A comparative study of the structural and mechanical properties of avian eggshells among hosts of obligate brood parasitic cowbirds (genus *Molothrus*)

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Obligate avian brood parasites depend on hosts for parental care, which in turn suffer fitness losses as a result of parasitism. Mechanisms by which brood parasitic cowbirds (*Molothrus* spp.) reduce host breeding success include the puncture (*M. rufoaxillaris* and *M. bonariensis*) or removal (*M. ater*) of the eggs of the host. Our working hypothesis is that the host eggs' mechanical strength and their size and shape in species with higher frequency of parasitism covaries with the cowbird's strategy to reduce host clutch size. Our results, obtained through phylogenetic analyses based on egg 2D geometric morphometry and eggshell mechanical and ultrastructural measurements, suggest that egg-puncturer behaviour has led to an increase in the strength of the host's eggshell, which might make them more difficult to be pierced. We also characterized larger, more rounded and asymmetrical eggs in frequent hosts of *M. ater*, which might be more difficult to be removed. These interspecific host egg and shell traits were also positively affected by the frequency of parasitism, indicating that species-specific patterns of parasitic costs select for respective anti-parasitic defences in hosts.

ADDITIONAL KEYWORDS: brood parasitism – cowbirds – egg-puncturer – egg-removal – eggshell strength – eggshell thickness – eggshell ultrastructure – quasi-static punctures.

INTRODUCTION

Obligate avian brood parasites lay their eggs in nests of other avian species, the hosts; the latter provide all parental care to the parasitic eggs and chicks. Brood parasites depend entirely on the hosts for reproduction, which in turn suffer fitness losses as a result of parasitism (Ortega, 1998; Davies, 2000). Therefore, it is expected that natural selection has favoured the evolution of defence mechanisms in hosts that reduce the negative impact, and adaptations in the parasites that counteract these defences, through coevolutionary arms races (Payne, 1977; Dawkins & Krebs, 1979; Rothstein, 1990; Davies, 2000; Krüger, 2007; Soler, 2017).

Cowbirds (Icteridae: *Molothrus* spp.) are obligate brood parasites with have different strategies in their use of hosts. Thus, the screaming cowbird (*Molothrus rufoaxillaris* Cassin, 1866) is a specialist which almost exclusively parasitizes the greyish baywing (*Agelaioides badius* Vieillot, 1819), whereas the shiny cowbird (*Molothrus bonariensis* Gmelin, 1789) and the brown-headed cowbird (*Molothrus ater* Boddaert, 1783) are extreme generalists that each use over 200 hosts species covering a wide range of body sizes (Lowther, 2018). The initial

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mechanism by which parasitic cowbirds reduce host reproductive fitness also varies, ranging from the pecking and destruction of host eggs (through puncturing) or their removal (through either grasp-ejecting or puncture-ejecting). The screaming and shiny cowbird typically peck host and parasite eggs already in the nest until one or more are damaged. They then lay an egg of their own (Hudson, 1874; Fraga, 1998; Gloag *et al.*, 2012, 2014). In contrast, the brown-headed cowbird typically removes one host egg when it lays its own, by either grasp- or puncture-ejection (Sealy, 1992, 1994). Overall, the frequency of egg puncture behaviour by the brown-headed cowbird is much lower than that of the other two congeneric cowbirds in our analyses. This remains true, even taking into account that some localized brown-headed cowbird populations (Peer *et al.*, 2013) also engage in host-egg and -chick destruction, through mafia (Hoover & Robinson, 2007) or farming behaviours (Swan *et al.*, 2015), when encountering host nests from which parasitic eggs had been removed or were not present. Typically, brown-headed cowbirds remove one host egg for each parasite egg they lay, while leaving the other host eggs unpecked (Hauber, 2003). However, for some host species, the brown-headed cowbird does not regularly remove a host egg or peck host eggs when laying (Antonson *et al.*, 2020).

Several hypotheses have been proposed to explain the benefits of these parasitic behaviours. For example, the reduction of host's clutch size could increase the incubation efficiency of the parasitic egg(s) (Peer & Bollinger, 2000), the feeding efficiency of parasitic nestlings (Fraga, 1979), reduce the number of host young with which the parasitic hatchling needs to compete (Sealy, 1992; Kilner *et al.*, 2004), and/or induce the hosts to abandon their nests when the parasite finds the nest too late into the nesting cycle (Swan *et al.*, 2015).

Although the adaptiveness of egg puncture and egg removal is not fully understood, it is clear that these behaviours may have significantly negative effects on host fitness (Peer & Sealy, 1999; Nakamura & Cruz, 2000; Tewksbury *et al.*, 2002; Peer *et al.*, 2005). The mechanical strength of the host egg should affect the success of egg puncture attempts by screaming and shiny cowbirds, because eggs with stronger shells would reduce the likelihood of damage. Conversely, eggshell strength should have a relatively low influence on the success of the egg removal by the brown-headed cowbird. Instead, egg size and shell shape should affect the success of egg ejection. Our working hypothesis is therefore that the egg's mechanical strength, and its size and shape in host species with higher frequency of parasitism, covaries with the parasite's own strategy of reducing the host's clutch size. If so, then we must ask which characteristics of the host egg and shell are associated with these behavioural differences in their brood parasites. Our predictions are (1) eggs of host species frequently parasitized by the mainly egg-puncturing screaming and shiny cowbirds are mechanically stronger and stiffer than eggs of host species (1a) with lower frequency of parasitism by these two cowbirds and (1b) that are parasitized by the egg-remover brown-headed cowbird; (2) eggs of host species frequently parasitized by the brown-headed cowbird are larger and have a more rounded shape (making them more difficult to be grasped or punctured) than eggs of host species (2a) with a lower frequency of parasitism, and (2b) frequently or infrequently parasitized by the two egg-puncturer cowbird species.

We also complemented our research by studying the egg and eggshell traits of two populations of the house wren (*Troglodytes aedon* Vieillot, 1809), that despite being the same species, are parasitized by different species of cowbirds and suffer different egg destruction behaviours at intra-specific and inter-specific levels. House wrens in North America are rarely parasitized by brown-headed cowbirds (Pribil & Picman, 1997) but have a high frequency of conspecific egg punctures (Belles-Isles & Picman, 1986), whereas house wrens in South America are frequently parasitized by shiny cowbirds (Kattan, 1997; Tuero *et al.*, 2007; de la Colina *et al.*, 2016) but have a low frequency of egg punctures by both parasites and conspecifics (Kattan, 1997; Fiorini *et al.*, 2009; Llambías & Fernández, 2009).

MATERIAL AND METHODS

STUDY AREA AND EGG COLLECTIONS

Fieldwork was conducted in Buenos Aires Province, Argentina, during the southern breeding seasons (October to January of 2014/15 and 2015/16), and in New York State, United States, during the northern breeding season (May to July of 2015). We collected fresh (non-incubated) eggs (one egg per nest) of three cowbird species (the screaming cowbird, hereafter: ScCo, the shiny cowbird, hereafter: ShCo, and the brown-headed cowbird, hereafter: BrCo) and a total of forty of their respective host species that vary in their frequencies of parasitism and body size. We also included data from published studies on eggs belonging to eight host species of the brown-headed cowbird. For more details see the Supporting Information.

Geometrical characteristics of eggs

Egg dimensions [length and breadth (mm)] and mass (g) of the full egg were measured using a caliper (Mitutoyo, accuracy: ± 0.02 mm) and a digital scale (Precisa 200A, accuracy: ± 0.001 g). Eggshell mass was also measured after performing the mechanical puncture tests (see below). The egg volume and the eggshell ratio were estimated from the following equations:

Egg volume = $498 \times 10^{-6} \times L \times B^2$ (mL) (Spaw & Rohwer, 1987)
Eggshell ratio = $100 \times Ms/Me$

where L: egg length (mm), B: egg breadth (mm), Ms: shell mass (g) and Me: egg mass (g).

The egg shapes were estimated through variables generated from 2D geometric morphometric analyses using egg outline images. For each egg image, chain codes were registered on the contour to calculate the elliptical Fourier descriptors (EFDs) of the first eleven harmonics using the 'Momocs' package in R [v.1.3.0.9000 (Bonhomme *et al.*, 2014)]. A matrix of harmonic coefficients was used for subsequent statistical analyses. For methodological details, see Supporting Information.

Mechanical properties of eggshells

The mechanical properties of eggshells were evaluated by puncture tests that were performed on the equatorial section of the eggs. We measured both the force needed to fracture the eggshell (breaking strength or puncture resistance) and egg deformation until failure. From the force vs. displacement diagrams (e.g. Supporting Information, Fig. S1), we estimated two mechanical properties necessary to compare eggs of different sizes and species: eggshell puncture stiffness (N/mm²) and eggshell puncture energy (J).

Eggshell puncture resistance (N) describes the response of the eggshell as a composite complex structure (Voisey & Hunt, 1974) and is used as an index of eggshell strength (Bain, 1990). Puncture stiffness provides a relative and standard measure of the overall eggshell stiffness characteristics, and puncture energy provides a measure of the amount of energy that eggshell material can absorb during a puncture test within the elastic range (Mohsenin & Mittal, 1977). For more details, see Supporting Information.

Ultrastructural characteristics of eggshells

The ultrastructure of shell cross-sections was analysed from images obtained by scanning electron microscopy (SEM; Zeiss Supra 40), using a technique based on the in-lens secondary electron detector (applying high voltage, 5kV). The total thickness (μm) of each specimen was measured as the distance from its outermost surface to the point where the basal mammillary cones inserted into the organic membranes. The thickness of the mammillary layer was measured as the distance from the basal cones to the point at which the palisade columns fuse. The outer palisade and inner palisade layers were identified according to the descriptions of Mikhailov (1987) and Dennis *et al.* (1996). They are predicted to be involved in the strength of the eggshell (Carnarius *et al.*, 1996), whereas the mammillary layer probably does not contribute to the stiffness characteristics of the eggshell (Bain, 1990). We calculated the palisade layer ratio as 100×palisade layer thickness/total shell thickness.

