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

S. J. Portugal<sup>1</sup>, M. E. Hauber<sup>2</sup>, G. Maurer<sup>3</sup>, B. G. Stokke<sup>4</sup>, T. Grim<sup>5</sup> & P. Cassey<sup>3</sup>

<sup>1</sup> Structure and Motion Laboratory, Royal Veterinary College, University of London, Hatfield, UK

<sup>2</sup> Department of Psychology, Hunter College and the Graduate Center of the City University of New York, New York, NY, USA

<sup>3</sup> School of Earth and Environmental Sciences, University of Adelaide, Adelaide, SA, Australia

<sup>4</sup> Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway

<sup>5</sup> Department of Zoology and Laboratory of Ornithology, Palacký University, Olomouc, Czech Republic

## Keywords

*Cuculus canorus*; conductance; eggshell porosity; parasite; permeability.

## Correspondence

Steven J. Portugal, Structure and Motion Laboratory, Royal Veterinary College, University of London, Hawkshead Lane, Hatfield, Herts, AL9 7TA, UK.  
Email: SPortugal@rvc.ac.uk

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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 of a higher porosity of the parasite's eggshell, allowing greater gaseous exchange, potentially supporting more rapid development. We compared the water vapour conductance ( $G_{H_2O}$ ) 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  $G_{H_2O}$  than eggs of their hosts, and lower  $G_{H_2O}$  than predicted for their egg size and phylogeny. A potential advantage for the cuckoo egg of having a lower  $G_{H_2O}$  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 (Metcalf *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 (Metcalf *et al.*, 1981; Stock & Metcalfe, 1987; Tullet &

**Table 1** Mean ( $\pm$ standard deviation) values of water vapour conductance ( $G_{H_2O}$ ) for each species measured in the current study, with sample sizes, egg mass and incubation length

Species	<i>N</i>	$G_{H_2O}$ (mg day <sup>-1</sup> torr <sup>-1</sup> )	Egg mass (g)	Incubation (days)	$G_{H_2O}$ (per g egg mass)
<b><i>Cuculus canorus</i></b>	9	0.6 $\pm$ 0.1	3.2 $\pm$ 0.1	12	0.19 $\pm$ 0.01
<i>Anthus pratensis</i>	1	1.0	2.1	14	0.46
<i>Anthus trivialis</i>	1	2.2	2.8	13	0.81
<i>Erithacus rubecula</i>	1	1.6	2.8	15	0.58
<i>Prunella modularis</i>	3	1.7 $\pm$ 0.1	2.8 $\pm$ 0.2	14	0.6 $\pm$ 0.02
<i>Sturnus vulgaris</i>	3	1.4 $\pm$ 0.2	6.8 $\pm$ 0.7	14	0.2 $\pm$ 0.02
<i>Turdus merula</i>	3	1.9 $\pm$ 0.2	7.2 $\pm$ 0.1	14	2.7 $\pm$ 0.01
<i>Turdus philomelos</i>	2	3.7 $\pm$ 0.2	2.2 $\pm$ 0.4	14	1.7 $\pm$ 0.07
<i>Carduelis cannabina</i>	1	1.9	2.9	13	0.66
<i>Emberiza citrinella</i>	2	1.2 $\pm$ 0.1	3.3 $\pm$ 0.4	14	0.36 $\pm$ 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, the higher porosity has been argued to enable eggs laid later within a clutch to hatch more quickly, 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 ( $G_{H_2O}$ , mg day<sup>-1</sup> torr<sup>-1</sup>), 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 ( $G_{H_2O}$ ). We compare these measured values of  $G_{H_2O}$  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 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  $G_{H_2O}$  (mg day<sup>-1</sup> torr<sup>-1</sup>), 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  $G_{H_2O}$  data from the literature for 11 additional passerine species and 56 non-passerine species (Table 2).

