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Rapid development of brood-parasitic cuckoo embryos cannot be explained by increased gas exchange through the eggshell

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Abstract

Obligate avian brood parasites lay their eggs in hosts' nests and play no role in the provisioning of the progeny. Many parasites, including *Cuculus* cuckoos, hatch before their hosts and the altricial chick evicts hosts eggs and nestlings. A hypothesized, but so far untested parasite adaptation is that the embryos of cuckoos develop more quickly than the hosts' because the higher porosity of the parasite's eggshell allows greater gaseous exchange, potentially supporting more rapid development. We compared the water vapour conductance (G_{H2O}) of common cuckoo (*Cuculus canorus*) eggshells and those of several passerines, including various cuckoo host species, and non-passerine species. Contrary to the prediction, the cuckoo eggs had lower G_{H2O} than eggs of their hosts, and lower G_{H2O} than predicted for their egg size and phylogeny. A potential advantage for the cuckoo egg of having a lower G_{H2O} may be that the yolk is depleted at a slower rate, allowing more reserves to remain at the end of incubation, assisting the embryo with the energetically demanding tasks of hatching from a thicker eggshell, and evicting host eggs and nestmates.

Introduction

Common cuckoos (*Cuculus canorus*: hereafter cuckoo) are obligate avian brood parasites that exploit a range of passerine host species (Brooke & Davies, 1988). Female cuckoos lay their eggs in host nests, and the costs of incubating the egg and rearing the chick are undertaken by the host parents (Schulze-Hagen, Stokke & Birkhead, 2009). Upon hatching, the cuckoo chick evicts the eggs and nestlings from the hosts' nest (e.g. Anderson *et al*., 2009; Grim *et al*., 2009). The eggs of cuckoos have consistently thicker shells than similarly sized host eggs (Davies, 2000; Spottiswoode, 2010; Igic *et al*., 2011), and are smaller in dimensions relative to adult body size than non-parasites (Payne, 1974; Krüger & Davies, 2004). Cuckoo eggs require *c.* 12-day long incubation, shorter than that of their hosts, which typically need 13+ days from laying to hatching (Davies, 2000; Schulze-Hagen *et al*., 2009). This fast development rate of cuckoo embryos has been attributed to: (1) internal incubation by the female cuckoo prior to laying (Birkhead *et al*., 2011); (2) larger energy stores in the yolk (Török *et al*., 2010); (3) a higher number of pores in the eggshell than host species (Hargitai *et al*., 2010); which in turn is proposed to (4) allow more intensive gas exchange, potentially supporting rapid embryonic development (Metcalfe *et al*., 1981; Stock & Metcalfe, 1987; Tullet & Burton, 1987; Christensen, Donaldson & Nestor, 1993), including elevated growth rates (Kleven *et al*., 1999; Grim, 2006, see Friedmann, 1927; Kattan, 1995; Jaeckle *et al*., 2012, for other species).

Cuckoo eggs have a higher number of pores than their host species (Hargitai *et al*., 2010). A greater numbers of pore openings, however, does not necessarily equate to a higher rate of gas exchange because individual pore canals may be furcated so that a single canal will have more than one opening on the outside (Board & Scott, 1980). Counting pore openings can result in an overestimation of the number of pore canals, and may overestimate porosity and intensity of gas exchange (Board & Scott, 1980). This may be more prevalent in cuckoo eggs, the pores of which are known to have a branched plugged structure (Board & Scott, 1980). As such, whether this greater number of pores in cuckoo eggs translates to a higher rate of gaseous exchange remains unknown. The rate of embryonic development is constrained by conductance of metabolic gases across the eggshell (Ar *et al*., 1974; Mortolo, 2009), and embryonic development can be stimulated or delayed by protracted increased or decreased oxygen supply (Metcalfe *et al*., 1981; Stock & Metcalfe, 1987; Tullet &

Table 1 Mean (±standard deviation) values of water vapour conductance (G_{H2O}) for each species measured in the current study, with sample sizes, egg mass and incubation length