Life history and host morphology data

For each host species, we collected data from published studies on parasitism frequency (parasitized nests/ total nests monitored), and body mass (g) and tarsus length (mm) of adult females.

The host species were grouped using two criteria: (1) according to host egg size in relation to the egg size of its respective parasite (four groups: large egg host of BrCo, small egg host of BrCo, large egg host of ScCo and ShCo, and small egg host of ScCo and ShCo); and (2) according to the parasitism frequency (four groups: frequent hosts of BrCo, infrequent hosts of BrCo, frequent hosts of ScCo and ShCo, infrequent hosts of ScCo and ShCo). We classified a host species as a "large host" when the mass of its egg was greater than the mass of the respective parasite's egg. We considered a host species as a "frequent host" when the overall parasitism rate was greater than 20%. For explanatory details of these thresholds, see Supporting Information.

Statistical analyses

Phylogenetic hypothesis

A set of 1000 topologies was obtained from the Bayesian posterior distribution provided in http://www.birdtree. org (Jetz *et al.*, 2012). A single phylogenetic consensus tree with branch lengths expressing divergence time was then generated [via the 'phytools' package, v.0.7.47 (Revell, 2009)] and used as a framework for performing all the analyses.

Host egg shape

The main components of egg shape variability were calculated by principal component analysis (PCA) on the matrix of harmonic coefficients using the 'Momocs'

package [v.1.3.0 (Bonhomme *et al.*, 2014)]. Egg shape variation along the PC axes were visualized using the *PCcontrib* function. Scores from the first PCs were used in the following analyses (see below, and Supporting Information). We also performed a phylogenetic principal component analysis (phylogenetic P-PCA) on the average values (one value per species) of the harmonic coefficients using the 'phytools' package [v.0.7.47 (Revell, 2009)]. We tested for a difference between host species (grouped according to parasitism frequency) regarding the distribution of the first three phylogenetic P-PC scores performing a phylogenetic multivariate analysis of variance (P-MANOVA) with Pillai's statistic, using the 'geiger' package [v.2.0.7 (Harmon *et al.*, 2008; Pennell *et al.*, 2014)]. Significance was evaluated on 1000 simulations under a Brownian-motion model of evolution. Phylogenetic post hoc *t*-tests [with Holm sequential Bonferroni correction for multiple levels (Holm, 1979; Revell, 2012)] were used to identify which host egg groups were different for each P-PC while controlling for phylogenetic effects ['phytools' package (Revell, 2009)].

P-MANOVA and P-LDA on host egg and shell traits

We tested for multivariate differences in the egg and shell traits among the host species (grouped according to their egg size and the parasitism frequency), using P-MANOVA with Pillai's statistic, via the 'geiger' package [v.2.0.7 (Harmon *et al.*, 2008; Pennell *et al.*, 2014)]. Significance was evaluated on 1000 simulations under a Brownian-motion model of evolution. The egg and shell traits used were eggshell puncture stiffness, eggshell puncture toughness, egg ellipticity (PC1), egg asymmetry (PC2), eggshell ratio, and palisade layer ratio. Each variable was log-transformed and then tested for evidence of phylogenetic signal using Pagel's λ (Pagel, 1999) implemented in the *phylosig* function ['phytools' package (Revell, 2012)]. Phylogenetic linear discriminant analysis (P-LDA) was performed using "phylo.fda.v0.2.R" script and the *phylo.fda* function (Schmitz & Motani, 2010). Standardized coefficients of discriminant functions were used to evaluate the relative contribution of the traits to discrimination.

Lambda values of some individual traits did not take extreme values close to 0 or 1, and the optimal lambda obtained by both discriminant function analyses were close to 0. Therefore, we also conducted standard multivariate analyses of variance and linear discriminant analyses, including data from parasite species and data from each of the egg samples (several individuals per species), with the function *lda* in the R package 'MASS' [v.7.3.52 (Venables & Ripley, 2002)]. For details, see Supporting Information.

Covariate-adjusted residuals

Typically, egg size variables (i.e. volume, mass) are positively correlated with adult body size and eggshell strength (Rahn *et al.*, 1975; Ar *et al.*, 1979; Thompson *et al.*, 1981; Spaw & Rohwer, 1987), although these relationships can also be affected by phylogeny (Rahn *et al.*, 1975; Deeming, 2007). These confounding variables that must be controlled for. An alternative method to remove the effect of a covariate on the response variable is by the use of residuals (Garcia-Berthou, 2001; Ceyhan & Goad, 2009). For assessing the differences in eggshell strength and egg size, we performed evolutionary associations through the phylogenetic generalized linear mixed-effects models (P-GLMMs) via the 'MCMCglmm' package in R [v.2.29 (Hadfield, 2010)]. We fitted the models using as response and fixed effect continuous variables: (a) eggshell strength vs. egg volume, (b) egg volume vs. female body mass, and (c) egg volume vs. female tarsus length. Phylogeny (given by the phylogenetic variance-covariance matrix) and species (as species-specific effect) were the random effect variables. We estimated R^2, an indicator of goodness of fit within a Bayesian framework, as the amount of variance explained by each model through alternative formulation of R^2 within a frequentist framework (following Gelman *et al.* 2017). We calculated the posterior probability of the phylogenetic signal of models, across the entire posterior distribution of model variances, using Lynch's phylogenetic heritability (h^2) equation, equivalent to Pagel's λ in generalized least-squares (GLS) models of phylogenetic signal inference (Lynch, 1991; Pagel, 1999; Hadfield & Nakagawa, 2010, but see Garamszegi, 2014). The phylogenetic signal is defined as the tendency for related species to resemble each other more than they resemble species drawn at random from the tree, and it can range from 0 (no phylogenetic signal, equivalent to a 'star' phylogeny) to 1 (consistent with Brownian Motion) (Blomberg & Garland, 2002). Then host species, grouped according their parasitism frequencies, were compared using Bayesian analysis of variance [via the 'MCMCglmm' package, v.2.29 (Hadfield, 2010)] on these residuals. All Bayesian models were run for 5 000 000 iterations with a burn-in of 10 000 and a thinning interval of 500. This generated 10 000 samples from each chain from which parameters were estimated. Finally, the Bayesian contrasts were tested using the *tidyMCMC* function from the 'broom.mixed' package in R [v.0.2.6 (Bolker & Robinson, 2020)]. Fitted models and contrasts were considered statistically significant when the 95% credible interval (CrI $_{95\%}$) did not span 0 or PMCMC < 0.05 (Hadfield, 2010). The phylogenetic signal in our models had values of ~0.5 (range: 0–1). Therefore we also conducted standard Bayesian

linear models (BMLs), including data from parasite species and data from each of the egg samples (several individuals per species). For details, see [Supporting Information](#).

Evolutionary associations using parasitism rate as a predictor variable

To assess patterns of evolutionary associations between egg and shell traits on the frequency of parasitism, we fitted P-GLMMs (for model details, see above). The log-transformed response variables were eggshell puncture stiffness, eggshell puncture energy, egg ellipticity (PC1), egg asymmetry (PC2), eggshell ratio and palisade layer ratio. The fixed effect continuous variables were parasitism frequency by the egg-remover cowbird (BrCo) and parasitism frequency by the egg-puncturer cowbirds (ScCo and ShCo). Phylogeny and species were the random effect variables. We estimated R^2 and h^2 (see above).

All statistical computations were conducted with R software [v.3.6.3 ([R Core Team, 2017](#))]. The plots were generated using the R package 'ggplot2' [v. 3.3.2 ([Wickham, 2016](#))].

Data availability

The life history and host morphology data are available in [Supporting Information, Table S5](#), and the data set of egg and shell mechanical and structural traits is given in [Supporting Information, Table S5](#). These and additional data may be downloaded from figshare.com ([López *et al.*, 2021](#)).

RESULTS

HOST EGG SHAPES

The variation in egg shapes was well distributed among the phylomorpho-space axes. The first dimension (P-PC1) accounted for 94.26% of the variation among samples, and primarily described the egg ellipticity. The second dimension (P-PC2) accounted for 2.83% of the variation and described primarily the egg asymmetry ([Fig. 1](#); [Supporting Information, Table S6](#)). The egg shape determined by 2D geometric morphometry varied among the host eggs grouped by parasitism frequencies (P-MANOVA: Pillai's trace = 0.759, $F_{5,42} = 2.847$, $P < 0.001$, $P_{phylo} < 0.003$, where P-PC axes 1 to 3 were retained). However, the model was significant only when P-PC1 was the dependent variable (P-ANOVA: $F_{5,42} = 4.321$, $P = 0.003$, $P_{phylo} = 0.018$). Phylogenetic post hoc *t*-tests showed pairwise differences between the frequent hosts of BrCo and the remaining three groups of host eggs: the

infrequent hosts of BrCo ($P = 0.048$), the frequent hosts of ScCo and ShCo ($P = 0.006$), and the infrequent hosts of ScCo and ShCo ($P = 0.013$). Overall, the phylomorpho-space projection of the first two main components indicated that the host species frequently parasitized by the egg-remover cowbird (BrCo) were located towards the negative values of P-PC1, indicating a rounded shape ([Fig. 1](#)).

Phylogeny had a low impact on the egg ellipticity (P-PC1; $\lambda = 0.38$, $P = 0.547$). We complemented these results by performing standard principal component analysis (PCA) on the matrix of harmonic coefficients to include data from parasitic cowbirds and intraspecific variability ([Supporting Information, Figs S2, S3](#)). Overall, the morpho-space projections also indicated that the group of the host species frequently parasitized by BrCo showed the most rounded egg shapes ([Supporting Information, Fig. S3](#)).