Previously, we had measured  $G_{H_2O}$  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 ( $\mu$ m) was measured on the sampled eggs following conductance measurements, according to a previously published protocol (Maurer, Portugal & Cassey, 2012). Briefly, length and width of eggshell and blowhole diameters were measured to 0.1 mm using Mitutoyo ABS Digimatic Callipers CD-6" C. 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

**Table 2** Mean ( $\pm$ standard deviation where available) values of water vapour conductance ( $G_{H_2O}$ ) for species taken from the literature, with sample sizes (where available), egg mass and incubation length

Species	<i>N</i>	$G_{H_2O}$ (mg day <sup>-1</sup> torr <sup>-1</sup> )	Egg mass (g)	Incubation (days)	Reference
Passerines					
<i>Troglodytes aedon</i>	27	0.65 $\pm$ 0.4	1.3 $\pm$ 0.1	16	Ar <i>et al.</i> (1974)
<i>Dendroica petechia</i>	3	0.45 $\pm$ 0.3	1.6 $\pm$ 0.9	13	Ar <i>et al.</i> (1974)
<i>Tachycineta bicolor</i>	5	0.5 $\pm$ 0.1	1.7 $\pm$ 0.1	14	Ar <i>et al.</i> (1974)
<i>Passer domesticus</i>	21	0.88 $\pm$ 0.3	2.6 $\pm$ 0.1	14	Ar <i>et al.</i> (1974)
<i>Agelaius phoeniceus</i>	18	1.73 $\pm$ 0.6	3.5 $\pm$ 0.4	14	Ar <i>et al.</i> (1974)
<i>Quiscalus quiscula</i>	3	2.98 $\pm$ 0.9	6.3 $\pm$ 0.0	13	Ar <i>et al.</i> (1974)
<i>Turdus migratorius</i>	6	1.42 $\pm$ 0.3	6.5 $\pm$ 0.1	14	Ar <i>et al.</i> (1974)
<i>Cinclus cinclus</i>	39	0.85 $\pm$ 0.3	4.5 $\pm$ 0.4	17	Ar <i>et al.</i> (1974)
<i>Poephila guttata</i>	14	0.25 $\pm$ 0.1	0.9 $\pm$ 0.8	14	Vleck, Hoyt & Vleck (1979)
<i>Ploceus cucullatus</i>	12	0.84 $\pm$ 0.1	2.8 $\pm$ 0.4	12	Vleck <i>et al.</i> (1979)
<i>Ficedula hypoleuca</i>	32	0.68 $\pm$ 0.4	1.6 $\pm$ 0.2	13	Kern, Cowie & Yeager (1992)
Non-passerines					
<i>Coturnix japonica</i>	12	3.1 $\pm$ 0.7	9.6 $\pm$ 0.7	18	Ar <i>et al.</i> (1974)
<i>Meleagris gallopavo</i>	11	13.5 $\pm$ 1.3	87.8 $\pm$ 4.3	29	Ar <i>et al.</i> (1974)
<i>Gallus gallus</i>	12	14.4 $\pm$ 2.4	53.9 $\pm$ 2.1	21	Ar <i>et al.</i> (1974)
<i>Pluvialis apricaria</i>	3	5.0 $\pm$ 4.7	32.6 $\pm$ 0.1	29	Ar <i>et al.</i> (1974)
<i>Phasianus colchicus</i>	12	6.6 $\pm$ 0.8	33.8 $\pm$ 2.3	24	Ar <i>et al.</i> (1974)
<i>Lophura nycthemera</i>	3	9.3 $\pm$ 0.7	39.9 $\pm$ 1	24	Ar <i>et al.</i> (1974)
<i>Phalacrocorax auritus</i>	8	5.6 $\pm$ 3.2	49.9 $\pm$ 3.4	29	Ar <i>et al.</i> (1974)
<i>Numenius phaeopus</i>	4	9.7 $\pm$ 1.5	53.5 $\pm$ 0.4	27	Ar <i>et al.</i> (1974)
<i>Haematopus ostralegus</i>	2	6.8 $\pm$ 0.2	41.4 $\pm$ 0.4	25	Ar <i>et al.</i> (1974)