Species	N	G_{H2O} (mg day^{-1} torr^{-1})	Egg mass (g)	Incubation (days)	G_{H2O} (per g egg mass)
Cuculus canorus	9	0.6 ± 0.1	3.2 ± 0.1	12	0.19 ± 0.01
Anthus pratensis	1	1.0	2.1	14	0.46
Anthus trivialis	1	2.2	2.8	13	0.81
Erithacus rubecula	1	1.6	2.8	15	0.58
Prunella modularis	3	1.7 ± 0.1	2.8 ± 0.2	14	0.6 ± 0.02
Sturnus vulgaris	3	1.4 ± 0.2	6.8 ± 0.7	14	0.2 ± 0.02
Turdus merula	3	1.9 ± 0.2	7.2 ± 0.1	14	2.7 ± 0.01
Turdus philomelos	2	3.7 ± 0.2	2.2 ± 0.4	14	1.7 ± 0.07
Carduelis cannabina	1	1.9	2.9	13	0.66
Emberiza citrinella	2	1.2 ± 0.1	3.3 ± 0.4	14	0.36 ± 0.02

The parasitic cuckoo is in bold.

Burton, 1987). Similarly, individual eggs with higher rates of gas exchange hatch, on average, earlier than is typical (Christensen *et al*., 1993; Stanishevskaya, 2006). Moreover, (1) intraspecifically, higher porosity has been argued to enable eggs laid later within a clutch to hatch more quickly than earlier laid eggs in the clutch, thus increasing the degree of hatching synchrony (Massaro & Davis, 2005; Boersma & Rebstock, 2009; Clark *et al*., 2010); (2) interspecifically, among Alcidae, their higher eggshell porosity has been suggested in some species as a mechanism of hatching more quickly than predicted from egg size (Zimmermann & Hipfner, 2007).

A higher rate of gas exchange across the eggshell can increase developmental rate, but would have to be mediated by other factors to ensure embryonic survival. A carefully controlled gas exchange across the eggshell is essential for the development of the avian embryo (Ar *et al*., 1974). Despite the fundamental differences in avian species' phylogenetic affinities and/or nesting environment, bird eggs as a rule across taxa typically lose 15–18% of their initial mass as water vapour during incubation (Drent, 1975; Ar & Rahn, 1980; Booth & Rahn, 1999; Zicus, Rave & Riggs, 2004; but see Ar *et al*., 1974). This proportionally constant amount of water loss during incubation appears to be optimal, as eggs that lose more or less water than the optimal rate show reduced hatching success, both within and between species (Snyder & Birchard, 1982; Rahn, 1984, Meir & Ar, 1987; Davis, Shen & Ackerman, 1988; Mortolo, 2009). The rate of water loss can be estimated across the eggshell as water vapour conductance (G_{H2O}, mg day^{-1} torr^{-1}), and physiologically must be balanced in such a way that desiccation does not endanger the embryo, while sufficient water is lost for embryo growth and air cell formation (Barrott, 1937; Romjin & Roos, 1938; Ar & Rahn, 1980).

Here, we provide the first measurements of the rate of gas exchange in common cuckoo eggs, and a range of their most frequent hosts, in terms of water vapour conductance (G_{H2O}). We compare these measured values of G_{H2O} to those available in the literature for other passerine and non-passerine species, to test the hypothesis that the eggs of cuckoos show a greater rate of gas exchange than those of host and/or other non-parasitic birds with similar sized eggs.