P-MANOVA AND P-LDA ON THE HOST EGG AND SHELL TRAITS

P-MANOVAs showed centroids of host species were well separated with the egg and eggshell traits, when these were grouped by their relative egg sizes (Pillai's trace = 1.137, $F_{3,34} = 3.152$, $P < 0.001$, $P_{phylo} < 0.001$), and their parasitism frequencies (Pillai's trace = 0.842, $F_{3,34} = 2.014$, $P < 0.016$, $P_{phylo} = 0.029$).

When the host species were grouped according to their relative egg sizes, 68.25%, 27.18% and 4.57% of the variance of the group centroids were explained by the first axis (P-LD1), second axis (P-LD2) and third axis (P-LD3), respectively. The most important contributors that explain the differences between the groups were: eggshell puncture stiffness, palisade layer ratio (for P-LD1), eggshell puncture energy, egg ellipticity (for P-LD2), and eggshell ratio (for P-LD3) ([Fig. 2](#); [Supporting Information, Table S1](#)). Overall, the scatter plots of P-LDA show a clear separation of the large hosts of the two more frequently egg-puncturer cowbirds and the small hosts of the egg-remover cowbirds groups, and the variables that best contributed to this separation were: eggshell puncture stiffness, eggshell puncture energy and egg ellipticity ([Fig. 2](#)).

When the host species were grouped according to their parasitism frequencies, 82.35%, 11.38% and 6.27% of the variance of the group centroids were explained by the first axis (P-LD1), second axis (P-LD2) and third axis (P-LD3), respectively. The most important contributors were eggshell puncture stiffness, egg ellipticity (for P-LD1), eggshell puncture energy (for P-LD2), eggshell ratio and palisade layer ratio (for P-FD3) ([Fig. 3](#); [Supporting Information, Table S1](#)).
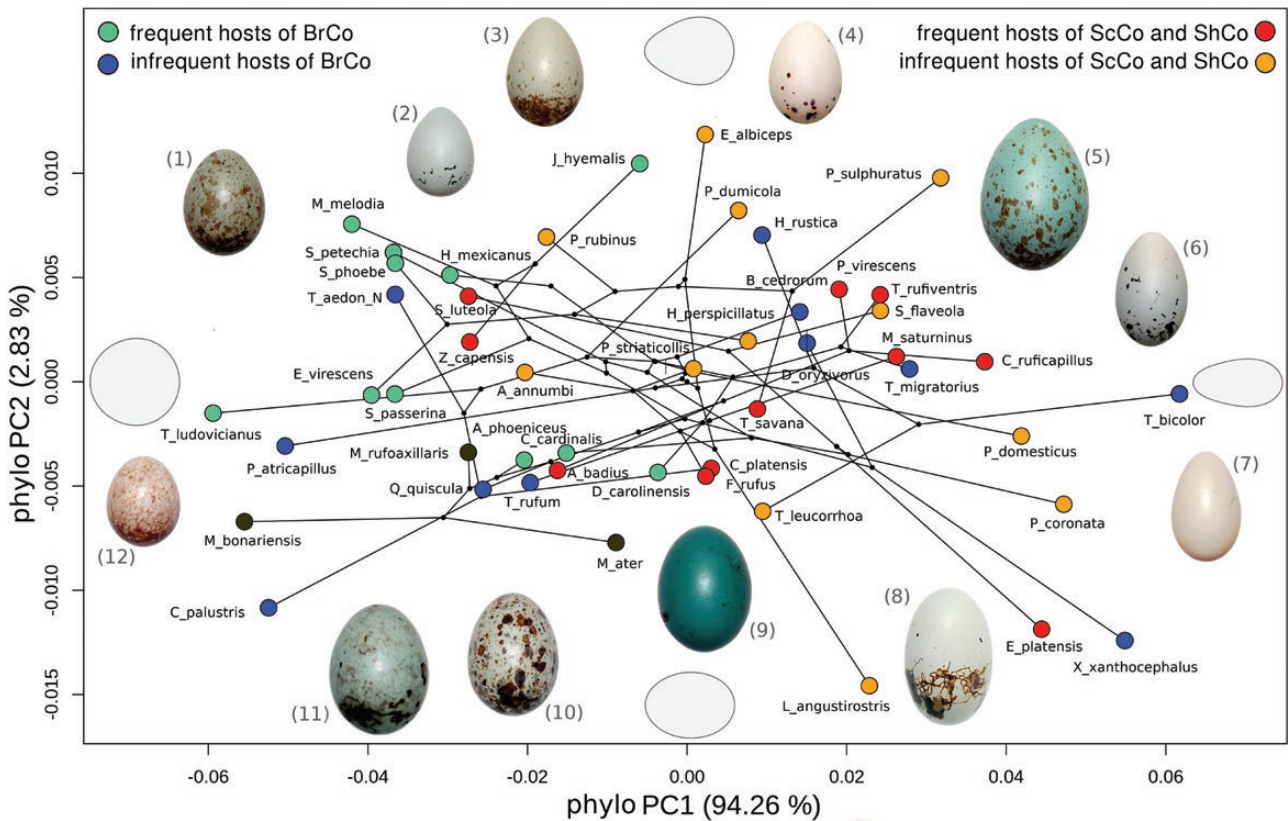
Figure 1. Phylomorpho-space projection of the first two principal components performed on shape descriptors of egg outlines. Each dot represents one species (i.e. PC scores from the harmonic coefficients mean value for each species), coloured according to their frequencies of parasitism by the egg-remover cowbird (BrCo) and egg-puncturer cowbirds (ScCo and ShCo). The dots are connected by lines that indicating phylogeny. Illustrations of some egg species representing extreme points: (1) *Melospiza melodia*, (2) *Haemorhous mexicanus*, (3) *Junco hyemalis*, (4) *Elaenia albiceps*, (5) *Turdus rufiventris*, (6) *Chrysomus ruficapillus*, (7) *Tachycineta bicolor*, (8) *Embernagra platensis*, (9) *Dumetella carolinensis*, (10) *Cardinalis cardinalis*, (11) *Agelaius phoeniceus* and (12) *Thryothorus ludovicianus*.

Overall, the scatter plots of P-LDA show a clear separation of the frequent hosts of the BrCo, and the egg ellipticity was the variable that best contributed to this separation (Fig. 3). Eggs from this group had a more rounded shape (PC1) than infrequent hosts of BrCo (post hoc *t*-tests: *P* = 0.042), frequent hosts of ScCo and ShCo (post hoc *t*-tests: *P* = 0.024), and infrequent hosts of ScCo and ShCo (post hoc *t*-tests: *P* = 0.025). On the other hand, eggs from frequent hosts of ScCo and ShCo required greater puncture energy than eggs from frequent and infrequent hosts of BrCo (post hoc *t*-tests: *P* = 0.051 and *P* = 0.024, respectively; Supporting Information, Table S2). In addition, the eggs from frequent hosts of BrCo had a lower eggshell ratio than the frequent (post hoc *t*-tests: *P* = 0.012) and infrequent (post hoc *t*-tests: *P* = 0.015) hosts of ScCo and ShCo (Supporting Information, Table S2).

Phylogeny had a moderate influence on the variables eggshell puncture stiffness, eggshell

puncture energy, and egg ellipticity (Supporting Information, Table S2). We complemented our results by performing standard MANOVA and LDA to include data from parasite cowbirds and intraspecific variability (Supporting Information, Table S3; Figs S4, S5). Overall, eggs from large and small host species of the two more frequently egg-puncturer cowbirds (ScCo and ShCo) showed greater eggshell mechanical puncture stiffness and energy than host species of egg-ejecter cowbirds (BrCo) (Supporting Information, Tables S4 and S6). When the host species were grouped according to their parasitism frequencies, eggs from frequent hosts of the egg-puncturer cowbirds (ScCo and ShCo) required greater eggshell puncture energy; whereas eggs from frequent hosts of the egg-ejecter cowbirds (BrCo) had a more rounded shape (PC1) and showed a lower proportion of the eggshell amount (eggshell ratio) (Supporting Information, Table S4).
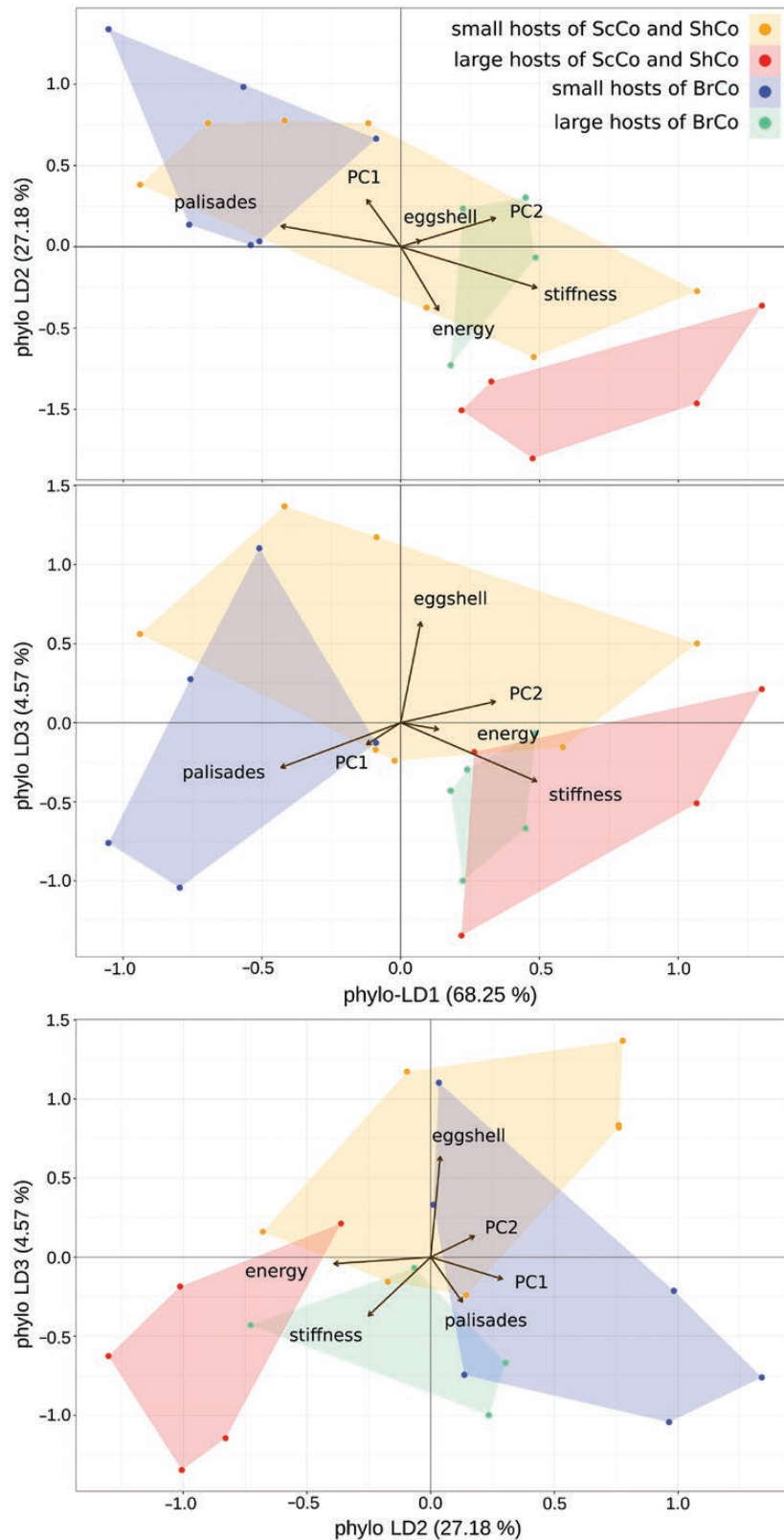
Figure 2. Phylogenetic linear discriminant analysis of mechanical and structural characteristics of eggs and shells (traits) and egg host species grouped by relative egg size. Convex hulls, which connect the most distant points of each

