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Comparison of regional eggshell porosity between the brood parasitic Brown-headed Cowbird (*Molothrus ater*) and its hosts: the Dickcissel (*Spiza americana*), and two non-parasitic relatives, the Red-winged Blackbird (*Agelaius phoeniceus*) and the Common Grackle (*Quiscalus quiscula*).

BY

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RESEARCH HONORS THESIS

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Abstract

The Brown-headed Cowbird (*Molothrus ater*) is a generalist brood parasite that lays eggs in the nests of many host species, including the Dickcissel (*Spiza americana*) and two non-parasitic relatives: the Red-winged Blackbird (*Agelaius phoeniceus*) and the Common Grackle (*Quiscalus quiscula*). Cowbird eggs reportedly hatch sooner than equivalently-sized host eggs, presumably via accelerated embryonic development enabled by a greater eggshell porosity and consequently greater gas exchange. However, the distribution of pores among apical, equatorial and basal eggshell regions within cowbirds and host species is undetermined. I tested the hypothesis that equatorial porosity would be greatest because respiratory gases primarily cross the eggshell pores and enter or exit the embryo's circulatory system via the chorioallantoic membrane located in that region. I found that the equatorial region of eggs within species had significantly greater pore density, pore area, and porosity than the basal or apical regions of eggshells from cowbirds ($P \leq 0.006$), Red-winged Blackbirds ($P \leq 0.002$), and Dickcissels ($P \leq 0.005$). Grackle eggshells did not follow this regional pattern, and porosity characteristics did not differ significantly between the equator and base ($P > 0.05$). Notably, cowbird eggshells had significantly greater pore area and porosity in equatorial regions compared to its three hosts ($P \leq 0.012$). Cowbird eggshells had a greater apical pore area than that of the Dickcissel ($P < 0.001$) and grackle ($P = 0.003$), and did not have significantly greater eggshell basal pore area or porosity compared to either of its relatives. These observations demonstrate region-specific rather than global increases in eggshell porosity, which may further explain the accelerated embryonic development of cowbirds compared to host species.

Introduction

The Brown-headed Cowbird (*Molothrus ater*, hereafter cowbird) is a generalist brood parasitic species (Passeriformes: Icteridae) which utilizes a variety of bird species as hosts to raise its young. Cowbird eggs hatch earlier than expected based on their egg size (Briskie and Sealy 1990, Peer and Bollinger 2000) and cowbirds have also been reported to hatch earlier than host species (Briskie and Sealy 1990, McMaster and

Sealy 1997). Asynchronous hatching between host and parasite benefits the parasitic cowbird nestling by allowing it to outcompete host nestlings for resources, thus leading to decreased reproductive success of the host (Payne 1977, Hauber 2003). We discovered in a previous study that hatching asynchrony may be due to an accelerated rate of embryonic development that is, in part, explained by increased porosity (i.e., pore area divided by eggshell thickness) in the cowbird eggshell (Jaekle et al. In Review). Egg incubation periods are inversely proportional to the gas conductance across an eggshell (Rahn and Ar 1974), which, in turn, is influenced by the level of eggshell porosity (Paganelli 1980, Ar and Rahn 1985, Booth and Seymour 1987, Booth 1989, Stein and Badyaev 2011). Ar et al. (1974) defined gas conductance as the total egg porosity multiplied by the constant 23.41. Embryos from eggshells with high porosity are able to maintain higher rates of metabolism (Burton and Tullett 1983) due to greater exchange of metabolic gases than eggs with lower porosity (Rahn et al. 1974, Ar and Rahn 1978, Paganelli 1980, Vleck and Bucher 1998). Cowbird eggs exhibited greater overall eggshell porosity and estimated gas conductance through eggshell pores than that of two similarly-sized hosts: the Dickcissel (*Spiza americana*) and the Red-winged Blackbird (*Agelaius phoeniceus*), a non-parasitic icterid (Jaekle et al. In Review). Therefore, differences in porosity, and thus development rate, may be the mechanism by which cowbirds hatch earlier than their hosts (Jaekle et al. In Review).

While the eggshell porosity of cowbirds is known to be greater than that of these hosts, the distribution of porosity across different regions of the eggshell has not been previously determined. Rokitka and Rahn (1987) found increased eggshell porosity in the basal region compared to the equator and apex in avian species from the taxonomic orders Galliformes (e.g. chickens and turkeys), Anseriformes (e.g. ducks and geese), Charadriiformes (e.g. terns), and Ciconiiformes (e.g. egrets). The base of avian eggs contains the air-cell, an air space between the inner and outer eggshell membranes, which expands as water is lost from the egg and serves as the site for embryonic gas exchange (Wangensteen 1972, Rahn and Ar 1974, Ackerman and Rahn 1981, Rokitka and Rahn 1987, Rahn and Paganelli 1990, Mao et al. 2007). The air-cell is most important during the later stages of development primarily directly before hatching during internal pipping, the process in which the

chick punctures the air-cell and pulmonary respiration begins (Mao et al. 2007). Mao et al. (2007) suggested that porosity in the basal region is important for the hatchability of an egg. However, during the majority of embryonic development, gas exchange essential to growth and survival of the embryo occurs primarily via the chorioallantoic membrane, which is a specialized vascular membrane (Patten 1951, Wangenstein 1972, Ackerman and Rahn 1981, Rokitka and Rahn 1987). During development, the chorioallantoic membrane grows primarily in the equatorial and apical regions of the egg, although it eventually expands to cover nearly the entire inner surface of the eggshell (Patten 1951, Paganelli 1980, Tazawa 1980; See Appendix I).

In this study, I tested the hypothesis that the degree of eggshell porosity is directly related to egg region due to the location of the chorioallantoic membrane. I predicted that eggshell porosity would be greatest in the equatorial and apical regions of the egg. To test this hypothesis, I compared eggshell porosity in the apical, equatorial, and basal regions of eggshells within and among the eggs of the cowbird and three of its potential host species: the Red-winged Blackbird, the Common Grackle (*Quiscalus quiscula*, hereafter grackle), and the Dickcissel. Two of the host species studied, the Red-winged Blackbird and the grackle, are non-parasitic relatives of the cowbird. Comparisons among these three icterid species may give insights about whether observed differences among species represent global or region-specific eggshell characters that evolved as an adaptation for the cowbird's unique parasitic reproductive strategy. If cowbird eggs have greater porosity in the equator and apex than these hosts, it would further explain the accelerated embryonic development observed in the cowbird.

Materials and Methods

Eggs of Brown-headed Cowbirds, Dickcissels, Red-winged Blackbirds, and grackles (N = 20 eggs per species) in different stages of development were collected from nests in McDonough County, Illinois, Riley County, Kansas, and Scott County, Iowa between 2006 and 2009. The eggs were stored at -20 °C in a laboratory freezer. After removal from the freezer, the length and width of each egg were measured using a

Vernier digital caliper (Neiko, +/- 0.02 mm) before preparation of the eggshells for analysis of porosity. We utilized eggs with no visible cracks or only thin fractures and avoided eggshells that were visibly damaged during freezing. All egg dimensions were within published ranges for all four species (Yasukawa and Searcy 1995, Peer and Bollinger 1997, Lowther 1993, Temple 2002).

Preparation of Eggshell Fragments

Measurements of eggshell fragments from cowbirds, Dickcissels, and Red-winged Blackbirds were made by one observer (Miranda Kiefer, MK). I (BC) completed all of the measurements of pore count and pore area for each eggshell fragment from grackles. I checked the precision of pore counts by MK by independently examining the same eggshell fragments and found no difference between the number of pores observed by both individuals (one-way ANOVA $F_{1,106} = 0.00$, $P = 1.00$, $n = 108$ measurements).