Materials and methods

Whole eggshells of British breeding birds were obtained from the class II collection at the Natural History Museum, Tring (NHM, UK), which are suitable for destructive scientific sampling (see Russell *et al*., 2010). The class II collection has been used previously for several studies (e.g. Cassey *et al*., 2010; Portugal *et al*., 2010a; Cassey *et al*., 2012). All eggs were collected shortly after they were laid, as demonstrated by the very small size of the blow hole (Scharlemann, 2001), but without detailed collection history, the host species of each individual cuckoo could not be ascertained (see also Moksnes *et al*., 2008). Each cuckoo egg ($n = 9$), however, was from a different collector and independent location. We selected the eggshells of host passerine species based on the availability within the destructive museum collection. Eggshells of the following hosts were selected (sample sizes are provided in Table 1), *Anthus pratensis* (meadow pipit), *Anthus trivialis* (tree pipit), *Erithacus rubecula* (European robin) and *Prunella modularis* (dunnock) (Davies, 2000). For their similarity in size and incubation periods to the cuckoo eggs and for general 'cuckoo versus other birds' comparison of G_{H2O} (mg day^{-1} torr^{-1}), we included *Carduelis cannabina* (common linnet), *Emberiza citrinella* (yellowhammer), *Sturnus vulgaris* (common starling), *Turdus merula* (common blackbird) and *Turdus philomelos* (song thrush), which are occasional, but not regular cuckoo hosts (Moksnes & Røskaft, 1995; Davies, 2000). We also included G_{H2O} data from the literature for 11 additional passerine species and 56 non-passerine species (Table 2).

Previously, we had measured G_{H2O} of museum eggs using small eggshell fragments (Portugal, Maurer & Cassey, 2010b, see also Portugal *et al*., 2014). Here, because of the smaller size of the eggshells, we instead use whole eggs. Eggshell thickness (μm) was measured on the sampled eggs following conductance measurements, according to a previously published protocol (Maurer, Portugal & Cassey, 2012). Briefly, length

Table 2 Mean (±standard deviation where available) values of water vapour conductance (G_{H2O}) for species taken from the literature, with sample sizes (where available), egg mass and incubation length