COVARIATE-ADJUSTED RESIDUALS

As expected, eggshell strength increased with egg volume (P-GLMM: slope = 0.640, PMCMC < 0.001, R^2 = 0.540, h^2 = 0.550); and egg volume increased with body mass (P-GLMM: slope = 0.710, PMCMC < 0.001, R^2 = 0.882, h^2 = 0.607) and with tarsal length of the adult female (P-GLMM: slope = 1.450, PMCMC < 0.001, R^2 = 0.686, h^2 = 0.867). Thus, the analyses of variance were performed on covariate-adjusted residuals (Fig. 4). Residual eggshell strength differed significantly between host species of ScCo and ShCo and host species of BrCo (Bayesian contrast: none of the CrI$_{95\%}$ included zero in the four pairwise comparisons). Overall, residual eggshell strength values of both host groups of ScCo and ShCo were positives without showing differences (Bayesian contrast: CrI$_{95\%}$ = -0.217 to 0.129), while these values of both host groups of BrCo were negatives without showing differences (Bayesian contrast: CrI$_{95\%}$ = -0.269 to 0.101) (Fig. 4). Residual egg volume of the frequent hosts of BrCo was positive and differed from the remaining three host eggs' groups when the covariate was female body mass (Bayesian contrast: CrI$_{95\%}$ did not include zero in the three pairwise comparisons). However, residual egg volume did not differ significantly between host species groups when the covariate was tarsal length of female (Bayesian contrast: CrI$_{95\%}$ included zero in the three pairwise comparisons) (Fig. 4).

We complemented these results through standard generalized linear mixed-effects models to include intraspecific variability data (see Supporting Information). In this case, residual eggshell strength of the frequent hosts of egg-puncturer cowbirds was positive and differed from the three host eggs groups (Bayesian contrast: CrI$_{95\%}$ did not overlap with zero in any of the three pairwise comparisons) (Supporting Information, Fig. S6).

EVOLUTIONARY ASSOCIATIONS USING PARASITISM
RATE AS A CONTINUOUS PREDICTOR

We found evidence of a positive association between eggshell mechanical traits (puncture stiffness and puncture energy) and the frequency of parasitism by of egg-puncturer cowbirds (P-GLMM: slope = 0.007, PMCMC = 0.024; and slope = 0.007, PMCMC = 0.011, respectively; Fig. 5). There was no evidence of an association between the eggshell mechanical

traits and the parasitism frequency of egg-remover cowbirds (P-GLMM: slope = -0.002, PMCMC = 0.491; and slope = -0.006, PMCMC = 0.134, respectively; Fig. 5). Regarding the two variables that describe the egg shape (ellipticity and asymmetry), we found evidence of a positive association between the egg ellipticity and the parasitism frequency of egg-remover cowbirds (P-GLMM: slope = 0.001, PMCMC < 0.001) (Fig. 6). Finally, regarding the variables that related to eggshell ultrastructure, there was a positive association between the palisade layer ratio and the parasitism frequency of egg-puncturer cowbirds (P-GLMM: slope = 0.007, PMCMC = 0.026) (Fig. 7).

DISCUSSION

Generalist brood parasitic cowbirds both in North and South America usually lay their eggs from elevated positions, thereby damaging some of the host eggs (Fraga, 2011; López *et al*., 2018). Cowbird species differ in the use of their beaks to reduce the clutch size of their hosts. Some of them damage host eggs through repeatedly puncturing host and parasite eggs already present in the nest, whereas others remove a host egg by either grasp- or puncture-ejection.

Observers who have described the method by which brown-headed cowbirds carry eggs have indicated that they can spike them on their opened mandibles (Hann, 1941; Norris, 1944; Harrison, 1975; Sealy, 1994). Brown-headed cowbirds typically remove one egg from nests they parasitize (Sealy, 1992, 1994) and replace it with one of their own, so that total clutch size remains stable (Hauber, 2003). However, the presence of pecked host eggs is the exception and not the rule in host nests of the brown-headed cowbird (Antonson *et al*., 2020). Furthermore, Dubina & Peer (2013) and Llambías *et al*. (2006) examined the egg-pecking behaviour of captive brown-headed, shiny and screaming cowbirds by presenting them with nests containing artificial eggs. Overall, 49% of captive brown-headed cowbird individuals pecked eggs, with the number of pecks per individual as 11 ± 3 (mean ± SE; Dubina & Peer, 2013), while more than 70% and 60% of shiny and screaming cowbird individuals pecked eggs, with a number of

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level of both sets of groups, are shown; internal points were omitted from the plot. The length of arrows represents the contribution of the traits to discrimination. For canonical coefficients of the phylogenetic discriminant function analyses, see Supporting Information, Table S1. Arrow references: stiffness = eggshell puncture stiffness; energy = eggshell puncture energy, PC1 = egg ellipticity (increasing values of PC1 indicate greater roundness of the egg; Supporting Information, Fig. S2), PC2 = egg asymmetry (increasing values of PC2 indicate greater symmetry of the egg; Supporting Information, Fig. S2), eggshell = ratio of the eggshell mass relative to the egg mass (eggshell ratio), palisades = ratio of palisade layer thickness in relation to total thickness of eggshell (palisade layer ratio).
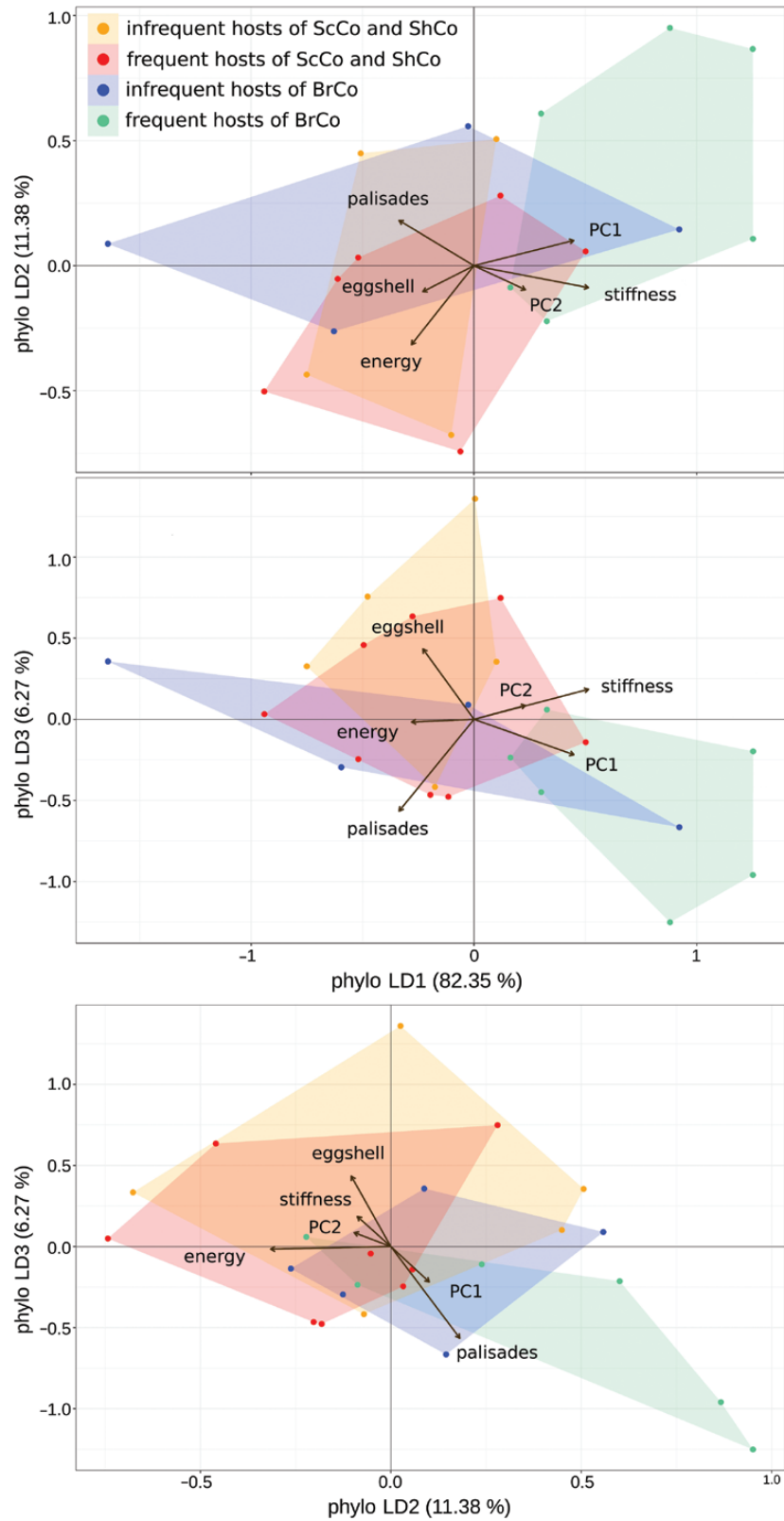
Figure 3. Phylogenetic linear discriminant analysis of mechanical and structural characteristics of eggs and shells (traits) and egg host species grouped by their frequency of parasitism. Convex hulls, which connect the most distant points of each