Eggshells were thawed for 1 min and broken into fragments using a scalpel. Fragments were then placed into boiling 5% NaOH for approximately 15 min to remove the inner eggshell membrane and outer cuticle. The pieces of eggshell were air-dried for 1-14 days before analysis. The eggshell fragments were broken into smaller pieces during the boiling process as well as manually broken to facilitate viewing with a compound microscope. We examined the outer surface of eggshell fragments ($n = 18$ fragments each for equatorial, basal, and apical regions of the eggshell; each fragment was 3.07 mm^2 in size) at a 400x magnification to count the number of pores and measure pore diameters. A calibrated ocular micrometer ($\pm 2.5 \text{ }\mu\text{m}$) was used to measure pore diameters. Observations of pore shape during analysis indicated that pores were mostly circular in shape. Therefore, for purposes of this analysis each eggshell pore was assumed to be circular in shape with a surface area (SA) calculated as $SA (\text{ }\mu\text{m}^2) = \pi(\text{diameter}/2)^2$. Although the eggs for all species collected were in various stages of embryonic development, a factor that could influence the eggshell thickness or pore diameter on the inner eggshell surface (Booth and Seymour 1987), this would have no effect on the pores we analyzed on the outer surface of the eggshell. We also measured eggshell thickness ($n = 3$

independent measures each for equatorial, basal, and apical regions of eggshell, ± 0.01 mm) using a Starrett model 2600-1 thickness indicator after the inner eggshell membrane and outer cuticle were removed.

Statistical Analyses

All statistical comparisons were performed using SPSS (version 20.0; IBM Corporation, Somers, New York). Nonparametric tests were utilized for all comparisons as data did not fit the assumptions of parametric tests. Multiple transformations were attempted but homogeneous variances (tested via the Levene Test for equality of variance) were not achieved. We used Kruskal-Wallis tests to reveal if differences existed among groups. If a significant difference was detected, Mann-Whitney U tests were performed to compare specific sample pairs to determine where the differences occurred. A Bonferroni correction ($\alpha = 0.05 \div N$ comparisons) was used to determine significance for individual Mann-Whitney comparisons. To normalize the data to egg size, we used published average egg masses for each species (cowbird [Rahn et al. 1988]; Dickcissel [Gross 1968]; Red-winged Blackbird [Manning 1981]; and grackle [Howe 1976]) as fresh weight was not taken during collection.

Comparisons of whole eggshells

To test for differences among species, mean values for the focal eggshell characteristics (i.e., pore number, pore area, and porosity) were used for each egg. I was able to calculate the total pore number, pore area, and porosity per egg based on estimated average values of pore number per mm^2 and pore area (μm^2) per mm^2 collected for each species in conjunction with calculated surface area (mm^2) values. I estimated egg surface area as egg volume^{0.666} $\times 4.951$, where egg volume = egg length \times egg width² $\times 0.51$ (Hoyt 1976).

Comparisons of egg regions

I tested differences among species for average pore number per mm², average pore area per mm², and average porosity per mm² of eggshell regions (i.e., base, apex, or equator). The measured eggshell characteristics were also compared among eggshell regions within each species.

Results

Interregional, Intraegg Comparisons

The majority of samples from all four species showed no significant differences among regions (i.e., apical, equator, base), and any differences that were observed were inconsistent among eggs (results not shown).

Interregional, Intraspecies Comparisons

Within species, there were significant differences in all studied eggshell characteristics among eggshell regions (Table 1). Post-hoc analyses revealed that equatorial regions of cowbird, Red-winged Blackbird, and Dickcissel eggshells had significantly greater eggshell pore densities, pore areas, and porosities than either the basal or apical regions (Table 2). In these three species, the base and apex eggshell pore density, pore area, and porosity did not differ significantly (Table 2). However, regional comparisons in the grackle eggshells revealed that the equatorial porosity was significantly greater than that of the apex and did not differ significantly from that of the base (Table 2). The basal region of grackle eggshells also had significantly greater pore density, pore area, and porosity than the apical region (Table 2). For the results of the individual Mann-Whitney analyses, see Appendix II.

Interspecies, Intraregional Comparisons

When each region was compared among species, there were significant differences in pore density, pore area, and porosity (Table 3). Comparisons of pore density among basal and equatorial eggshell regions revealed

that cowbird eggshells were not significantly different from the eggshells of Dickcissels ($P \geq 0.029$), but were significantly less than those of Red-winged Blackbirds ($P \leq 0.009$; Table 4, Fig. 1A). Cowbird eggshells had a significantly greater equatorial pore density than that of the grackle ($P < 0.001$), but had significantly less porosity than the grackle in the basal region ($P = 0.008$; Table 4, Fig. 1A). The cowbird eggshells had significantly greater apical pore density than that of the Dickcissel ($P = 0.005$) and grackle ($P = 0.002$); however cowbird eggshell pore density in the apex did not differ significantly from that of the Red-winged Blackbird ($P = 0.059$; Table 4).

Post-hoc analyses revealed that cowbird eggshells had significantly greater pore area ($P \leq 0.003$) and porosity ($P \leq 0.012$) in the equator than the three host species (Fig. 1B-C). Similarly, the pore area of apical regions in cowbird eggs was greater than that of Dickcissels ($P < 0.001$) and grackles ($P = 0.003$), although cowbird apical eggshell pore area did not differ significantly from the eggshells of Red-winged Blackbirds ($P = 0.154$; Table 4). The porosity of apical eggshells from cowbirds was significantly greater than that of Dickcissels and grackles ($P \leq 0.001$); however, there was no significant difference between the apical porosity of cowbird and Red-Winged Blackbird eggshells ($P = 0.293$; Table 4). The pore area of the basal eggshell region in cowbird eggs was significantly greater than only the Dickcissel ($P = 0.007$; Table 4). Cowbird basal eggshell pore area was significantly less than that of grackles ($P = 0.001$) and did not differ significantly from eggshells of Red-winged Blackbirds ($P = 0.439$; Table 4). Similarly, there was no significant difference between basal eggshell porosity of cowbirds and Red-winged Blackbirds ($P = 0.227$) or Dickcissels ($P = 0.024$), and cowbird eggshells had significantly less basal porosity than grackles ($P = 0.003$; Table 4). For further comparisons among host species see Table 4 and Figure 1.

Whole Egg Comparisons among Species

Results from eggshell comparisons (i.e. pore density, pore area, and porosity) among whole eggs of the cowbird, Red-winged Blackbird, and Dickcissel were consistent with conclusions from Jaeckle et al. (In Review). All characteristics analyzed per egg were first normalized by egg mass. There was a significant

difference among species in average pore density per mm^2 ($\chi^2 = 15.54$, $df = 3$, $P = 0.001$). Likewise, there was a significant difference among species in average pore density per egg ($\chi^2 = 18.24$, $df = 3$, $P < 0.001$). There were no significant differences between the cowbird and its three hosts in average pore density per mm^2 ($P \geq 0.014$; Table 5) or per egg ($P \geq 0.097$; Fig. 2A). However, the pore density per mm^2 and per egg of Red-winged Blackbird eggshells was significantly greater than that of the Dickcissel ($P \leq 0.005$) and the grackle ($P \leq 0.001$; Table 5, Fig. 2A). There was no significant difference in average pore density per mm^2 between Dickcissel and grackle eggs ($P = 0.904$; Table 5), nor were there differences in average pore density per egg between Dickcissel and grackle eggs ($P = 0.947$; Fig. 2A).

There was a significant difference among species in pore area per mm^2 ($\chi^2 = 32.49$, $df = 3$, $P < 0.001$; Table 5) and per egg ($\chi^2 = 29.57$, $df = 3$, $P < 0.001$; Fig. 2B). Post-hoc analyses revealed eggshell pore area per mm^2 and per egg did not differ significantly between Dickcissels and Red-winged Blackbirds ($P \geq 0.020$); however eggshells of both species had significantly less pore area per mm^2 and per egg than cowbirds ($P \leq 0.001$; Table 5, Fig. 2B). Grackle eggshell pore area per mm^2 was also greater than that of the Red-winged Blackbird ($P < 0.001$; Table 5), as was the pore area per egg ($P = 0.001$; Fig. 2B). Grackle eggs also had greater pore area than the Dickcissel, both per mm^2 ($P < 0.001$; Table 5) and per egg ($P < 0.001$; Fig. 2B).