<i>Larus marinus</i>	9	16.7 $\pm$ 2.7	110.8 $\pm$ 12.1	28	Ar <i>et al.</i> (1974)
<i>Larus canus</i>	8	15.0 $\pm$ 2.9	76.2 $\pm$ 4.5	25	Ar <i>et al.</i> (1974)
<i>Larus fuscus</i>	6	16.0 $\pm$ 1.6	84.9 $\pm$ 4.3	25	Ar <i>et al.</i> (1974)
<i>Larus argentatus</i>	3	16.5 $\pm$ 1.9	88.2 $\pm$ 4.0	29	Ar <i>et al.</i> (1974)
<i>Catharacta skua</i>	6	18.4 $\pm$ 0.3	95.5 $\pm$ 5.4	28	Ar <i>et al.</i> (1974)
<i>Fratercula arctica</i>	6	7.9 $\pm$ 1.2	59.6 $\pm$ 3.4	38	Ar <i>et al.</i> (1974)
<span style="border: 1px solid black; padding: 0 2px;">21</span> <i>Larus glaucescens</i>	21	22.6 $\pm$ 1.0	98.2 $\pm$ 1.9	28	Morgan <i>et al.</i> (1978)
<i>Rissa tridactyla</i>	11	9.7 $\pm$ 0.8	51.5 $\pm$ 1.0	27	Morgan <i>et al.</i> (1978)
<i>Anser erythropus</i>	7	20.6	122.9	25	Hoyt <i>et al.</i> (1979)
<i>Anser cygnoides</i>	7	26.7	146.4	28	Hoyt <i>et al.</i> (1979)
<i>Anser fabalis</i>	9	24.9	152.3	27	Hoyt <i>et al.</i> (1979)
<i>Anser brachyrhynchus</i>	3	23.4	139.4	27	Hoyt <i>et al.</i> (1979)
<i>Anser anser</i>	3	33.2	162.5	27	Hoyt <i>et al.</i> (1979)
<i>Anser indicus</i>	2	8.4	110.1	28	Hoyt <i>et al.</i> (1979)
<i>Branta sandvicensis</i>	3	33.4	154.4	30	Hoyt <i>et al.</i> (1979)
<i>Branta leucopsis</i>	7	19.6	106.6	24	Hoyt <i>et al.</i> (1979)
<i>Branta ruficollis</i>	5	12.9	67.8	25	Hoyt <i>et al.</i> (1979)
<i>Cyanochen cyanoptera</i>	2	14.7	82.8	30	Hoyt <i>et al.</i> (1979)
<i>Chen rossii</i>	3	18.6	91.8	22	Hoyt <i>et al.</i> (1979)
<i>Chen canagica</i>	7	27.4	136.1	24	Hoyt <i>et al.</i> (1979)
<i>Chloephaga picta</i>	1	23.8	106.1	30	Hoyt <i>et al.</i> (1979)
<i>Chloephaga poliocephala</i>	1	13.9	100	30	Hoyt <i>et al.</i> (1979)
<i>Cairina moschata</i>	4	12.3	80.2	35	Hoyt <i>et al.</i> (1979)
<i>Chloephaga rubidiceps</i>	3	11.7	84.1	30	Hoyt <i>et al.</i> (1979)
<i>Tadorna ferruginea</i>	2	16.6	79.1	29	Hoyt <i>et al.</i> (1979)
<i>Tadorna variegata</i>	6	14.1	89.6	30	Hoyt <i>et al.</i> (1979)
<i>Tadorna tadorna</i>	4	15.3	79.9	28	Hoyt <i>et al.</i> (1979)
<i>Cairina scutulata</i>	1	22.8	99.1	30	Hoyt <i>et al.</i> (1979)
<i>Callonetta leucophrys</i>	10	6.1	31.6	23	Hoyt <i>et al.</i> (1979)
<i>Aix sponsa</i>	5	8.4	43.4	30	Hoyt <i>et al.</i> (1979)
<i>Aix galericulata</i>	2	8	27.4	29	Hoyt <i>et al.</i> (1979)
<i>Dendrocygna bicolor</i>	7	17.1	54.4	25	Hoyt <i>et al.</i> (1979)
<i>Dendrocygna arcuata</i>	4	6.1	36.5	30	Hoyt <i>et al.</i> (1979)
<i>Dendrocygna arborea</i>	9	11.6	59.8	30	Hoyt <i>et al.</i> (1979)
<i>Dendrocygna autumnalis</i>	10	11.6	42.8	27	Hoyt <i>et al.</i> (1979)