39 ± 19 and 49 ± 20 pecks per individual, respectively (Llambías *et al.*, 2006). Thus, egg-puncturer cowbirds performed between 3.5 and 4.5 times more pecks than the brown-headed cowbirds. Furthermore, brown-headed cowbirds peck host or fake eggs much more frequently in captivity than they do in the wild (White *et al.*, 2009). Finally, although some populations of brown-headed cowbirds engage in host-egg destruction through mafia (Hoover & Robinson, 2007) or farming (Swan *et al.*, 2015) behaviours, these patterns seem to be localized and may not be representative of the species as a whole (Peer *et al.*, 2013).

In turn, the host's eggs are frequently punctured by shiny and screaming cowbirds in parasitized and non-parasitized nests in the wild, and the high frequency of this egg-puncturer behaviour is well documented (see supplementary videos in Gloag *et al.*, 2012, 2014) and has been mentioned by several authors (e.g. Hudson, 1874; Hoy & Ottow, 1964; Post & Wiley, 1977; Fraga, 1978, 1985, 1998; Carter, 1986; Mermoz & Reboreda, 1994; Lichtenstein, 1998; Massoni & Reboreda, 1999, 2002; Nakamura & Cruz, 2000; Astié & Reboreda, 2006, 2009; Gloag *et al.*, 2014), who reported that it is one of the most important costs of brood parasitism, leading to reduced host clutch and brood sizes, and also reduced numbers of fledglings.

What defences have hosts evolved to counter the egg-puncturer and grasp-rejecting behaviours of adult brood parasites (Peer, 2006)? No hypothesis is likely to be all-encompassing, because these behaviours vary among parasitic species, and even within single species. Egg puncture and grasp-removal may be behaviourally plastic with multiple functions. Our results suggest that egg-removal behaviour by brown-headed cowbirds results in the evolution of anti-parasitic defences in the eggs of their frequent hosts, through imposing strong selection pressures that affect the shape and size of host eggs. Larger, more rounded and asymmetrical eggshells of the more frequent hosts may be more difficult to be ejected. In parallel, the high frequencies of egg-puncturer behaviours of screaming and shiny cowbirds may have also influenced the egg evolution of their frequent hosts, by imposing selection pressure to increase the stiffness and strength of the eggshells. A greater eggshell mass in relation to the internal egg mass, also them more difficult to be damaged by pecks from the parasites.

The eggshells of the screaming and shiny cowbird's are extremely strong, stiff and are able to absorb energy elastically, that is, without being damaged (Supporting Information, Table S4). These mechanical properties are the result of the underlying characteristics in the ultra- and microstructure of the calcite crystals that make up the parasitic eggshells (López *et al.*, unpublished data). However, these egg and eggshell traits of the two egg-puncturer parasitic species are not shared with the egg-removing brown-headed cowbird (Supporting Information, Table S4). Our results confirm, through direct and quantitative measurements, the hypothesis that eggs of screaming and shiny cowbirds have a reduced likelihood of being punctured by other parasitic females visiting the same nest (Hudson, 1874; Brooker & Brooker, 1991).

Multiple parasitism is very common in hosts of the two species of egg-puncturer cowbird but is far less common in most brown-headed cowbird hosts (Hauber, 2001). For example, up to 90% of greyish baywing, 65% of brown-and-yellow marshbird (*Pseudoleistes virescens*), 80% of chalk-browed mockingbird (*Mimus saturninus*) and 60% of rufous-bellied thrush (*Turdus rufiventris*) nests are parasitized by two or more screaming and/or shiny cowbird eggs, with an intensity of parasitism between 2-5 eggs per parasitized nest (Fernández & Mermoz, 2000; Sackmann & Reboreda, 2003; De Mársico *et al.*, 2010; Gloag *et al.*, 2012). Multiple parasitism may affect the success of large hosts less than that of smaller hosts because many large hosts can care for broods containing parasitic young and their own young (Peer & Bollinger, 1997; Mermoz & Reboreda, 2003; Sackmann & Reboreda, 2003). In contrast, large hosts are also significantly affected by egg puncture (Astié & Reboreda, 2006), rather than by losses incurred when cowbird nestlings outcompete their own (Lorenzana & Sealy, 1999). In addition, egg puncture can be costly behaviour for the cowbirds themselves, because it may increase the probability of total nest abandonment by the hosts (Fraga, 1978; Massoni & Reboreda, 1998), Therefore, the benefits of egg puncturer by cowbirds in large hosts may outweigh the costs of the increasing probability of nest abandonment (Fiorini *et al.*, 2009).

Accordingly, the scatter plots of P-LDA and LDA (Fig. 2; Supporting Information, Fig. S4) support

level of both sets of groups, are shown; internal points were omitted from the plot. The length of arrows represents the contribution of the traits to discrimination. For canonical coefficients of the phylogenetic discriminant function analyses, see Supporting Information, Table S1. Arrow references: stiffness = eggshell puncture stiffness; energy = eggshell puncture energy, PC1 = egg ellipticity (increasing values of PC1 indicate greater roundness of the egg; Supporting Information, Fig. S3), PC2 = egg asymmetry (increasing values of PC2 indicate greater symmetry of the egg; Supporting Information, Fig. S3), eggshell = ratio of the eggshell mass relative to the egg mass (eggshell ratio), palisades = ratio of palisade layer's thickness in relation to total thickness of eggshell (palisade layer ratio).
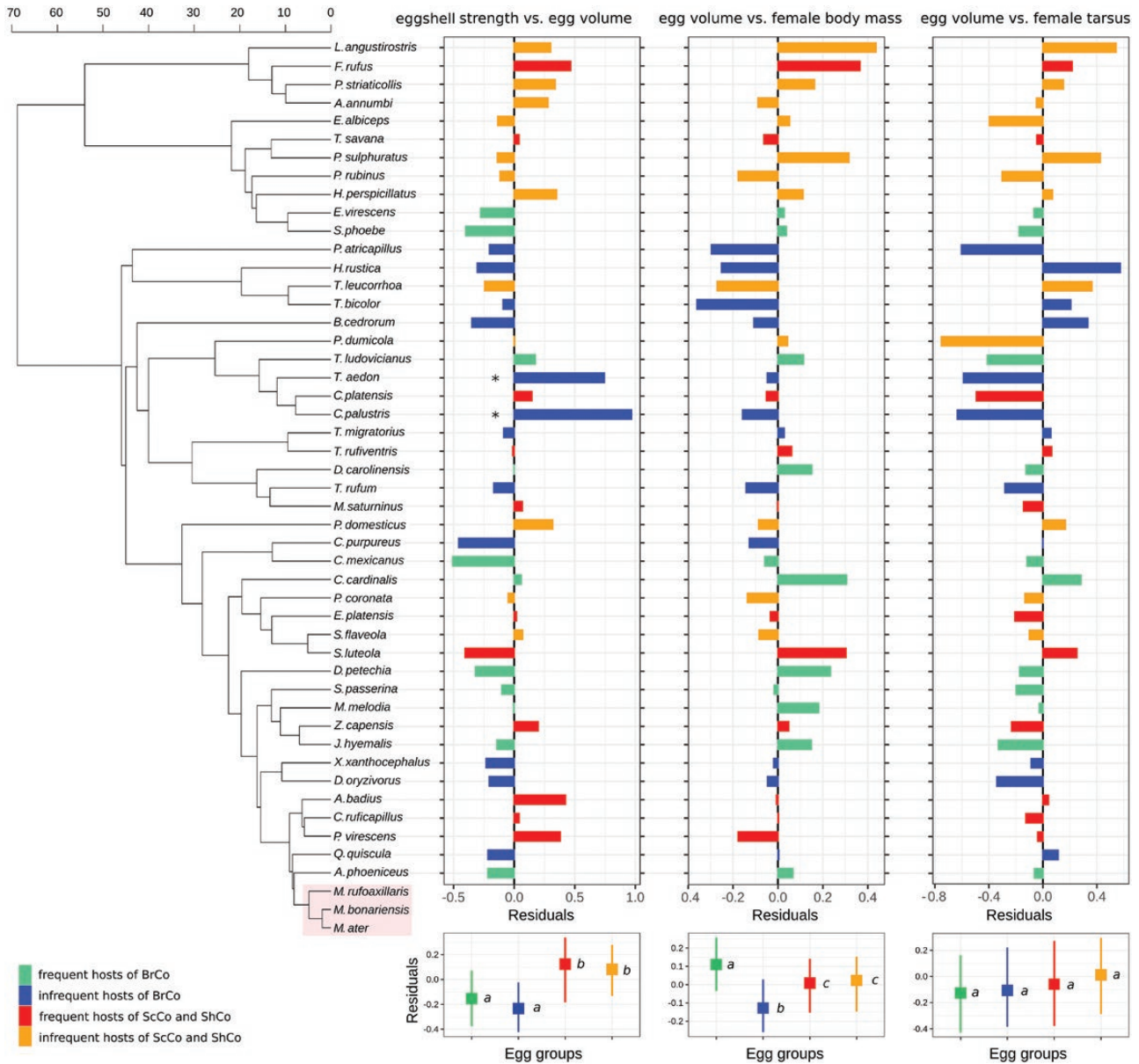
Figure 4. Phylogeny showing the species of the parasite cowbirds and hosts used in the comparative analyses. Residual eggshell breaking strength (using egg volume as a covariate) and residual egg volume (using body mass and tarsus length of female as covariates) for the host species are indicated to the right of the phylogeny. Plots of posterior mean and 95% credible interval associated with each host species groups are indicated below. Different letters indicate there is evidence that the response differs between the groups. (*) *Troglodytes aedon* and *Cistothorus palustris* (both in North America) exhibit intraspecific egg puncture behaviours and low frequency of BrCo parasitism (e.g. Belles-Isles & Picman, 1986; Picman *et al.*, 1996). These two showed exceptionally high values of eggshell breaking strength and eggshell puncture stiffness (this study), possibly as a counter-defence to high frequencies of intraspecific egg punctures (Picman *et al.*, 1996; this study).