There was a significant difference among species in average porosity per mm^2 ($\chi^2 = 20.70$, $df = 3$, $P < 0.001$) and per egg ($\chi^2 = 18.570$, $df = 3$, $P < 0.001$), which revealed results similar to comparisons of pore area. Eggshell porosity of grackles did not differ significantly from cowbirds in comparisons per mm^2 ($P = 0.769$; Table 5) or per egg ($P = 0.129$; Fig. 2C). Both the cowbird and the grackle had significantly greater eggshell porosity per mm^2 than the eggshells of the Red-winged Blackbird ($P \leq 0.003$) and Dickcissel ($P \leq 0.003$; Table 5). Similarly, cowbird eggshells had significantly greater porosity per egg than Red-winged Blackbirds ($P = 0.004$) and Dickcissels ($P < 0.001$); however, grackle eggs had significantly greater porosity per egg than those of Dickcissels ($P = 0.003$) but not Red-winged Blackbirds ($P = 0.060$; Fig. 2C). There was no significant

difference in porosity per mm^2 or per egg between the Red-winged Blackbird and the Dickcissel ($P \geq 0.072$; Table 5, Fig. 2C).

Discussion

Cowbird, Red-winged Blackbird, and Dickcissel eggs all showed greater levels of pore density, pore area, and porosity in equatorial eggshell regions compared to basal or equatorial regions. Although these results agree with the predictions of this study, they are in conflict with the findings of Rokitka and Rahn (1987). Rokitka and Rahn (1987) found the basal eggshell region was most porous and described a steady decrease in porosity from the base to the apex of the egg. This discrepancy in regional porosity characterization may be due to differences in chick development among species in the two studies. For example, some of the species studied by Rokitka and Rahn (1987) produced precocial nestlings (i.e., the young are mature and mobile at hatching) while those utilized in this study produce altricial nestlings (i.e., the young are helpless at hatching). The basal region of eggs contains the air-cell, a structure essential to internal pipping and therefore chick hatchability (Mao et al. 2007). This later phase of development when pulmonary respiration is initiated may be especially important for the success of precocial or semi-precocial nestlings directly before hatching as they may require a higher level of basal eggshell porosity compared to altricial or semi-altricial species. However, we found that the eggshell porosity of grackles, a species with altricial young, did not differ significantly between the equatorial and basal eggshell regions. Regional porosity comparisons between the eggs of precocial and altricial nestlings are required to adequately test this nestling maturation-specific porosity distribution hypothesis.

The greater equatorial eggshell porosity (i.e. pore area per thickness) compared to basal or apical egg regions observed in cowbirds, Red-winged Blackbirds, and Dickcissels correlates to the location of the chorioallantoic membrane, a vascularized structure which is the primary site of embryonic gas exchange during development (e.g., Patten 1951, Wangensteen 1972). Ackerman and Rahn (1981) found that during the

majority of embryonic development, metabolic gas flux is confined to areas of the eggshell which cover the chorioallantoic membrane. Although over time this structure expands to cover the entire inner eggshell surface (Paganelli 1980, Tazawa 1980), it is principally situated in the equatorial region of the egg. Since porosity is proportional to permitted gas flux (e.g., Ar et al. 1974), it is logical that eggshell regions significant for respiration would also have increased porosity. A study of turkey regional eggshell porosity by Christensen (1983) revealed that successfully hatched eggs of that precocial species had significantly greater porosity in equatorial and basal eggshell regions than that of non-hatching eggs. These results support my explanatory hypothesis concerning the porosity distribution of eggshell porosity among different regions of the avian eggshell.

It is noteworthy that cowbirds had greater porosity (through an increased pore area) than the three studied host species in the equatorial region when taking into consideration the relationship between equatorial porosity and nestling development and hatchability. This region-specific increase would allow for greater rates of gas exchange in the specific location of the chorioallantoic membrane (Patten 1951). The cowbird also had significantly greater apical eggshell porosity than the Dickcissel and Grackle. This finding, too, supports the premise that the distribution of porosity in cowbird eggshells is correlated with the location of the chorioallantoic membrane. Furthermore, cowbird eggshell porosity in the base, a region where the chorioallantoic membrane is absent, was not greater than any of its host species. The greater porosity of cowbird eggshells than those of its hosts only in regions associated with the primary exchange membrane for allantoic respiration could be a factor which permits the apparent accelerated rate of development of the brood parasite compared to its hosts (Briskie and Sealy 1990, McMaster and Sealy 1997).

Comparisons between the cowbird and two of its non-parasitic relatives, the Red-winged Blackbird and the grackle, revealed that these observed differences in total porosity distribution among regions may be unique adaptations for the cowbird's brood parasitic reproductive strategy. Although the overall weight-specific porosity per egg did not differ significantly between cowbird and grackle eggshells, the cowbird had greater

regional porosity than the grackle in the equatorial and apical regions. Further, cowbird eggshells only had significantly greater porosity in the equatorial region when compared to those of the Red-winged Blackbird, even though the total porosity per egg of the cowbird was substantially greater. Together, these comparisons reveal that adaptations for region-specific rather than global increases in eggshell porosity may be more beneficial for the success of the cowbird as a brood parasite. This may offer insight into the evolution of porosity across the avian eggshell, as porosity in the basal region would have little effect on development and, hence, would not have significant selection pressure to confer a fitness benefit.

In summary, the brood parasitic Brown-headed cowbird has an accelerated rate of embryonic development compared to some host species, which may be explained by region-specific increases in eggshell porosity. I found through intraspecific egg comparisons among species that the equatorial region was especially important for gas exchange during development due to a greater proportion of total eggshell porosity located in this region in cowbird, Red-winged Blackbird, and Dickcissel eggs. Additionally, cowbird eggshells had significantly greater porosity than all three host species in the equator of the egg, but not the base. These data suggest that region-specific rather than global increases in eggshell porosity evolved in the cowbird to reduce its incubation period as an adaptation for brood parasitism.

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Literature Cited

- Ackerman, R. A. and H. Rahn. 1981. In vivo O₂ and water vapor permeability of the hen's eggshell during early development. *Respiration Physiology* 45(1): 1-8.
- Ar, A. and H. Rahn. 1978. Interdependence of gas conductance, incubation length and weight of the avian egg. In J. Piiper [ed.], *Respiratory functions in birds, adult and embryonic*. Springer-Verlag, Berlin.
- Ar, A. and H. Rahn. 1985. Pores in avian eggshells: gas conductance, gas exchange and embryonic growth rate. *Respiration Physiology* 61(1): 1-20.
- Ar, A., C. V. Paganelli, R. B. Reeves, D. G. Greene, and H. Rahn. 1974. The avian egg: water vapor conductance, shell thickness, and functional pore area. *Condor* 76: 153-58.
- Booth, D. T. 1989. Regional changes in shell thickness, shell conductance, and pore structure during incubation in eggs of Mute swan. *Physiological Zoology* 62: 607-620.
- Booth, D. T. and R. S. Seymour. 1987. Effect of eggshell thinning on water vapor conductance of Malleefowl eggs. *Condor* 89(3): 453-459.
- Briskie, J. V., and S. G. Sealy. 1990. Evolution of short incubation periods in the parasitic cowbirds, *Molothrus* spp. *Auk* 107: 789-794.
- Burton, F. G. and S. G. Tullett. 1983. A comparison of the effects of eggshell porosity on the respiration and growth of domestic fowl, duck and turkey embryos. *Comparative Biochemistry and Physiology Part A: Physiology* 75(2): 167-174.
- Christensen, V. L. 1983. Distribution of pores on hatching and nonhatching turkey eggs. *Poultry Science* 62(7): 1312-1316.
- Gross, A. O. 1968. Dickcissel. Pages 158-191 in *Life histories of North American cardinals, grosbeaks, buntings, towhees, finches, sparrows and their allies*. (Austin, OL., Ed.) U.S. National Museum Bulletin no. 237, Pt. 1.
- Hauber, M. E. 2003. Hatching asynchrony, nestling competition, and the cost of interspecific brood parasitism. *Behavioral Ecology* 14: 227-235.