Species	*N*	G_{H2O} (mg day^{-1} torr^{-1})	Egg mass (g)	Incubation (days)	Reference
Passerines					
Troglodytes aedon	27	0.65 ± 0.4	1.3 ± 0.1	16	Ar *et al*. (1974)
Setophaga petechia	3	0.45 ± 0.3	1.6 ± 0.9	13	Ar *et al*. (1974)
Tachycineta bicolor	5	0.5 ± 0.1	1.7 ± 0.1	14	Ar *et al*. (1974)
Passer domesticus	21	0.88 ± 0.3	2.6 ± 0.1	14	Ar *et al*. (1974)
Agelaius phoeniceus	18	1.73 ± 0.6	3.5 ± 0.4	14	Ar *et al*. (1974)
Quiscalus quiscula	3	2.98 ± 0.9	6.3 ± 0.0	13	Ar *et al*. (1974)
Turdus migratorius	6	1.42 ± 0.3	6.5 ± 0.1	14	Ar *et al*. (1974)
Cinclus cinclus	39	0.85 ± 0.3	4.5 ± 0.4	17	Nybo *et al*. (1997)
Taeniopygia guttata	14	0.25 ± 0.1	0.9 ± 0.8	14	Vleck, Hoyt & Vleck (1979)
Ploceus cucullatus	12	0.84 ± 0.1	2.8 ± 0.4	12	Vleck *et al*. (1979)
Ficedula hypoleuca	32	0.68 ± 0.4	1.6 ± 0.2	13	Kern, Cowie & Yeager (1992)
Non-passerines					
Coturnix japonica	12	3.1 ± 0.7	9.6 ± 0.7	18	Ar *et al*. (1974)
Meleagris gallopavo	11	13.5 ± 1.3	87.8 ± 4.3	29	Ar *et al*. (1974)
Gallus gallus	12	14.4 ± 2.4	53.9 ± 2.1	21	Ar *et al*. (1974)
Pluvialis apricaria	3	5.0 ± 4.7	32.6 ± 0.1	29	Ar *et al*. (1974)
Phasianus colchicus	12	6.6 ± 0.8	33.8 ± 2.3	24	Ar *et al*. (1974)
Lophura nycthemera	3	9.3 ± 0.7	39.9 ± 1	24	Ar *et al*. (1974)
Phalacrocorax auritus	8	5.6 ± 3.2	49.9 ± 3.4	29	Ar *et al*. (1974)
Numenius phaeopus	4	9.7 ± 1.5	53.5 ± 0.4	27	Ar *et al*. (1974)
Haematopus ostralegus	2	6.8 ± 0.2	41.4 ± 0.4	25	Ar *et al*. (1974)
Larus marinus	9	16.7 ± 2.7	110.8 ± 12.1	28	Ar *et al*. (1974)
Larus canus	8	15.0 ± 2.9	76.2 ± 4.5	25	Ar *et al*. (1974)
Larus fuscus	6	16.0 ± 1.6	84.9 ± 4.3	25	Ar *et al*. (1974)
Larus argentatus	3	16.5 ± 1.9	88.2 ± 4.0	29	Ar *et al*. (1974)
Catharacta skua	6	18.4 ± 0.3	95.5 ± 5.4	28	Ar *et al*. (1974)
Fratercula arctica	6	7.9 ± 1.2	59.6 ± 3.4	38	Ar *et al*. (1974)
Larus glaucescens	21	22.6 ± 1.0	98.2 ± 1.9	28	Morgan *et al*. (1978)
Rissa tridactyla	11	9.7 ± 0.8	51.5 ± 1.0	27	Morgan *et al*. (1978)
Anser erythropus	7	20.6	122.9	25	Hoyt *et al*. (1979)
Anser cygnoides	7	26.7	146.4	28	Hoyt *et al*. (1979)
Anser fabalis	9	24.9	152.3	27	Hoyt *et al*. (1979)
Anser brachyrhynchus	3	23.4	139.4	27	Hoyt *et al*. (1979)
Anser anser	3	33.2	162.5	27	Hoyt *et al*. (1979)
Anser indicus	2	8.4	110.1	28	Hoyt *et al*. (1979)
Branta sandvicensis	3	33.4	154.4	30	Hoyt *et al*. (1979)
Branta leucopsis	7	19.6	106.6	24	Hoyt *et al*. (1979)
Branta ruficollis	5	12.9	67.8	25	Hoyt *et al*. (1979)
Cyanochen cyanoptera	2	14.7	82.8	30	Hoyt *et al*. (1979)
Chen rossii	3	18.6	91.8	22	Hoyt *et al*. (1979)
Chen canagica	7	27.4	136.1	24	Hoyt *et al*. (1979)
Chloephaga picta	1	23.8	106.1	30	Hoyt *et al*. (1979)
Chloephaga poliocephala	1	13.9	100	30	Hoyt *et al*. (1979)
Cairina moschata	4	12.3	80.2	35	Hoyt *et al*. (1979)
Chloephaga rubidiceps	3	11.7	84.1	30	Hoyt *et al*. (1979)
Tadorna ferruginea	2	16.6	79.1	29	Hoyt *et al*. (1979)
Tadorna variegata	6	14.1	89.6	30	Hoyt *et al*. (1979)
Tadorna tadorna	4	15.3	79.9	28	Hoyt *et al*. (1979)
Cairina scutulata	1	22.8	99.1	30	Hoyt *et al*. (1979)
Callonetta leucophrys	10	6.1	31.6	23	Hoyt *et al*. (1979)
Aix sponsa	5	8.4	43.4	30	Hoyt *et al*. (1979)
Aix galericulata	2	8	27.4	29	Hoyt *et al*. (1979)
Dendrocygna bicolor	7	17.1	54.4	25	Hoyt *et al*. (1979)
Dendrocygna arcuata	4	6.1	36.5	30	Hoyt *et al*. (1979)
Dendrocygna arborea	9	11.6	59.8	30	Hoyt *et al*. (1979)
Dendrocygna autumnalis	10	11.6	42.8	27	Hoyt *et al*. (1979)

Table 2 Continued.

