaged significantly less often than large and small host eggs when cowbird eggs were "laid" onto host eggs in both North and South American systems. Likewise, cowbird eggs were damaged significantly less often than large and small host eggs when host eggs were laid onto cowbird eggs. These results support the hypothesis that the thick eggshells of shiny and brown-headed cowbirds decrease the likelihood that they will be damaged during laying and also support the hypothesis that these eggs inflict damage to host eggs when laid from an elevated position that is typical of cowbirds. This is similar to *Clamator* cuckoos that lay from the nest rim or an elevated position (Gaston 1976; Arias de Reyna et al. 1982). Their thick shells protect the eggs during laying, and host eggs are frequently damaged resulting in enhanced incubation of the parasite egg and fewer host nestmates to compete against (Soler et al. 1997). Cowbirds should likewise benefit from fewer nestmates to compete against (e.g., Astie and Reboreda 2009; Fiorini et al. 2009) and also by having enhanced incubation in smaller clutches (Peer and Bollinger 1997, 2000). Damage to host eggs after being struck by cowbird and other brood parasite eggs has been recorded frequently (Wyllie 1981; Blankespoor et al. 1982; Kattan 1998; Soler et al. 1998; Peer and Sealy 1999; Figure 2a-c). Parasitized clutches invariably have fewer eggs than unparasitized clutches and it has been assumed to be a result of female parasites removing or damaging host eggs (Sealy 1992; Peer and Bollinger 2000; Astié and Reboreda 2006). However, our results and others (Gaston 1976; Soler et al. 1998) indicate that some of these eggs could have been lost from damage during laying and subsequently removed by hosts.

We found no evidence to support the hypothesis that thick eggshells provide protection when eggs are jostled in nests, although

Red-winged blackbird (large host, 42 g)	36 % larger volume than brown-headed cowbird egg	11	3.8 ± 0.2 b	$3.9 \pm 0.2^{\text{ b}}$	$0.74 \pm 0.02 ^{d}$	$86 \pm 2^{\text{f}}$	-0.9 ± 0.2 ^a
Brown-headed cowbird (parasite, 39 g)	led	10	2.8 ± 0.3 °	2.8 ± 0.5 [€]	0.77 ± 0.02 c	$104 \pm 5^{\circ}$	$0.9 \pm 0.2^{\rm d}$
Northern house wren (small host, 11 g)	53% smaller volume than brown-head cowbird egg	6	$1.3 \pm 0.1 \mathrm{d}$	$1.4 \pm 0.1 ^{d}$	0.81 ± 0.04 b,c	83 ± 2 d	$0.5 \pm 0.1 ^{\circ}$
Southern house wren (small host, 11 g)	60% smaller volume than shiny cowbird egg	6	1.6 ± 0.1 c	1.7 ± 0.1 c	0.76 ± 0.04 c,d	$68 \pm 2^{\circ}$	-1.1 ± 0.2^{a}
Shiny cowbird (parasite, 47 g)	lan	12	4.0 ± 0.2 b	$4.3 \pm 0.4^{\text{b}}$	0.80 ± 0.01 b	121 ± 3^{b}	$1.3 \pm 0.3 ^{\rm b}$
Chalk-browed mockingbird (large host, 73 g)	45% larger volume th shiny cowbird egg	\mathcal{N} 10	$v [ml] 5.8 \pm 0.8 ^{a}$	m [g] 6.1 ± 0.6 ^a	w/l 0.70 ± 0.02 ^a	$t [\mu m] 97 \pm 5 ^{a}$	Residual -0.8 ± 0.3 ^a

 $v = \exp$ volume; $m = \exp$ mass; $w/t = \exp$ roundness; $t = \exp$ thickness; residual = standardized residuals of the regression between Log shell thickness and Log egg volume. Values are reported as mean \pm standard deviation. Different letters (superscript) indicate statistically significant differences (P < 0.05).

Table 2

Egg parameters of the parasitic cowbirds and their hosts

our damage estimates should be viewed as conservative because it is possible more violent jolts may occur at the nests. We did find support for the multiple parasitism hypothesis in that thick eggshells provide protection when additional cowbird eggs are laid onto them because cowbird eggs were rarely damaged during these trials. This would be an important benefit for female shiny cowbirds because they multiply parasitize nests more frequently than brownheaded cowbirds (Gloag et al. 2012; Supplementary Video 1; but see Rivers and Peer 2016) and this may explain our finding that shiny cowbird eggshells were thicker than brown-headed cowbird eggs. Female shiny cowbirds also puncture eggs during nest inspection and laying visits (Fiorini et al. 2014), so a thick eggshell may help prevent such damage in multiply parasitized nests (Brooker and Brooker 1991).

Interestingly, house wren eggs in North America sustained less damage during our simulated laying events and were also more thickly shelled than those in South America (Table 2, Supplementary Figure 1). House wrens puncture conspecific eggs and those of other cavity-nesting species as a result of competition for limited cavity nests (Johnson 1998) and this behavior is more common in North America than in South America. House wrens puncture eggs up to 84% of the time in North America (Belles-Isles and Picman 1986), but only at 3% of nests in South America (Llambias and Fernandez 2009). This could account for the thicker eggshells and greater puncture resistance in the northern house wren compared to the southern house wren.

In summary, our data suggest that the thick eggshells of shiny and brown-headed cowbirds serve several important functions including protecting them against damage during laying, causing damage to host eggs, and preventing damage when additional cowbird eggs are laid onto them when nests are multiply parasitized. Spaw and Rohwer (1987) "tentatively" concluded that protection during laying events was not important in the evolution of thick eggshells and instead argued thick eggs evolved to withstand puncture-ejection. Our direct test of the protection during laying hypothesis suggests otherwise and also raises doubt as to the significance of puncture resistance in the initial evolution of thick eggshells by cowbird hosts. Although a thick eggshell prevents egg-puncture in some small cuckoo hosts (Antonov et al. 2006, 2009; Li et al. 2016), there are relatively few shiny and brown-headed cowbird hosts that punctureeject (Peer and Sealy 2004; Reboreda et al. 2013) and punctureejection does not appear to be especially costly in species studied to date (Sealy 1996). In contrast, cowbirds invariably lay quickly (Sealy et al. 1995; Peer and Sealy 1999), they often do so from an elevated position (Rothstein 1990; Ellison et al. in review), their eggs are typically laid onto host eggs because they wait until host eggs are present to increase the likelihood their eggs will be accepted (Rothstein 1975), and additional cowbird eggs may be laid onto their eggs in the event a nest is multiply parasitized (Gloag et al. 2012). Therefore, the selection pressure to lay thick-shelled eggs to protect cowbird eggs from damage during laying events appears to be more consistent than the selection pressure from puncture-ejection.

SUPPLEMENTARY MATERIAL

Supplementary data are available at Behavioral Ecology online

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Data accessibility: Analyses reported in this article can be reproduced using the data provided by López et al. (2018).

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