- Howe, H. F. 1976. Egg size, hatching asynchrony, sex and brood reduction in the Common Grackle. *Ecology* 57: 1195-1207.
- Hoyt, D. F. 1976. The effect of shape on surface-volume relationships of birds eggs. *Condor* 78: 343-349.
- Jaeckle, W. B., M. Kiefer, B. Childs, R. G. Harper, J. W. Rivers, and B. D. Peer. In Review. Comparison of eggshell porosity and estimated gas flux between the Brown-headed Cowbird and two common hosts.
- Lowther, P. E. 1993. Brown-headed Cowbird (*Molothrus ater*), *The Birds of North America Online* (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: <http://bna.birds.cornell.edu/bna/species/047>
- Manning, T. H. 1981. Analysis of weight lost by eggs of eleven species of birds during incubation. *Canadian Field-Naturalist* 95: 63-68.
- Mao, K. M., A. Murakami, A. Iwasawa and N. Yoshizaki. 2007. The asymmetry of avian egg-shape: and adaptation for reproduction on dry land. *Journal of Anatomy* 210: 741-748.
- McMaster, D. G. and S. G. Sealy. 1997. Host-egg removal by Brown-Headed Cowbirds: A test of the host incubation limit hypothesis. *Auk* 114(2): 212-220.
- Paganelli, C. V. 1980. The physics of gas exchange across the avian eggshell. *American Zoology* 20(2): 329-338.
- Patten, B. M. 1951. *Early embryology of the chick*, 4th ed. New York, McGraw-Hill.
- Payne, R. B. 1977. The ecology of brood parasitism in birds. *Annual Review of Ecology and Systematics* 8: 1-28.
- Peer, B. D. and E. K. Bollinger. 1997. Explanations for the infrequent cowbird parasitism on Common Grackles. *Condor* 99(1): 151-161.
- Peer, B. D., and E. K. Bollinger. 2000. Why do female Brown-headed Cowbirds remove host eggs? A test of the incubation efficiency hypothesis. Pp. 187-192 in *Ecology and management of cowbirds and their hosts* (J.N.M. Smith, T. Cook, S. I. Rothstein, S. K. Robinson, and S. G. Sealy, eds.). University of Texas Press, Austin.

- Rahn, H. and A. Ar. 1974. The avian egg: incubation time and water loss. *Condor* 76(2): 147-152.
- Rahn, H., L. Curran-Everett, and D. T. Booth. 1988. Eggshell differences between parasitic and nonparasitic Icteridae. *Condor* 90: 962-964.
- Rahn, H. and C. V. Paganelli. 1990. Gas fluxes in avian eggs: Driving forces and the pathway for exchange. *Comparative Biochemistry and Physiology Part A: Physiology* 95(1): 1-15.
- Rahn, H., C. V. Paganelli, and A. Ar. 1974. The avian egg: air-cell gas tension, metabolism and incubation time. *Respiration Physiology* 22: 297-300.
- Rokitka, M. A. and H. Rahn. 1987. Regional differences in shell conductance and pore density of avian eggs. *Respiration Physiology* 68(3): 371-376.
- Stein, L. R. and A. V. Badyaev. 2011. Evolution of eggshell structure during rapid range expansion in a passerine bird. *Functional Ecology* 25: 1215-1222.
- Tazawa, H. 1980. Adverse effect of failure to turn the avian egg on embryo oxygen exchange. *Respiration Physiology* 41: 137-142.
- Temple, S. A. 2002. Dickcissel (*Spiza americana*), *The Birds of North America Online* (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: <http://bna.birds.cornell.edu/bna/species/703>
- Vleck, C. M., and T. L. Bucher. 1998. Energy metabolism, gas exchange, and ventilation. *Oxford Ornithology Series* 8: 89-116.
- Wangenstein, O. D. 1972. Gas exchange by a bird's embryo. *Respiration Physiology* 14(1-2): 64-74.
- Yasukawa, K. and Searcy, W. A. 1995. Red-winged Blackbird (*Agelaius phoeniceus*), *The Birds of North America Online* (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: <http://bna.birds.cornell.edu/bna/species/184>

Table 1. Comparison of pore density (per mm²), pore area (µm² per mm²), and porosity (µm²mm⁻¹ per mm²) among eggshell regions (base, apex and equator) within the cowbird and its hosts via Kruskal-Wallis tests.

Pore Density	χ^2	df	P-value
Cowbird	25.27	2	<0.001
Red-winged Blackbird	13.29	2	0.001
Grackle	15.26	2	<0.001
Dickcissel	15.83	2	<0.001
Pore Area			
Cowbird	27.68	2	<0.001
Red-winged Blackbird	17.40	2	<0.001
Grackle	22.78	2	<0.001
Dickcissel	13.56	2	0.001
Porosity			
Cowbird	28.14	2	<0.001
Red-winged Blackbird	16.16	2	<0.001
Grackle	21.98	2	<0.001
Dickcissel	12.59	2	0.002

Table 2. Comparison of median pore density (per mm²), pore area (µm² per mm²), and porosity (µm²mm⁻¹ per mm²) among eggshell regions within species. Superscripts of different letters indicate a statistically significant difference ($P \leq 0.0125$). 25% and 75% refer to percentiles.

<u>Cowbird</u>					
Eggshell pore density	n	Median	25%	75%	Maximum
Base	326	0.00 ^b	0.00	0.33	1.63
Equator	345	0.33 ^a	0.00	0.65	2.28
Apex	248	0.33 ^b	0.00	0.33	1.63
Eggshell pore area					
Base	326	0.00 ^b	0.00	6.40	159.89
Equator	345	3.20 ^a	0.00	15.99	148.70
Apex	248	0.30 ^b	0.00	6.40	102.33
Eggshell porosity					
Base	326	0.00 ^b	0.00	58.08	1332.45
Equator	345	29.07 ^a	0.00	151.45	1274.58
Apex	248	2.55 ^b	0.00	65.76	852.77
<u>Red-winged Blackbird</u>					
Eggshell pore density	n	Median	25%	75%	Maximum
Base	281	0.33 ^b	0.00	0.49	1.30
Equator	314	0.33 ^a	0.00	0.65	2.28
Apex	247	0.33 ^b	0.00	0.65	1.63
Eggshell pore area					
Base	281	0.40 ^b	0.00	2.00	41.57
Equator	314	0.55 ^a	0.00	5.20	41.97
Apex	247	0.10 ^b	0.00	2.00	45.17
Eggshell porosity					
Base	281	4.14 ^b	0.00	29.52	445.42
Equator	314	6.24 ^a	0.00	57.74	449.70
Apex	247	1.11 ^b	0.00	23.32	483.96

(Table 2 continued)