Species	N	G_{H2O} (mg day^{-1} torr^{-1})	Egg mass (g)	Incubation (days)	Reference
Eudocimus albus	30	7.8 ± 3.4	50.8 ± 5.7	24	Vleck *et al.* (1983)
Bubulcus ibis	19	5.4 ± 1.5	27.8 ± 2.8	22	Vleck *et al.* (1983)
Plegadis falcinellus	6	7.6 ± 2.0	37.4 ± 4.4	22	Vleck *et al.* (1983)
Egretta thula	6	3.8 ± 0.7	22.6 ± 1.5	23	Vleck *et al.* (1983)
Egretta tricolor	8	3.6 ± 1.9	26.6 ± 1.5	22	Vleck *et al.* (1983)
Nycticorax nycticorax	1	6.2	38.8	25	Vleck *et al.* (1983)
Sterna sandvicensis	6	8.3 ± 1.6	36.1 ± 2.6	29	Vleck *et al.* (1983)
Sterna maxima	17	13.3 ± 3.0	68.0 ± 5.2	25	Vleck *et al.* (1983)
Philomachus pugnax	1	3.62	19.1	22	Visser *et al.* (1995)
Tringa totanus	12	4.3 ± 0.4	22.3 ± 1.5	24	Visser *et al.* (1995)
Limosa limosa	26	9.5 ± 1.8	39.1 ± 3.3	23	Visser *et al.* (1995)
Numenius arquata	10	16.2 ± 1.9	82.8 ± 6.6	28	Visser *et al.* (1995)

Species are organized according to the source reference.

and width of eggshell and blowhole diameters were measured to 0.1 mm using Mitutoyo ABS Digimatic Callipers CD-6" C (Mitutoyo Coventry, Wickmans Drive Warwickshire, Coventry, Warwickshire, UK). We measured shell thickness of our samples in two different ways: (1) if the shell had an adequate equatorial blowhole, we measured the thickness of the shell in the opposite section of the egg through the blowhole; (2) shells were cut in half, following conductance measurements, along their long axis, using a diamond-tipped dentist drill (Milnes Bros., Surrey, UK). The thickness of one of the egg halves was measured to 1 μm three times each for the regions at the blunt end, the equator and the pointed end of the egg using a modified Mitutoyo micrometer (Series 227-203, Absolute Digimatic Mitutoyo, UK), at its 1.5-N constant pressure setting. Both anvils of the micrometer had been capped with an aluminium pin with a diameter of 1.35 mm (radius 0.35 mm). The repeatability (Lessells & Boag, 1987) of thickness measurements with the Series 207 micrometer was assessed previously on a sample of 20 helmeted guineafowl *Numida meleagris* eggs measured 10 times at the same location of the equator (Maurer *et al.*, 2012). Measurements showed a highly consistent repeatability (intra-class correlation coefficient $r > 0.99$, $n_0 = 10$, $a = 20$, see Maurer *et al.*, 2012 for full details).

The G_{H2O} of the eggs was measured following the same standard protocol (Board & Scott, 1980; Booth & Seymour, 1987; Portugal *et al.*, 2010a,b; Maurer, Portugal & Cassey, 2011a), that was used by studies that were the source of comparative literature data (see earlier). Briefly, the eggshells were filled with distilled water to capacity. As the eggs had been blown following collection, the blow hole was covered using impermeable plastic, cut to size to cover the hole and glued on using Loctite™ superglue (Loctite® Brand - Consumer Products Henkel Corporation, Ohio). The plastic covering the blow hole comprised, on average, less than 2.5% of the total surface area. The eggs were placed into desiccators, which in turn were housed in a constant temperature thermocabinet (Camlab, Cambridgeshire, UK) at 30 ± 1°C (Booth & Seymour, 1987; Portugal *et al.*, 2010b; Maurer *et al.*, 2011a). After 24 h, the eggs were weighed to the nearest 0.1 mg with an analytical balance (Sartorius, Göttingen, Germany) before being returned to the desiccators. The eggs were weighed at the same time of day on 3 successive days to provide two values of 24-h G_{H2O}, and a mean was taken. Any mass loss was assumed to be the result of water loss (Booth & Seymour, 1987; Portugal *et al.*, 2010b; Maurer *et al.*, 2011a). Calculation of G_{H2O} was as previously described (Booth & Seymour, 1987; Portugal *et al.*, 2010b; Maurer *et al.*, 2011a). Briefly, the water vapour conductance of a shell can be calculated as:

$$G_{H_2O} = \frac{M_{H_2O}}{P_{H_2O}} \tag{1}$$

Where G_{H2O} = water vapour conductance (mg day^{-1} torr^{-1}); M_{H2O} = the rate of mass loss (mg day^{-1}); and $\Delta\ P_{H2O}$ = water vapour pressure difference across the shell (torr).