a statistical separation of large hosts of the egg-puncturer cowbirds from the remnant host groups, and the variables eggshell puncture stiffness and puncture energy contributed to this separation. Thus,

despite our results from the phylogenetic univariate analysis not showing differences in eggshell puncture stiffness between groups of hosts, the magnitude of the phylogenetic signal influencing eggshell puncture
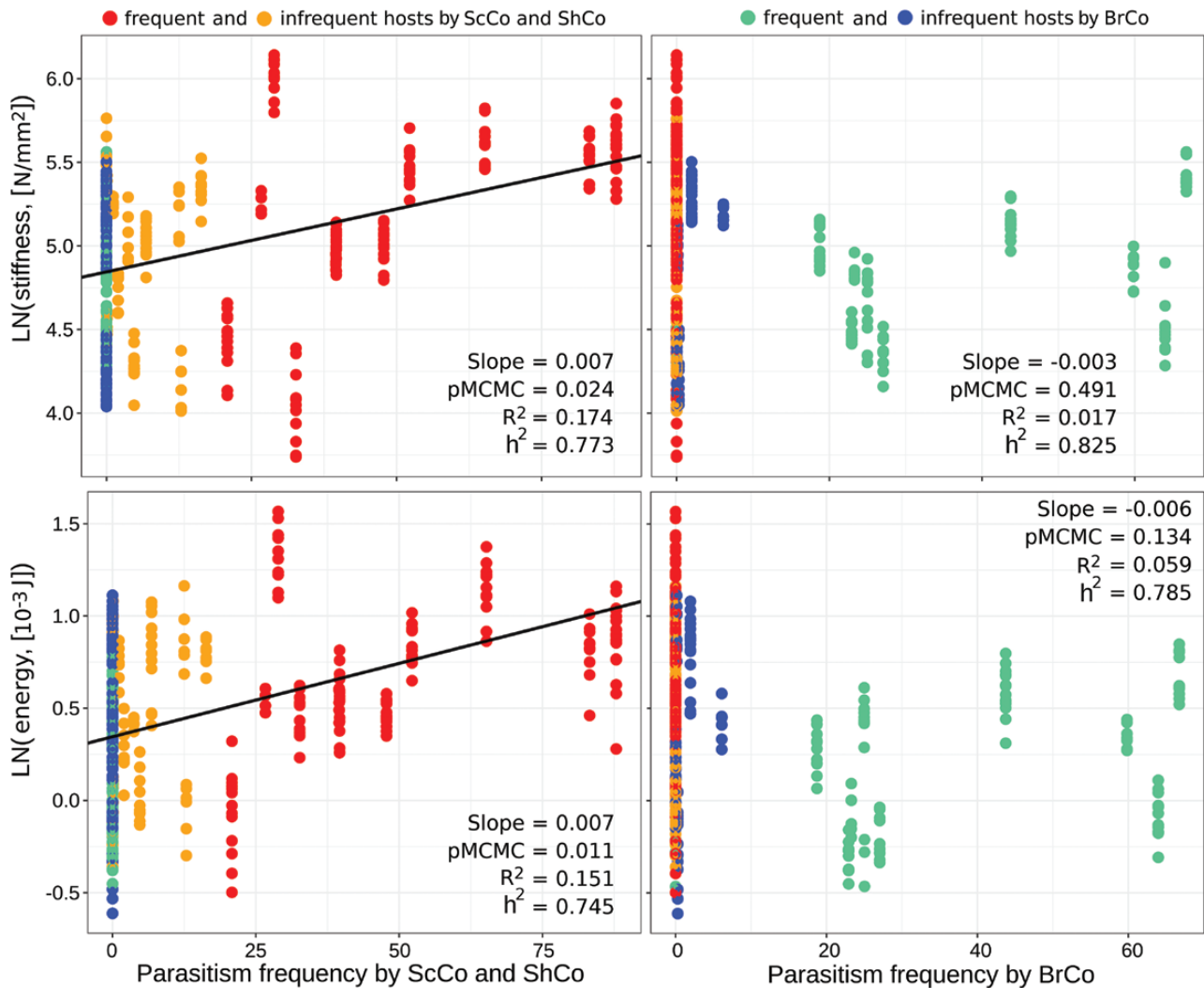
Figure 5. Phylogenetic linear regressions between host egg mechanical traits (eggshell puncture stiffness, and eggshell puncture energy) and parasitism frequency of the egg-puncturer (ScCo and ShCo) and egg-remover (BrCo) species of cowbirds.

stiffness was moderate ($\lambda = 0.58$, $P = 0.037$). This indicates that the trait has not evolved independently of phylogeny and has not evolved according to Brownian motion (i.e. we found no strong evidence that closely related species are more similar in their eggshell traits than distantly related species). Therefore, we complemented our analyses by performing standard tests and assessing intraspecific variability. The eggshells of large and frequent hosts of the egg-puncturer cowbirds were stiffer, and the eggs of this frequent host group also had stronger shells (according to our covariate-adjusted residual analyses) than the remaining host groups. The high frequencies of the egg-puncturer behaviour of screaming and shiny cowbirds thus seem to induce the evolution of strong anti-parasitic defences in the eggs of their large and common hosts, making their eggs to be difficult to damage by pecking.

Contrary to what is commonly suggested by previous studies using indirect estimates of host and parasitic eggshell strengths (e.g. Spaw & Rohwer, 1987; Rothstein, 1990), the brown-headed cowbird's eggs did not prove to have hells stronger and stiffer in our tests than those of their large hosts (Supporting Information, Table S4), or of any of their frequent and infrequent hosts (see Supporting Information). The only mechanical property of the brown-headed cowbird eggs that stood out relative to the eggs of their hosts analysed in our research was the formers' greater ability to absorb a higher amount
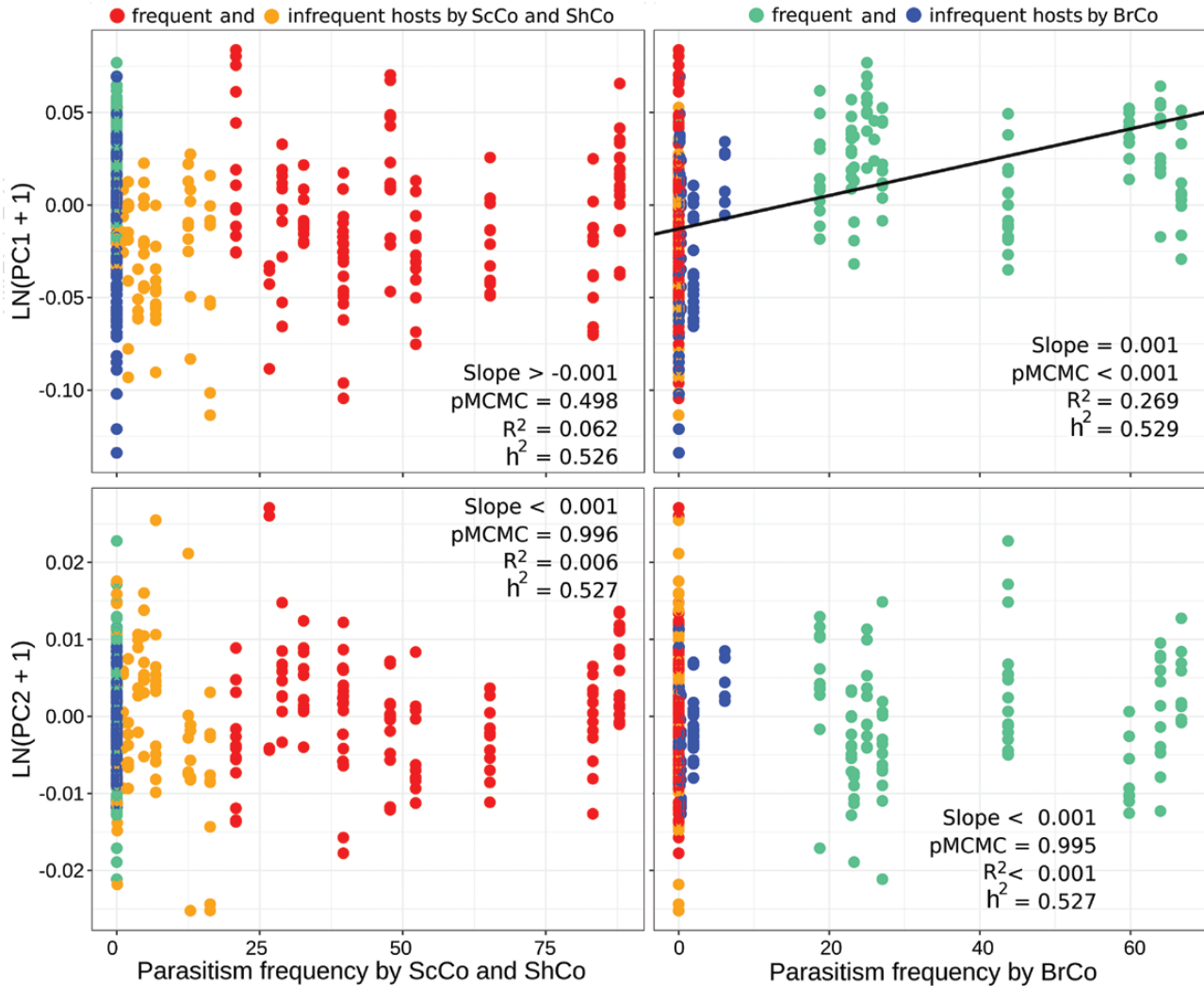
Figure 6. Phylogenetic linear regressions between host egg shape traits (PC1, and PC2) and parasitism frequency of the egg-puncturer (ScCo and ShCo) and egg-remover (BrCo) cowbirds. PC1 and PC2 are related to the ellipticity and asymmetry of the eggs, respectively. In these cases, increasing values of PC1 and PC2 indicate a more rounded and more symmetrical shape of the eggs (Supporting Information, Fig. S3).