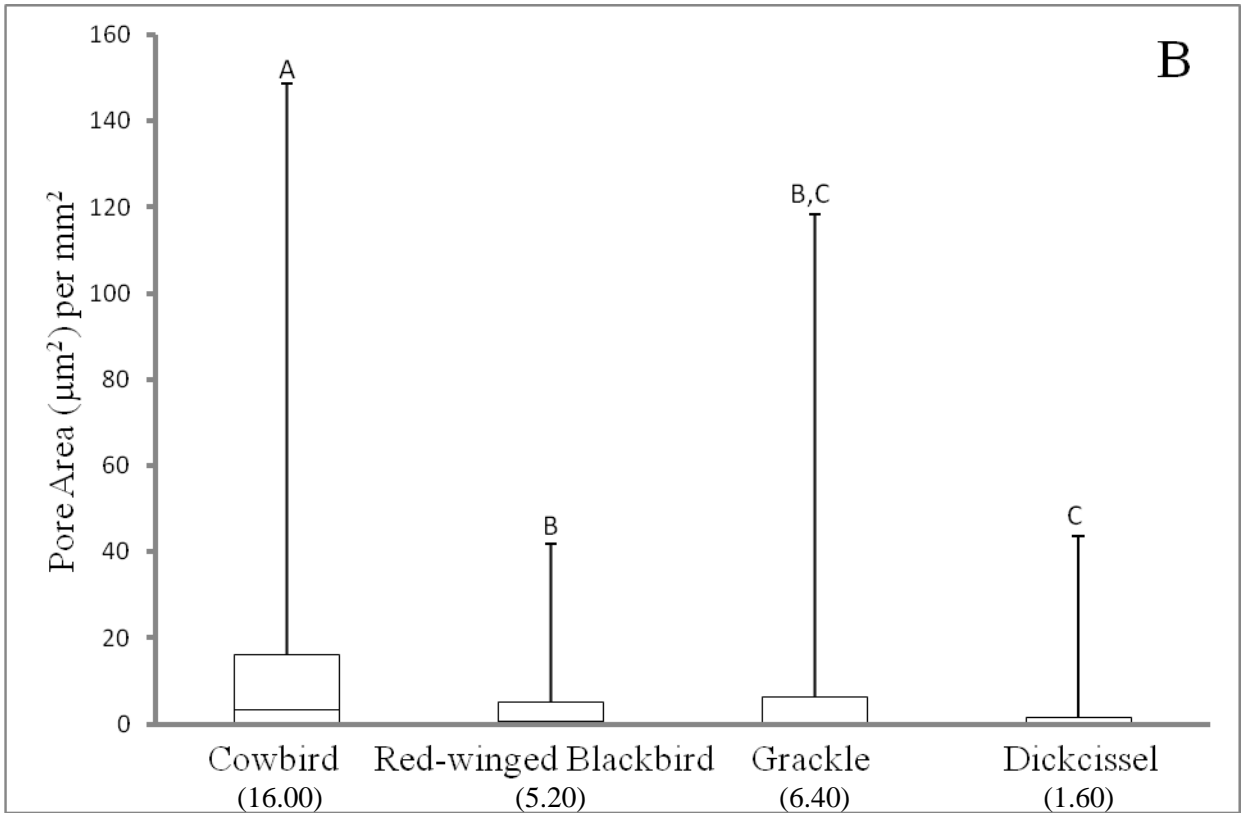
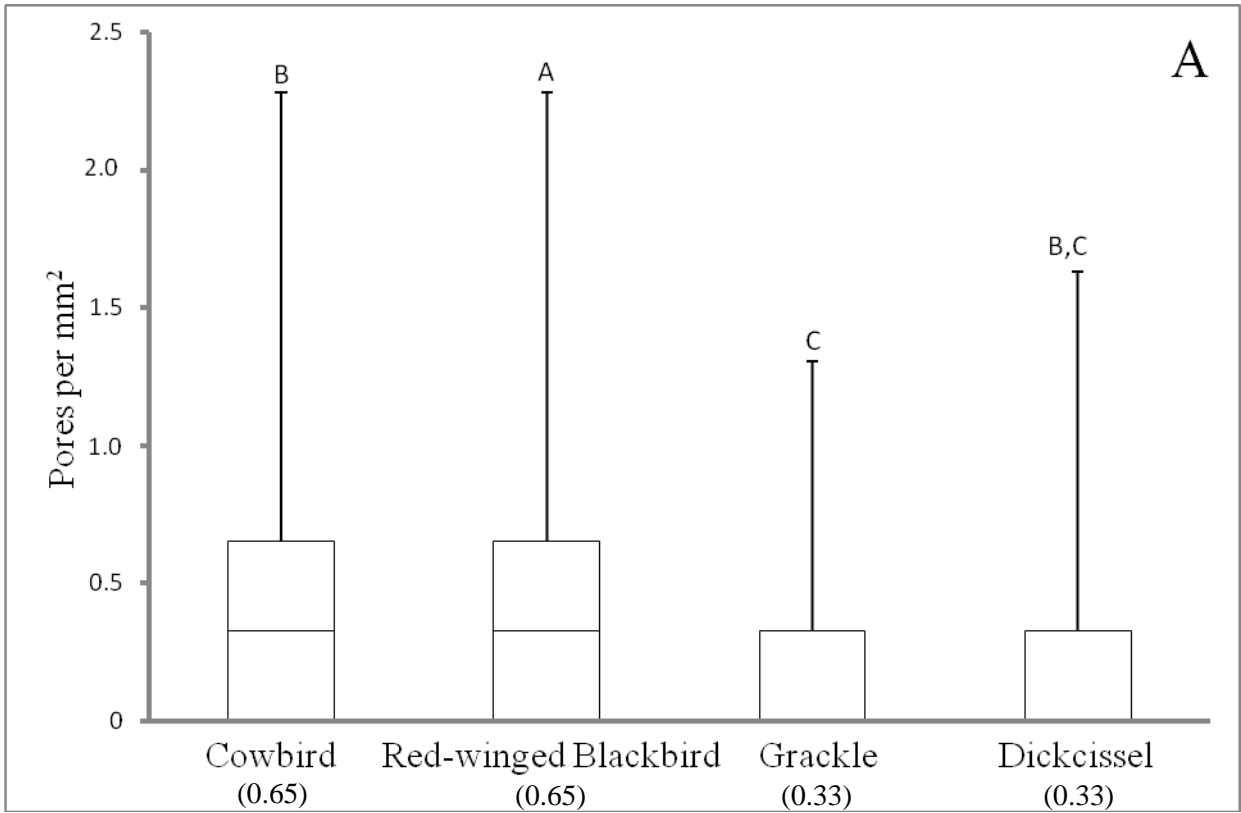
		<u>Grackle</u>			
Eggshell pore density	n	Median	25%	75%	Maximum
Base	360	0.32 ^a	0.00	0.33	1.30
Equator	360	0.00 ^a	0.00	0.33	1.30
Apex	360	0.00 ^b	0.00	0.33	1.30
Eggshell pore area					
Base	360	0.40 ^a	0.00	14.39	129.51
Equator	360	0.00 ^a	0.00	6.40	118.32
Apex	360	0.00 ^b	0.00	3.00	54.36
Eggshell porosity					
Base	360	3.64 ^a	0.00	119.12	1022.48
Equator	360	0.00 ^a	0.00	57.72	1044.01
Apex	360	0.00 ^b	0.00	25.85	440.79
		<u>Dickcissel</u>			
Eggshell pore density	n	Median	25%	75%	Maximum
Base	349	0.00 ^b	0.00	0.33	1.95
Equator	359	0.33 ^a	0.00	0.33	1.63
Apex	289	0.00 ^b	0.00	0.33	1.63
Eggshell pore area					
Base	349	0.00 ^b	0.00	0.50	78.35
Equator	359	0.10 ^a	0.00	1.60	43.57
Apex	289	0.00 ^b	0.00	0.50	44.87
Eggshell porosity					
Base	349	0.00 ^b	0.00	6.66	870.53
Equator	359	1.15 ^a	0.00	20.86	522.85
Apex	289	0.00 ^b	0.00	8.43	448.70

Table 3. Comparison of pore density (per mm²), pore area (μm² per mm²), and porosity (μm²mm⁻¹ per mm²) within eggshell regions among the cowbird and its hosts.

Base	χ^2	df	P-value
Eggshell pore density	27.72	3	<0.001
Eggshell pore area	43.23	3	<0.001
Eggshell porosity	34.24	3	<0.001
Equator			
Eggshell pore density	46.83	3	<0.001
Eggshell pore area	47.97	3	<0.001
Eggshell porosity	43.07	3	<0.001
Apex			
Eggshell pore density	33.04	3	<0.001
Eggshell pore area	27.55	3	<0.001
Eggshell porosity	25.15	3	<0.001

Table 4. Comparison of pore density (per mm²), pore area (µm² per mm²), and porosity (µm²mm⁻¹ per mm²) in two eggshell regions among the cowbird and its hosts. Superscripts of different letters indicate a statistically significant difference ($P \leq 0.0125$).