Previously, we had demonstrated that the G_{H2O} of eggshell fragments from museum and fresh black-headed gull *Chroicocephalus ridibundus* eggs did not differ significantly (Portugal *et al.*, 2010b). In addition to this previous work, we conducted a repeated G_{H2O} comparison using whole fresh and museum common quail *Coturnix coturnix* eggs, as these eggs were the closest in size (egg length) to the cuckoo eggs (quails: 31.05 ± 1.31 mm; cuckoos 23.12 ± 0.87 mm) that were both available in the museum collection and also freely available as fresh specimens. As shown for the black-headed gulls (Portugal *et al.*, 2010b), there was also no significant difference in G_{H2O} between the fresh ($n = 24$) and museum ($n = 6$) quail eggs [$t = -0.372$, degrees of freedom (d.f.) = 28, $P = 0.71$, 2.46 ± 0.68 and 2.68 ± 0.31 mg day^{-1} torr^{-1} for fresh and museum quail eggs, respectively, Fig. 1]. The collection dates of the museum quail eggs ranged from 1901–1963. The intra-specific variation in G_{H2O} was very low (Fig. 1). To ensure that the plastic covering the blow hole was a sufficient airtight seal, the fresh quail eggs measured previously for the museum and fresh G_{H2O} comparison were blown. The eggs were then filled with distilled water (as mentioned earlier), and the impermeable plastic cover was fitted, before G_{H2O} was measured. No significant difference was detected in G_{H2O} between the fresh quail eggs and the blown eggs fitted with

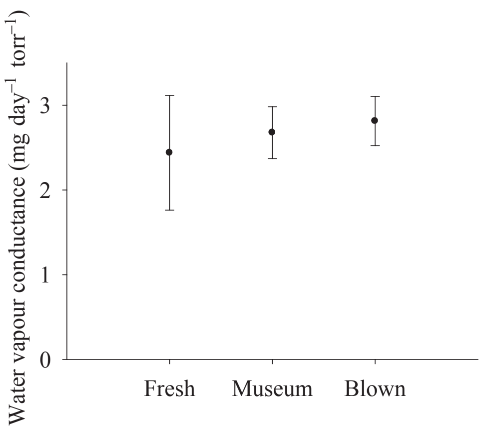

Figure 1 Comparison of water vapour conductance (mean ± standard error; mg day^{-1} torr^{-1}) in common quail *Coturnix coturnix* eggs between freshly collected ('fresh', $n = 24$), museum specimen ('museum', $n = 6$) and blown fresh eggs sealed with a plastic device ('blown') (see Materials and methods). There was no overall statistically significant difference in water vapour conductance between the museum and fresh eggs or between fresh eggs and those same eggs when blown, filled with water, and the blow hole sealed (see Materials and methods).

the plastic cover (paired *t*-test, $t = -2.012$, d.f. = 23, $P = 0.06$, 2.81 ± 0.29 mg day^{-1} torr^{-1}, for blown quail eggs, Fig. 1).

Avian phylogenetic trees were constructed online (http://www.birdtree.org) based on data from the complete avian phylogeny of Jetz *et al.* (2012), and using the primary backbone tree of Hackett *et al.* (2008). One thousand trees were constructed and a phylogenetic generalized least square model ($\text{Log}_{10}G_{H_2O} \sim \text{Log}_{10}\text{EggMass}$) was conducted using the package 'caper' (Orme *et al.*, 2011) in the statistical software program R, version 3.0.2 (R Software, Vienna, Austria, http://www.R-project.org). Phylogenetic signal was measured by Pagel's lambda (λ) (Pagel, 1999). Lambda indicates the strength of the phylogenetic relationship, where values lie between 0 and 1. Lambda values of or near 0 are indicative of phylogenetic independence and values of or near 1 indicate that the variable is fully explained by evolutionary history (Freckleton, Harvey & Pagel, 2002). Fitted values are shown as mean ± standard error.