of energy without breaking (eggshell puncture energy). This trait is still advantageous for the cowbirds due to their egg-laying behaviour from an elevated position (López *et al.*, 2018) and to the attempts of some of their smaller hosts to pierce-eject parasitic eggs when grasp-ejection is bill-gape size-wise impossible (Sealy, 1996). The cowbird's eggshell puncture energy is positively associated with ultrastructural properties of the shell, such as the proportion of the eggshell mass in relation to the total mass (López *et al.*, unpublished data). This is another trait of the brown-headed cowbird eggs that distinguishes them from those of its hosts (Supporting Information, Table S4).

On the other hand, the egg removal behaviour of host eggs by brown-headed cowbirds also seems to drive the evolution of anti-parasitic defences in their hosts, by affecting their shape and size characteristics of the eggs. Here we showed that small hosts and the frequently parasitized hosts had more rounded eggs, compared to the other host groups. Furthermore, the eggs of the frequent hosts of the brown-headed cowbirds had more rounded shapes. This result, added to the parasite egg's similar mechanical characteristics to those of the hosts (see above), contradicts the hypothesis that brown-headed cowbirds have more rounded (and thus stronger) than their hosts' eggshells (Lack, 1968; Picman, 1989; Brooker & Brooker, 1991). Indeed, brown-headed cowbird eggs are characterized by having a more symmetrical shape than those of their hosts (ellipsoid shapes; Fig. 1; Supporting Information, Table S4).
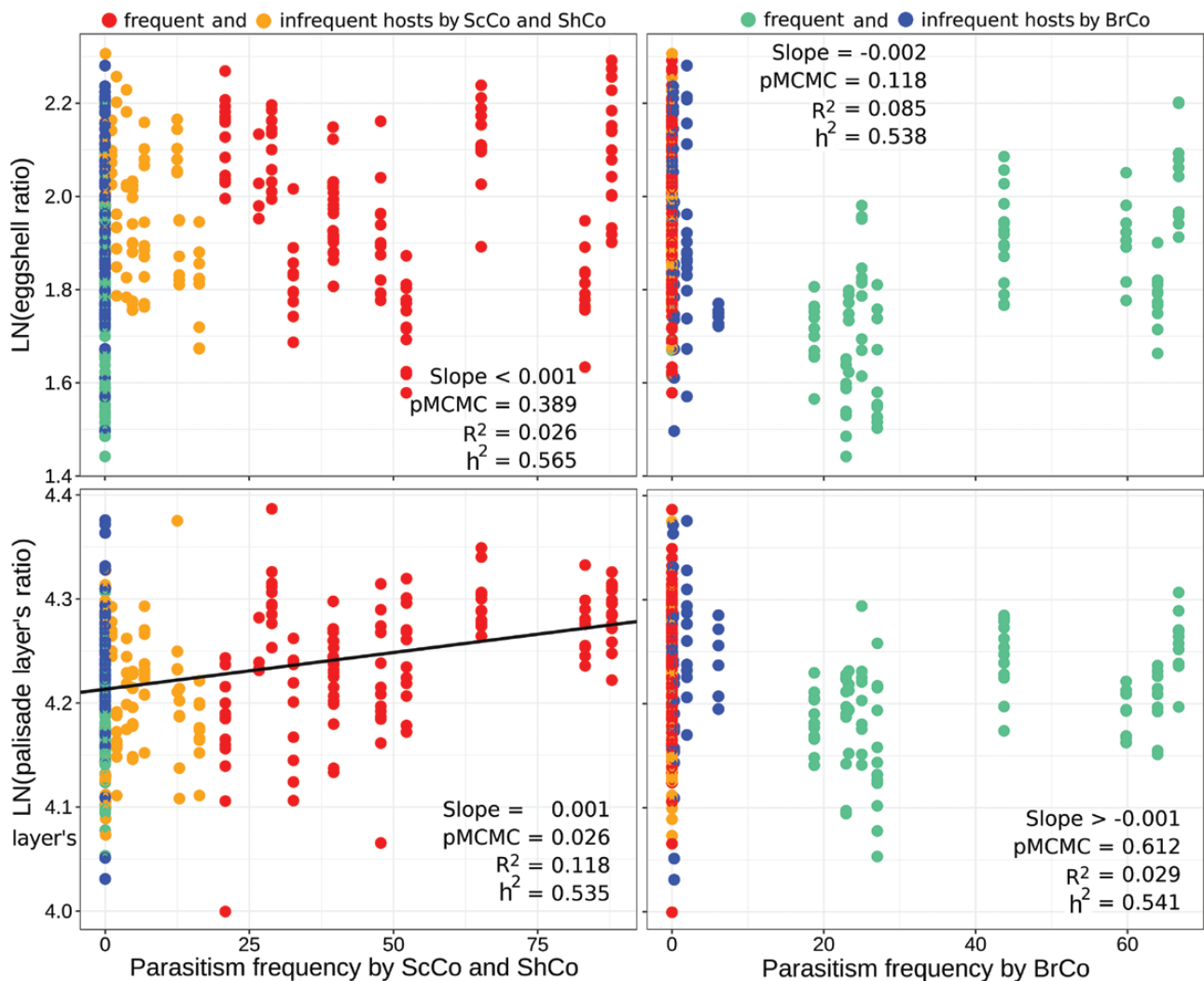
Figure 7. Phylogenetic linear regressions between host eggshell traits (ratio of the eggshell mass relative to the egg mass, and ratio of palisade layer thickness in relation to total thickness of eggshell) and parasitism frequency of the egg-puncturer (ScCo and ShCo) and egg-remover (BrCo) cowbirds.

Through the 2D geometric morphometry analyses, we were able to determine that there were differences in egg shape among the host and parasite egg groups. The aspect ratio [ratio between the length and width of the egg (Reichenow, 1870)] is the main index used to compare the egg shape between parasites and hosts (Picman, 1989; Brooker & Brooker, 1991; Spottiswoode & Colebrook-Robjent, 2007). However, this index cannot determine differences between asymmetric (conical) and symmetric (ellipsoid) shapes (this study, but see Hays *et al.*, 2020). Egg shape is usually ranked second among the egg macrostructural properties affecting breaking strength (Anderson *et al.*, 2004). It is predicted that the optimally strong avian egg shape should be spherical,

because this would provide the highest resistance against external forces (Bain, 1991) and because it would also provide the most effective gas transfer between the embryo and the outside environment (Ar *et al.*, 1979). However, these two approaches are not sufficient to explain the diversity of avian egg shapes found in nature (Stoddard *et al.*, 2017). According to Panheleux *et al.* (1999) and Nedomova *et al.* (2009), egg shape does not appear to be decisive in terms of deviation from the spherical shape for resistance to breakage (Bain, 1991). The eggs of the domestic hen (*Gallus gallus*) are more rounded than those of the guinea fowl (*Numida meleagris*); however, the pierce-strength was 2.5 times greater in guinea fowl than hen eggs (Petersen & Tyler, 1967). Our results on

the frequent hosts of brown-headed cowbirds showed them to possess more rounded egg shapes. Like the previous example, these eggs had no greater ability to absorb energy elastically, stiffness or strength than the remaining host groups. Two studies suggest that rupture force of eggs is independent of eggshell curvature (Nedomova *et al.*, 2016; Trnka *et al.*, 2016), and an alternative hypothesis suggests that egg shape may be an adaptation to utilize the brood patch area of the parents through which the heat of incubation is transmitted and could also be related to clutch size and nest type (Thompson, 1942; Drent, 1975; Andersson, 1978; Barta & Székely, 1997). Our study supports a new hypothesis, in that larger, more rounded (i.e. less elongated), and asymmetrical eggs of frequent hosts of the brown-headed cowbird may be an evolved defence against egg removal by these parasites.