<u>Base</u>					
Eggshell pore density	n	Median	25%	75%	Maximum
Cowbird	326	0.00 ^b	0.00	0.33	1.63
Red-winged Blackbird	281	0.33 ^a	0.00	0.49	1.30
Grackle	360	0.32 ^a	0.00	0.33	1.30
Dickcissel	349	0.00 ^b	0.00	0.33	1.95
Eggshell pore area					
Cowbird	326	0.00 ^b	0.00	6.40	159.89
Red-winged Blackbird	281	0.40 ^b	0.00	2.00	41.57
Grackle	360	0.40 ^a	0.00	14.39	129.51
Dickcissel	349	0.00 ^c	0.00	0.50	78.35
Eggshell porosity					
Cowbird	326	0.00 ^{b,c}	0.00	58.08	1332.45
Red-winged Blackbird	281	4.14 ^{a,b}	0.00	29.52	445.42
Grackle	360	3.64 ^a	0.00	119.12	1022.48
Dickcissel	349	0.00 ^c	0.00	6.66	870.53
<u>Apex</u>					
Eggshell pore density	n	Median	25%	75%	Maximum
Cowbird	248	0.33 ^a	0.00	0.33	1.63
Red-winged Blackbird	247	0.33 ^a	0.00	0.65	1.63
Grackle	360	0.00 ^b	0.00	0.33	1.30
Dickcissel	289	0.00 ^b	0.00	0.33	1.63
Eggshell pore area					
Cowbird	248	0.30 ^a	0.00	6.40	102.33
Red-winged Blackbird	247	0.10 ^{a,b}	0.00	2.00	45.17
Grackle	360	0.00 ^{b,c}	0.00	3.00	54.36
Dickcissel	289	0.00 ^c	0.00	0.50	44.87
Eggshell porosity					
Cowbird	248	2.55 ^a	0.00	65.76	852.77
Red-winged Blackbird	247	1.11 ^{a,b}	0.00	23.32	483.96
Grackle	360	0.00 ^{b,c}	0.00	25.85	440.79
Dickcissel	289	0.00 ^c	0.00	8.43	448.70



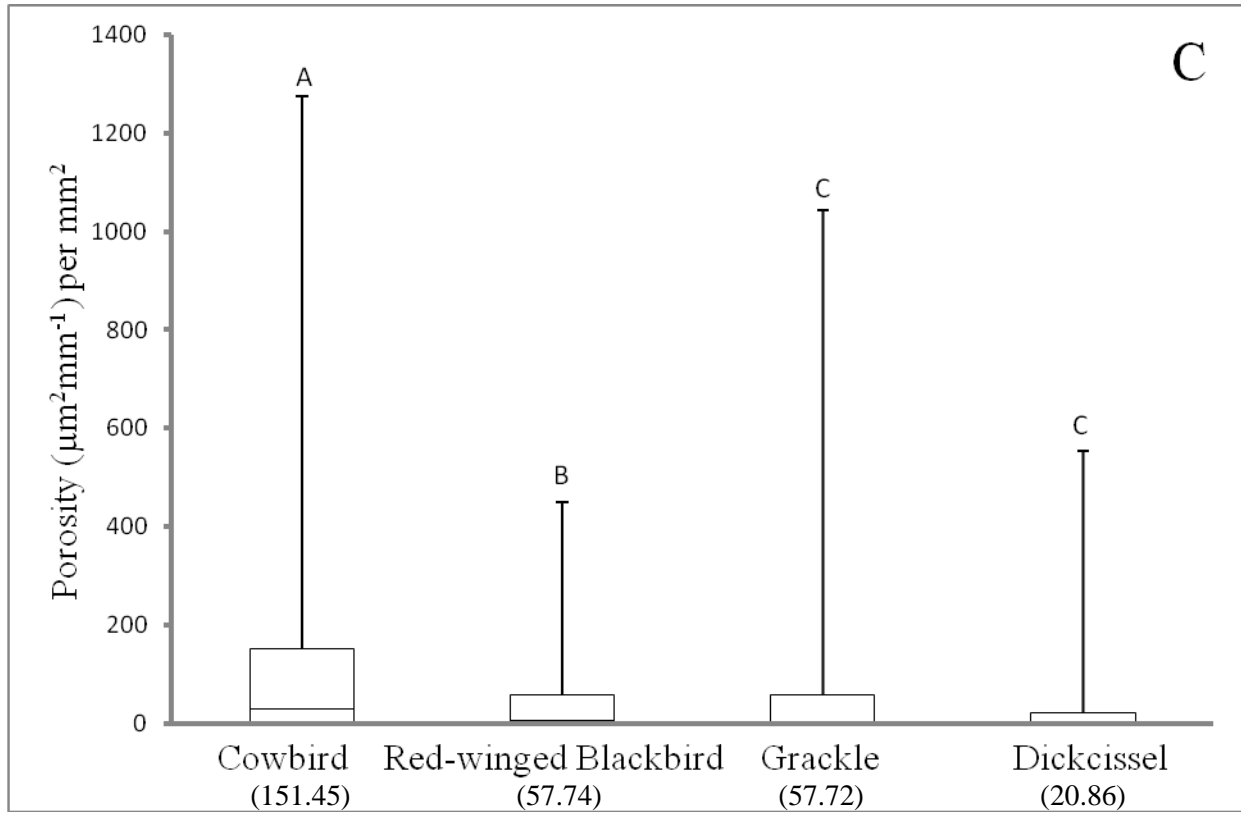
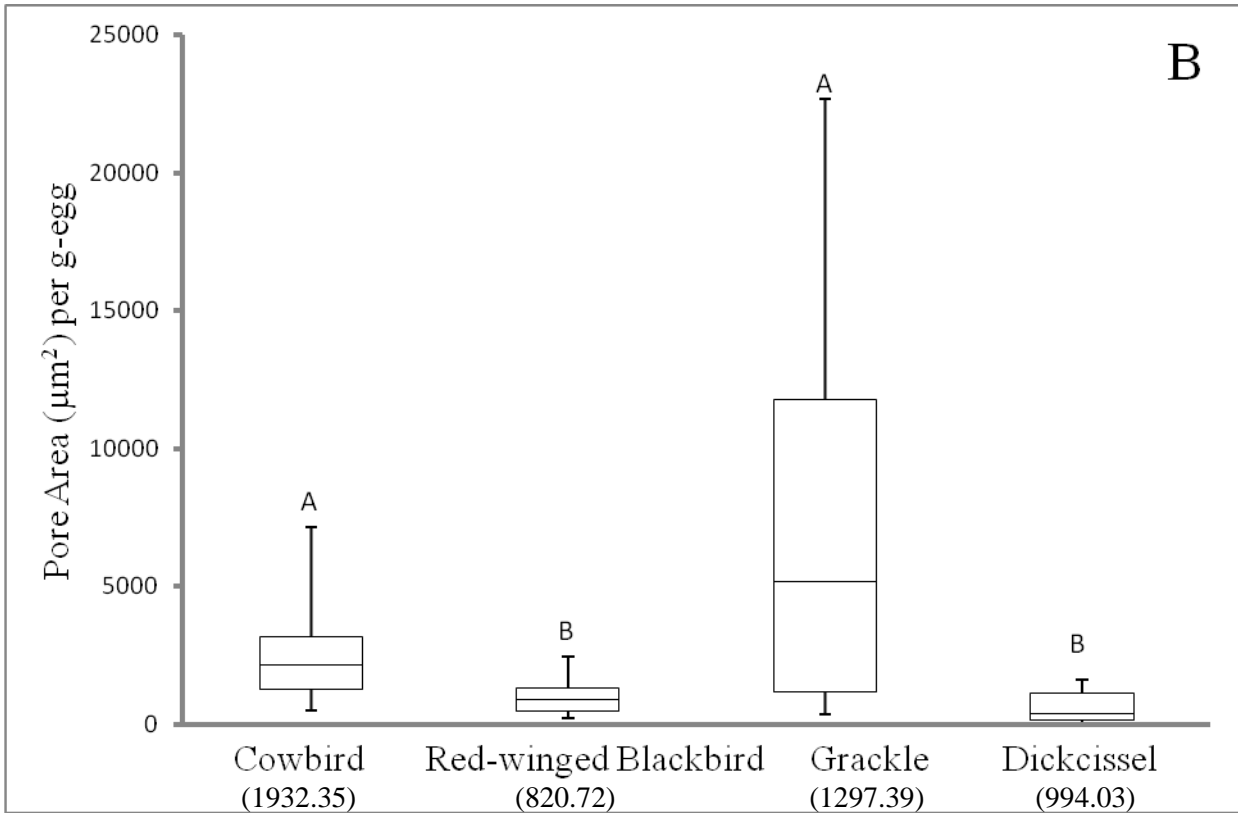
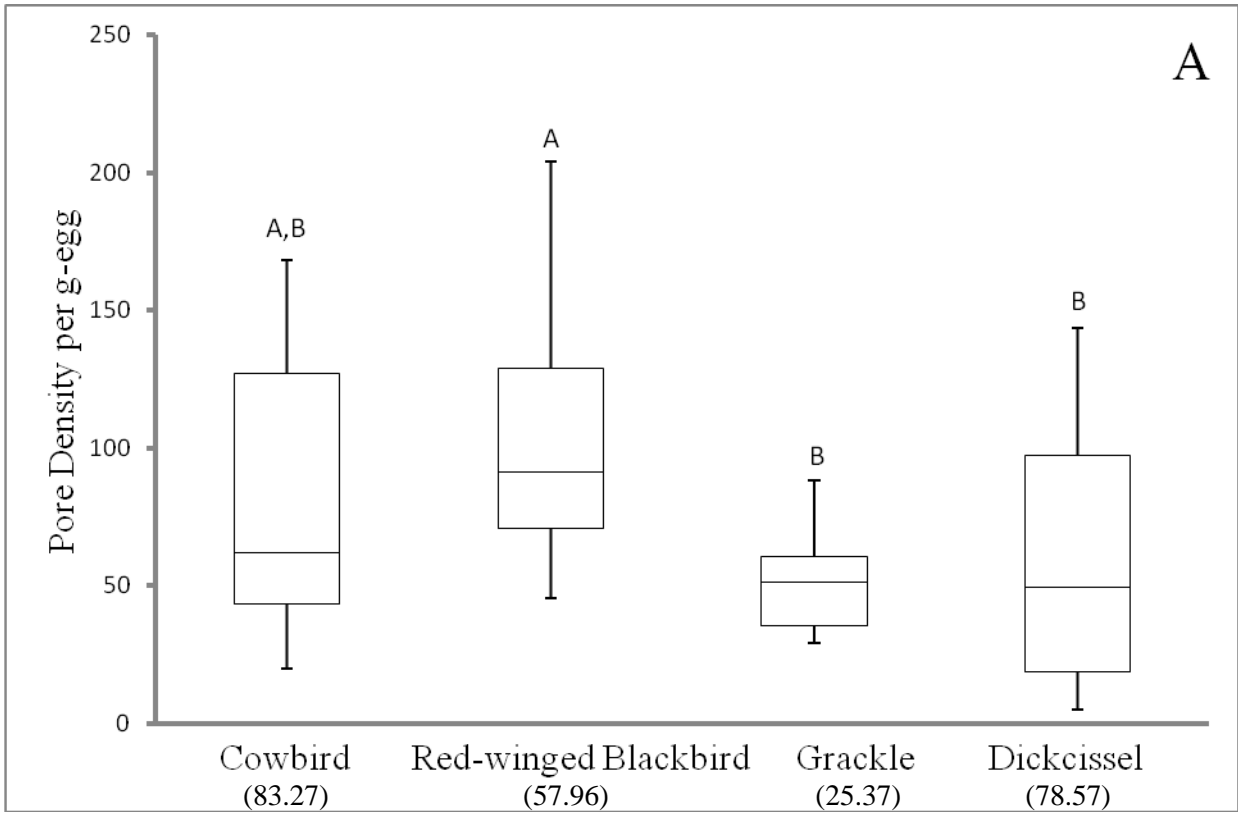