Results

We confirmed that cuckoo eggshells were thicker (0.88 ± 0.03 μm) than those of the host species (per species value, 0.72 ± 0.1 μm, *t*-test, $t = 98.46$, d.f. = 4, $P < 0.001$). The average G_{H_2O} for the cuckoo eggs (0.59 ± 0.07 mg day^{-1} torr^{-1}) was lower than that of all passerines combined (1.58 ± 0.91 mg day^{-1} torr^{-1}). The closest species in egg mass to the cuckoos (3.2 ± 0.1 g) had higher G_{H_2O} in comparison, 1.2 ± 0.1 and 1.9 mg day^{-1} torr^{-1} for *Emberiza citrinella* (3.3 ± 0.4 g) and *Carduelis cannabina* (2.9 g), respectively (Table 1). There was very little variation in G_{H_2O} between the cuckoo eggs (Table 1). Across species, not accounting for phylogeny, there was a strong significant positive correlation between G_{H_2O} and egg mass [log-transformed; Pearson's *r* [95% confidence interval] = 0.97 (0.95, 0.98), $n = 77$, $P < 0.001$; Fig. 2].

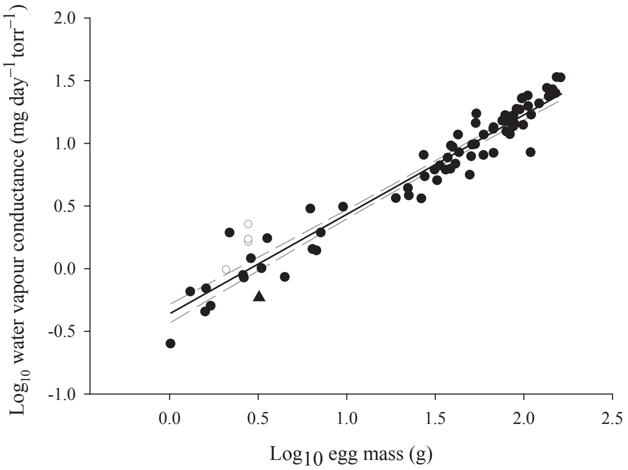

Figure 2 Regression (solid line) with 95% confidence intervals (grey-dashed line) of eggshell water vapour conductance ($\log_{10} G_{H_2O}$, mg day^{-1} torr^{-1}) on egg mass ($\log_{10}$ g). The regression line ($G_{H_2O} = 0.18 \times \text{egg mass} + 0.60$, $r^2 = 0.91$, $P < 0.001$) is calculated for all species, taken both from the host species measured in this study (open circles, $n = 4$) and from non-host species (closed circles, $n = 73$). Cuckoos (closed triangle) are not included in calculating the regression line.

Across the 77 species, for which data were available, G_{H_2O} possessed a strong phylogenetic signal across the 1000 resampled phylogenies [median Pagel's Lambda (95th percentiles) = 0.90 (0.88, 0.93)]. In a 1000 phylogenetic generalized least squares models the positive relationship between egg mass and G_{H_2O} was highly significant [median model estimate (95th percentiles) = 0.81 (0.79, 0.82)]. Across all 1000 phylogenies the cuckoo had, on average, the smallest (i.e. most negative) residual value in the phylogenetic generalized least squares model between egg mass and G_{H_2O} [median residual (corrected for phylogeny) = –0.57; average rank = 2.01], meaning cuckoo eggs have lower G_{H_2O} than the species' location within the avian phylogeny would predict. Interestingly, the species with the next most negative residual was the European dipper (*Cinclus cinclus*), which nests in the moist banks of fast moving montaine streams (median residual [corrected for phylogeny] = –0.46; average rank = 5.08).