We showed that the egg shape, via PC1 which describes the variation between more elongated to more rounded shapes, was significantly and positively correlated with the frequency of brown-headed cowbird parasitism. Although this parasite removes eggs from nests of large hosts (Sealy, 1992), no difference in egg shape was found between the large hosts of this species and the hosts of South American egg-puncturer cowbirds. A reason for this may be that the strength of parasitism selection pressure by brown-headed cowbirds is lower on the populations of its large hosts, as the small hosts are parasitized more often within this species (Ortega, 1998). In addition, the brown-headed cowbirds parasitize hosts larger than themselves less frequently when compared to screaming, shiny and bronzed (*Molothrus aeneus*) cowbirds (Carter, 1986; Mason, 1986; Peer & Sealy, 1999; Astié & Reboreda, 2006; Peer, 2006; De Mársico *et al.*, 2010). The strength of the selection pressure by shiny and screaming cowbirds should be greater in the populations of their hosts, since these hosts are typically parasitized far more often than most hosts of the brown-headed cowbirds (Carter, 1986; Mason, 1986; Peer & Sealy, 1999; Astié & Reboreda, 2006; Peer, 2006; De Mársico *et al.*, 2010). Indeed, our results showed that the frequent hosts of the egg-puncturer cowbirds had eggs with a greater ability to absorb energy without breaking (eggshell puncture energy) and a higher ratio of the shell mass.

Our interpretation of how the behaviours of highly frequent egg-puncturers, and laying from high position, affect the eggshell puncture stiffness and puncture energy are also supported by the current study's examination of the house wren's eggs from two distant populations with different life and parasitism histories. We found that northern house wren eggs were stronger and stiffer, with thicker shells and greater mass of the shells; whereas the southern house wren eggs had a greater capacity to deform elastically, 2.6 times more so relative to the northern house wrens (0.21 ± 0.03 mm vs. 0.08 ± 0.01 mm; Supporting Information, Fig. S1), implying a greater capacity to absorb energy without breaking. House wren nests in South America are frequently parasitized by shiny cowbirds (Kattan, 1997; Tuero *et al.*, 2007; de la Colina *et al.*, 2016), although there is a low incidence of punctured eggs in house wren nests despite its high frequency of cowbird parasitism (Kattan, 1997; Fiorini *et al.*, 2009; Tuero *et al.*, 2012). Shiny cowbird females lay from above the rim of small nests [typically < 5 cm internal diameter (de la Peña, 2013)], dropping their eggs from a height of 7 to 9 cm (López *et al.*, 2018; Ellison *et al.*, 2019). On the other hand, northern house wren nests are rarely parasitized by brown-headed cowbirds and according to Pribil & Picman (1997), this host may prevent parasitism by building nests in cavities with an entrance too small for the female cowbird to enter. Northern house wrens, however, frequently puncture conspecific eggs and those of other cavity-nesting heterospecifics as a result of competition for limited cavity nests (Johnson, 1998), and this behaviour 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 & Picman, 1986), but only at 3% of nests in South America (Llambías & Fernández, 2009). This could account for the thicker eggshells and greater puncture resistance in the northern house wren compared to the southern house wren and thus may be independent of cowbird parasitism pressure. Secondarily, the greater eggshell puncture stiffness of the northern house wren eggs may be favoured if they suffer less damage during the cowbird laying events (López *et al.*, 2018). However, brown-headed cowbird eggs are lighter and smaller than those of shiny cowbirds, which could be important for future experimental studies. These must use model eggs correctly reflecting the size and weight of those in the local population.

Overall, our results suggest that the high frequency of egg-puncturer behaviour of some brood parasitic cowbirds has resulted in an increase in the host eggshells' stiffness and strength of the eggshells of the host, making them more difficult to be pierced by shiny and screaming cowbirds. The larger more rounded, and more asymmetrical eggs in the common hosts of brown-headed cowbirds might make their host eggs more difficult to be ejected. All the host egg and shell traits were also positively affected by the frequency of parasitism, implying that specific parasitic costs may select for respective anti-parasitic defences in host eggshell phenotypes.

ETHICAL NOTE

Research in Argentina was permitted by Organismo Provincial para el Desarrollo Sostenible, Argentina (Permit number 71/16-O.P.D.S.) and Ministerio de Agroindustria - Dirección de Flora y Fauna - Buenos Aires Provincia, Argentina (Permit number 40/16), and complies with the current laws of Argentina. Research in the USA was permitted by a Hunter College IACUC protocol (MH 2/13-01), a New York State Fish and Wildlife License (78) and US Federal Permits (23681 & GATE-00270).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Methods S1. Eggshell specimens, egg shapes, eggshell mechanical properties, statistical analyses.
Results S1.
References S1.
Figure S1. Morpho-space projections of the first two principal components performed on shape descriptors of egg outlines. Convex hulls, which connect the most distant points of each level of both sets of groups, are shown. Representative force vs. displacement curves for eggs from two populations of the house wren (top) and barn swallow (bottom).
Figure S2. Morpho-space projections of the first two principal components performed on shape descriptors of egg outlines. Each dot represents an egg individual, coloured according to their largest or smallest size with respect to the egg size of its parasite species. The 95% confidence ellipses are also shown. BrCo = brown-headed cowbird egg scores, ScCo and ShCo = scores of the screaming cowbird and shiny cowbird eggs.

Figure S3. Morpho-space projections of the first two principal components performed on shape descriptors of egg outlines. Each dot represents an egg individual, coloured according to the frequencies of parasitism. Ninety-five percent confidence ellipses are shown. BrCo = scores of the brown-headed cowbird eggs, ScCo and ShCo = scores of the screaming cowbird and shiny cowbird eggs, respectively.

Figure S4. Standard linear discriminant analysis of mechanical and structural characteristics of eggs and shells (traits) and egg host species grouped by relative egg size. Ninety-five percent confidence ellipses are shown. The length of arrows represents the contribution of these respective traits to discrimination. BrCo = scores of the brown-headed cowbird eggs, ScCo and ShCo = scores of the screaming cowbird and shiny cowbird eggs, respectively. Arrow references: PC1 = egg ellipticity (increasing values of PC1 indicate greater roundness of the egg), PC2 = egg asymmetry (increasing values of PC2 indicate greater symmetry of the egg), eggshell = ratio of the eggshell mass relative to the egg mass (eggshell ratio), palisades = ratio of palisade layer thickness in relation to total thickness of eggshell (palisade layer ratio), stiffness = eggshell puncture stiffness, energy = eggshell puncture energy.

Figure S5. Standard linear discriminant analysis of mechanical and structural characteristics of eggs and shells (traits) and egg host species grouped by frequency of parasitism. Ninety-five percent confidence ellipses are shown. The length of arrows represents the contribution of these respective traits to discrimination. BrCo = scores of the brown-headed cowbird eggs, ScCo and ShCo = scores of the screaming cowbird and shiny cowbird eggs, respectively. Arrow references: PC1 = egg ellipticity (increasing values of PC1 indicate greater roundness of the egg), PC2 = egg asymmetry (increasing values of PC2 indicate greater symmetry of the egg), eggshell = ratio of the eggshell mass relative to the egg mass (eggshell ratio), palisades = ratio of palisade layer thickness in relation to total thickness of eggshell (palisade layer ratio), stiffness = eggshell puncture stiffness, energy = eggshell puncture energy.

Figure S6. Linear regressions and plots of posterior mean and 95% credible interval associated of the residual eggshell strength (using egg volume as covariate) and residual eggshell volume (using body mass and tarsus length of female as covariates) for the host species grouped according their parasitism frequencies. Different letters indicate that there is evidence that the responses statistically differ between the groups.

Table S1. Standardized coefficients of the phylogenetic lineal discriminant (P-LD) analyses grouped according their (A) relative egg sizes and (B) parasitism frequencies. References: stiffness = eggshell puncture stiffness, energy = eggshell puncture energy, ellipticity = egg ellipticity (PC1), asymmetry = egg asymmetry (PC2), eggshell = 100×shell mass/egg mass (eggshell ratio), palisade layer = 100×palisade layer thickness/total shell thickness (palisade layer ratio).

Table S2. Summary of phylogenetic ANOVAs and *post hoc t*-tests, using the host species grouped according (A) their relative egg sizes and (B) their parasitism frequencies. References: stiffness = eggshell puncture stiffness (N/mm²); energy = eggshell puncture energy (10^{-3}J); ellipticity = egg ellipticity (PC1); asymmetry = egg asymmetry (PC2), eggshell = 100×shell mass/egg mass (eggshell ratio), palisade layer = 100×palisade layer thickness/total shell thickness (palisade layer ratio), LH–BrCo = large hosts of brown-headed cowbird, SH–BrCo = small hosts of brown-headed cowbird, LH–SCo = large hosts of screaming and shiny cowbirds, and SH–SCo = small hosts of screaming and shiny cowbirds. FH–BrCo = frequent hosts of brown-headed cowbirds, IH–BrCo = infrequent hosts of brown-headed cowbirds, FH–SCo = frequent hosts of screaming and shiny cowbirds, and IH–SCo = infrequent hosts of screaming and shiny cowbirds.

Table S3. Standardized and structure coefficients of the linear discriminant (LD) analyses on the host egg species grouped according their (A) relative egg sizes and (B) parasitism frequencies. References: stiffness = eggshell puncture stiffness, energy = eggshell puncture energy, ellipticity = egg ellipticity (PC1), asymmetry = egg asymmetry (PC2), eggshell = 100×shell mass/egg mass (eggshell ratio), palisade layer = 100×palisade layer thickness/total shell thickness (palisade layer ratio).

Table S4. ANOVAs' summary, post hoc *t*–test and trait mean (±SE) values of host egg species grouped according their (A) relative egg sizes and (B) parasitism frequencies. References: see Table S2 for descriptions. Pairwise comparisons' tests with different letters, in parentheses, indicate differences between means of the egg host species groups. Pairwise comparisons tests with different letters, in parentheses, indicate differences between means of the egg host species groups.

Table S5. Life history and host morphology data. For each host species, we collected data from published studies on body mass (g) and tarsus length (mm) of adult females, and on parasitism frequency (parasitized nests/total nests monitored).

Table S6. Data set of the egg and shell mechanical and structural traits. For each egg species, we calculated the following variables: egg volume (mL), relative egg size, eggshell puncture resistance (N), eggshell puncture stiffness (N/mm²), eggshell puncture energy (10^{-3}J), egg ellipticity (Phylo-PC1), egg asymmetry (Phylo-PC2) and sample size (n).