Figure 1: Differences in (A) pores per mm², (B) pore area (µm²) per mm² and (C) porosity (µm²mm⁻¹) per mm² of the equatorial eggshell region among cowbird and host eggshells. Each box plot shows 25th and 75th percentiles (box), median (line in box), and maximum (top whisker) values. Minimum values for all species in all comparisons were zero. Interquartile ranges are listed in parentheses beneath species name. Significant differences (P ≤ 0.0125) are represented by different letters located above the upper whisker.

Table 5. Comparison of overall pore density (per mm²), pore area (μm² per mm²), and porosity (μm²mm⁻¹ per mm²) among cowbird and host eggshells. Superscripts of different letters indicate a statistically significant difference ($P \leq 0.0125$).

Eggshell pore density	n	Median	25%	75%	Minimum	Maximum
Cowbird	14	0.21 ^{b,c}	0.15	0.36	0.07	0.50
Red-winged Blackbird	20	0.31 ^{a,b}	0.27	0.41	0.18	0.69
Grackle	20	0.20 ^c	0.15	0.26	0.13	0.33
Dickcissel	20	0.20 ^c	0.12	0.33	0.06	0.49
Eggshell pore area						
Cowbird	14	6.36 ^a	4.07	9.68	1.66	19.72
Red-winged Blackbird	20	3.00 ^b	1.78	4.35	0.94	4.35
Grackle	20	6.90 ^a	4.64	9.96	1.54	15.49
Dickcissel	20	1.20 ^b	0.75	3.89	0.11	7.98
Eggshell porosity						
Cowbird	14	61.49 ^a	43.09	87.99	14.12	167.44
Red-winged Blackbird	20	33.98 ^b	19.57	51.01	10.59	73.88
Grackle	20	58.99 ^a	40.88	80.33	14.39	122.81
Dickcissel	20	17.38 ^b	10.36	50.14	1.44	102.57