Discussion

Contrary to our prediction, the cuckoo eggs in the present study did not have a higher G_{H_2O} than their hosts, or other non-host passerine species with similar sized eggs. Instead, cuckoo eggs exhibited a significantly lower G_{H_2O}. Thus, despite higher counts of external pore openings (Hargitai *et al.*, 2010), cuckoo eggs do not have an increased permeability of the eggshell, which suggests that this is not a contributing factor to the rapid development of the cuckoo embryo. This is contrary to what has been previously established in parasitic cowbirds (*Molothrus* spp.), which had higher G_{H2O} compared with that of their hosts and G_{H2O} predicted for their egg mass (Jaeckle *et al*., 2012).

Cuckoo eggshells are thicker and stronger than their hosts (Igic *et al*., 2011). A clear link between avian eggshell thickness and gas conductance has never been established, but the exceptional thickness of the cuckoo eggshell, which functions initially to both protect the egg from breakage during laying (Lack, 1968) and prevent host puncture (Swynnerton, 1918; Spottiswoode, 2010), may contribute to its lower G_{H2O}. However, it has been demonstrated that a relatively thick eggshell does not act as a barrier to gas exchange, and can be counteracted by either larger pores, or a higher pore density (Hargitai *et al*., 2010; Jaeckle *et al*., 2012). As such, a thicker eggshell does not necessarily equate with low G_{H2O}. A potential explanation for how cuckoo eggs achieve the intense gas exchange required for rapid embryonic development in the course of incubation is that the shell is subsequently thinned (Booth & Seymour, 1987), both substantially and rapidly, during incubation to provide calcium for the embryo's growth (Ar *et al*., 1974; Handrich, 1989). A more rapid thinning of the cuckoo eggshell during incubation could serve a dual function of providing calcium for stronger bone development and increasing G_{H2O} for faster development (e.g. Carey, 1979; Hanka *et al*., 1979; Oviedo-Rondón *et al*., 2008; Maurer, Portugal & Cassey, 2011b). This increase in G_{H2O} may be achieved either by increasing interstitial gas exchange through the shell itself or by 'activating' more pores to open to and provide gas exchange through the shortening of the furcated pore channels, which are characteristic of cuckoo eggshells. Stronger bones and muscles would also provide the structure and strength required by the cuckoo chick to hatch earlier (Honza *et al*., 2001; Igic *et al*., 2011), and for the energetically and physiologically costly eviction of host eggs and nestlings, accomplished by the naked and blind cuckoo chick within days after hatching (Anderson *et al*., 2009; Grim *et al*., 2009; Hargitai *et al*., 2012). Differences in the skeletal strength of hatchling hosts and parasites may offer an intriguing additional explanation for increased eggshell thickness in cuckoos.

We were limited in the present study by the availability of host species in the destructive collection of the NHM. The nature of the specimens meant that there was no certainty from which host each cuckoo egg came from. Therefore, we did not make a direct paired comparison between a specialist cuckoo's egg and the host clutch that it was part of. As a result, we cannot investigate the specific relationships of G_{H2O} between different female cuckoos and their preferred hosts. However, values of cuckoo G_{H2O} are (1) lower compared with both host and non-host passerines (Fig. 2); (2) show remarkably small variation (Table 1), suggesting that a comparison with additional host species values would not change our conclusions. Therefore, we propose that the mechanism by which a cuckoo egg hatches earlier than its host is not a simple consequence of a more permeable shell at laying and a higher rate of gaseous exchange. Instead, we suggest that the process of eggshell thinning during incubation in the cuckoos may be more extreme compared with its hosts, and the interaction between G_{H2O} and eggshell thickness more complex than initially described. It is generally assumed that a low conductance across the shell will deplete yolk reserves at a slower rate than a high conductance (Ar *et al*., 1974). Because of the high energetic costs for the cuckoo embryo (e.g. greater cumulative number of pecks) of hatching from a stronger, thicker eggshell (Honza *et al*., 2001), a slower conductance may allow more yolk reserves to remain at the end of incubation, to assist with the energetically demanding event of hatching. This may be an alternate explanation for why cuckoo eggs have larger energy stores in the yolk (e.g. Török *et al*., 2010) when first laid.

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