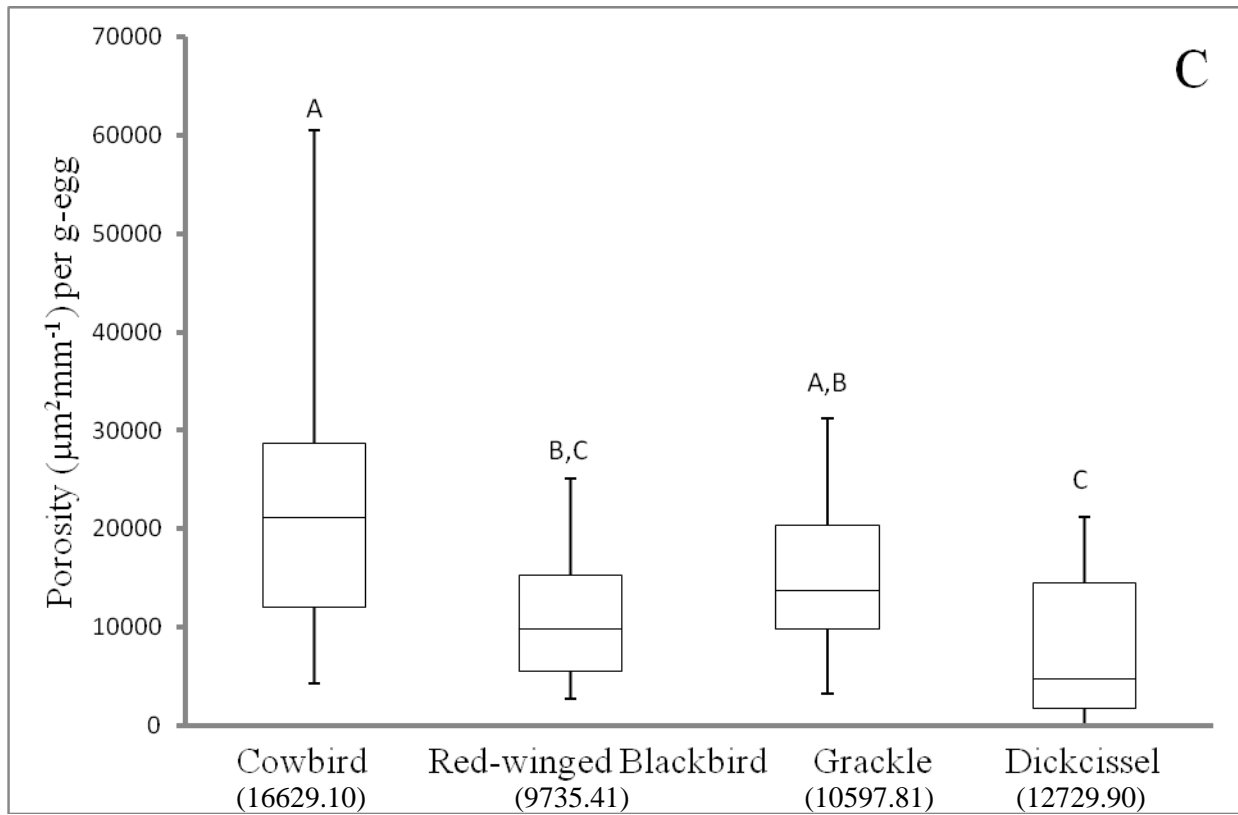
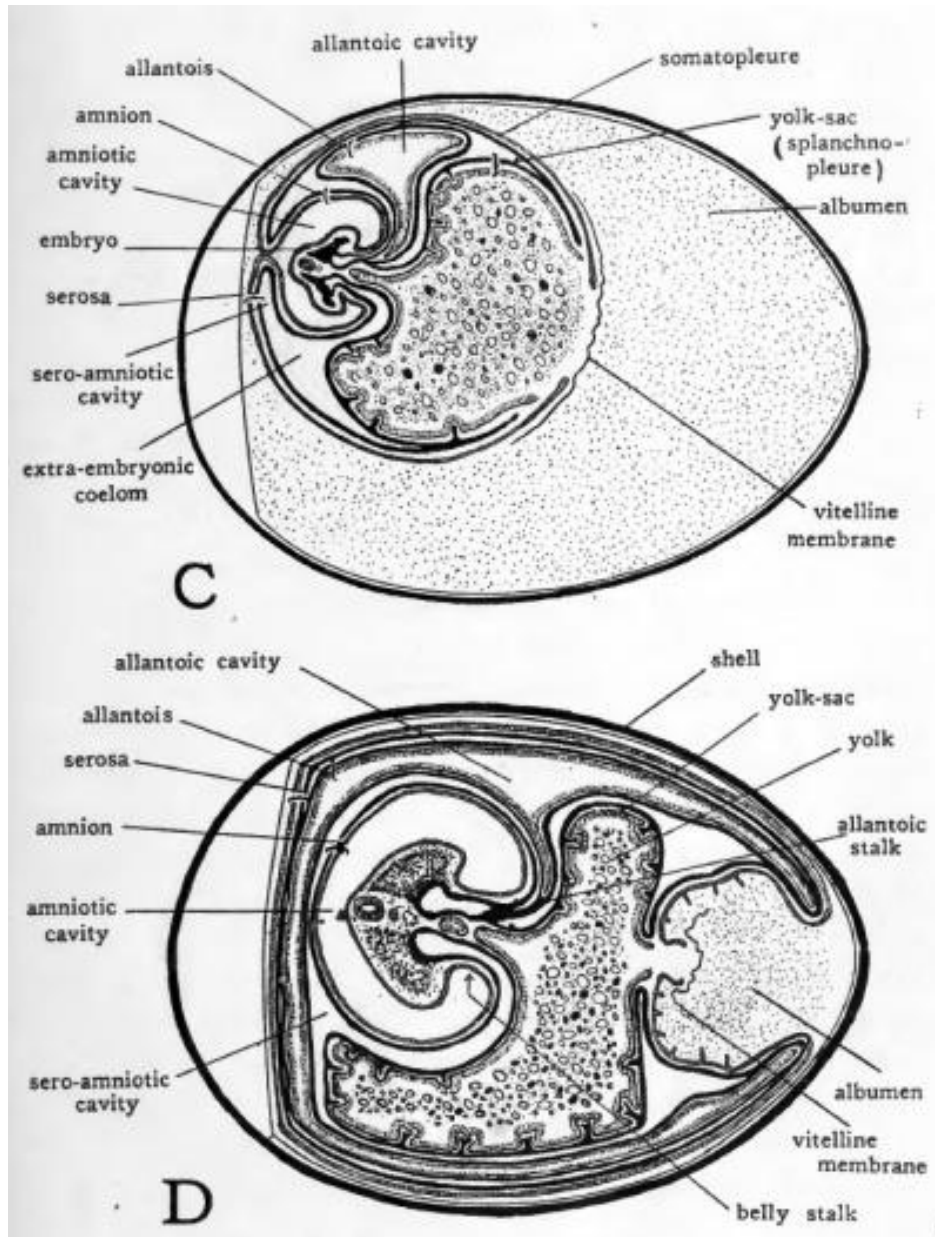


Figure 2. Differences in (A) estimated pore density per g-egg, (B) estimated pore area (µm²) per g-egg and (C) estimated porosity (µm²mm⁻¹) per g-egg among cowbird and host eggshells. All values represent entire eggshell estimates which were normalized by mass. Each box plot shows 25th and 75th percentiles (box), median (line in box), minimum (bottom whisker), and maximum (top whisker) values. Interquartile ranges are listed in parentheses beneath species name. Significant differences ($P \leq 0.0125$) are represented by different letters located above the upper whisker.

Appendices

Appendix I. The development of the chorioallantoic membrane in (C) an embryo of about 5 days incubation and in (D) an embryo of about 14 days incubation. The chorioallantoic membrane (here labeled simply "allantois") is produced by the embryo and develops in the equatorial egg region before expanding to cover the entire inner eggshell surface. The air-cell is unlabeled but can be be seen in the basal egg region as the physical space between the eggshell and the embryo. Figure from Patten (1951).



Appendix II. Comparison of pore density (per mm²), pore area (μm² per mm²), and porosity (μm²mm⁻¹ per mm²) among eggshell regions within the cowbird and its hosts via Mann-Whitney U tests.

<u>Cowbird</u>		
Apex v. Base	U	P-value
Eggshell pore density	36886.50	0.046
Eggshell pore area	37093.00	0.087
Eggshell porosity	37106.00	0.088
Apex v. Equator		
Eggshell pore density	37499.50	0.006
Eggshell pore area	36572.50	0.002
Eggshell porosity	36438.50	0.001
Base v. Equator		
Eggshell pore density	44780.50	<0.001
Eggshell pore area	43840.00	<0.001
Eggshell porosity	43773.50	<0.001
<u>Red-winged Blackbird</u>		
Apex v. Base	U	P-value
Eggshell pore density	34489.00	0.896
Eggshell pore area	33948.00	0.652
Eggshell porosity	33945.00	0.651
Apex v. Equator		
Eggshell pore density	33121.00	0.002
Eggshell pore area	31855.50	<0.001
Eggshell porosity	32090.00	<0.001
Base v. Equator		
Eggshell pore density	37978.50	0.002
Eggshell pore area	37321.50	0.001
Eggshell porosity	37576.00	0.001

(Appendix II continued)

	<u>Grackle</u>	
Apex v. Base	U	P-value
Eggshell pore density	55088.00	<0.001
Eggshell pore area	52525.00	<0.001
Eggshell porosity	52739.00	<0.001
Apex v. Equator		
Eggshell pore density	58483.00	0.011
Eggshell pore area	57709.00	0.005
Eggshell porosity	57862.50	0.006
Base v. Equator		
Eggshell pore density	61486.50	0.194
Eggshell pore area	59660.00	0.050
Eggshell porosity	59673.50	0.051
	<u>Dickcissel</u>	
Apex v. Base	U	P-value
Eggshell pore density	49238.00	0.552
Eggshell pore area	49346.50	0.592
Eggshell porosity	49243.00	0.557
Apex v. Equator		
Eggshell pore density	44260.00	<0.001
Eggshell pore area	44838.00	0.001
Eggshell porosity	45012.50	0.001
Base v. Equator		
Eggshell pore density	54943.50	0.002
Eggshell pore area	55349.00	0.003
Eggshell porosity	55734.00	0.005