**Table 2** Continued.

Species	N	$G_{H_2O}$ (mg day <sup>-1</sup> torr <sup>-1</sup> )	Egg mass (g)	Incubation (days)	Reference
<span style="border: 1px solid black; padding: 0 2px;">21</span> <i>Eudocimus albus</i>	30	7.8 ± 3.4	50.8 ± 5.7	24	Vleck <i>et al.</i> (1983)
<i>Bubulcus ibis</i>	19	5.4 ± 1.5	27.8 ± 2.8	22	Vleck <i>et al.</i> (1983)
<i>Plegadis falcinellus</i>	6	7.6 ± 2.0	37.4 ± 4.4	22	Vleck <i>et al.</i> (1983)
<i>Egretta thula</i>	6	3.8 ± 0.7	22.6 ± 1.5	23	Vleck <i>et al.</i> (1983)
<i>Egretta tricolor</i>	8	3.6 ± 1.9	26.6 ± 1.5	22	Vleck <i>et al.</i> (1983)
<i>Nycticorax nycticorax</i>	1	6.2	38.8	25	Vleck <i>et al.</i> (1983)
<i>Sterna sandvicensis</i>	6	8.3 ± 1.6	36.1 ± 2.6	29	Vleck <i>et al.</i> (1983)
<i>Sterna maxima</i>	17	13.3 ± 3.0	68.0 ± 5.2	25	Vleck <i>et al.</i> (1983)
<i>Philomachus pugnax</i>	1	3.62	19.1	22	Visser <i>et al.</i> (1995)
<i>Tringa tetanus</i>	12	4.3 ± 0.4	22.3 ± 1.5	24	Visser <i>et al.</i> (1995)
<i>Limosa limosa</i>	26	9.5 ± 1.8	39.1 ± 3.3	23	Visser <i>et al.</i> (1995)
<span style="border: 1px solid black; padding: 0 2px;">23</span> <i>Numenius arquata</i>	10	16.2 ± 1.9	82.8 ± 6.6	28	Visser <i>et al.</i> (1995)

Species are organized according to the source reference.

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  $\mu$ m 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), 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).

21 The  $G_{H_2O}$  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. 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  $G_{H_2O}$ , 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  $G_{H_2O}$  was as previously described (Booth & Seymour, 1987; Portugal *et al.*,

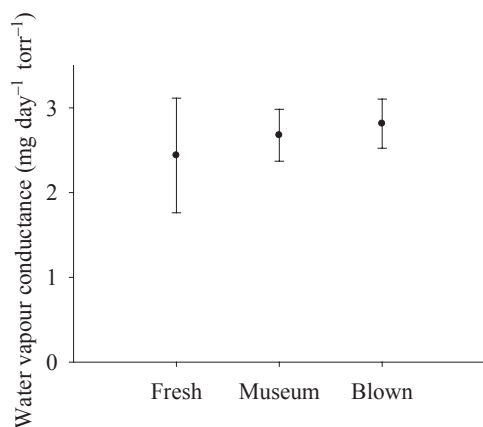
2010b; Maurer *et al.*, 2011a). Briefly, the water vapour conductance of a shell can be calculated as:

$$G_{H_2O} = \frac{M_{H_2O}}{P_{H_2O}} \quad (1)$$

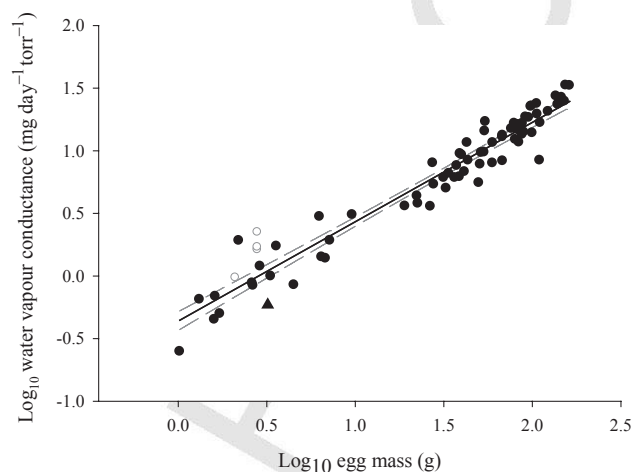
Where  $G_{H_2O}$  = water vapour conductance (mg day<sup>-1</sup> torr<sup>-1</sup>);  $M_{H_2O}$  = the rate of mass loss (mg day<sup>-1</sup>); and  $\Delta P_{H_2O}$  = water vapour pressure difference across the shell (torr).

Previously, we had demonstrated that the  $G_{H_2O}$  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  $G_{H_2O}$  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  $G_{H_2O}$  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<sup>-1</sup> torr<sup>-1</sup> 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  $G_{H_2O}$  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  $G_{H_2O}$  comparison were blown. The eggs were then filled with distilled water (as mentioned earlier), and the impermeable plastic cover was fitted, before  $G_{H_2O}$  was measured. No significant difference was detected in  $G_{H_2O}$  between the fresh quail eggs and the blown eggs fitted with the plastic cover (paired  $t$ -test,  $t = -2.012$ , d.f. = 23,  $P = 0.06$ , 2.81 ± 0.29 mg day<sup>-1</sup> torr<sup>-1</sup>, 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



**Figure 1** Comparison of water vapour conductance (mean  $\pm$  standard error;  $\text{mg day}^{-1} \text{ torr}^{-1}$ ) 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).



**Figure 2** Regression (solid line) with 95% confidence intervals (grey-dashed line) of eggshell water vapour conductance ( $\log_{10} G_{\text{H}_2\text{O}}$ ,  $\text{mg day}^{-1} \text{ torr}^{-1}$ ) on egg mass ( $\log_{10} \text{g}$ ). The regression line ( $G_{\text{H}_2\text{O}} = 0.18 \times \text{egg mass} + 0.60$ ,  $r^2 = 0.91$ ,  $P < 0.001$ ) 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.

constructed and a phylogenetic generalized least square model ( $\log_{10} G_{\text{H}_2\text{O}} \sim \log_{10} \text{EggMass}$ ) 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 ( $\lambda$ ) (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  $\pm$  standard error.

## Results

We confirmed that cuckoo eggshells were thicker ( $0.88 \pm 0.03 \mu\text{m}$ ) than those of the host species (per species value,  $0.72 \pm 0.1 \mu\text{m}$ ,  $t$ -test,  $t = 98.46$ ,  $\text{d.f.} = 4$ ,  $P < 0.001$ ). The average  $G_{\text{H}_2\text{O}}$  for the cuckoo eggs ( $0.59 \pm 0.07 \text{ mg day}^{-1} \text{ torr}^{-1}$ ) was lower than that of all passerines combined ( $1.58 \pm 0.91 \text{ mg day}^{-1} \text{ torr}^{-1}$ ). The closest species in egg mass to the cuckoos ( $3.2 \pm 0.1 \text{ g}$ ) had higher  $G_{\text{H}_2\text{O}}$  in comparison,  $1.2 \pm 0.1$  and  $1.9 \text{ mg day}^{-1} \text{ torr}^{-1}$  for *Emberiza citronella* ( $3.3 \pm 0.4 \text{ g}$ ) and *Carduelis cannabina* ( $2.9 \text{ g}$ ), respectively (Table 1). There was very little variation in  $G_{\text{H}_2\text{O}}$  between the cuckoo eggs (Table 1). Across species, not accounting for phylogeny, there was a strong significant positive correlation between  $G_{\text{H}_2\text{O}}$  and egg mass [log-transformed; Pearson's  $r$  [95% confidence interval = 0.97 (0.95, 0.98),  $n = 77$ ,  $P < 0.001$ ; Fig. 2].

Across the 77 species, for which data were available,  $G_{\text{H}_2\text{O}}$  possessed a strong phylogenetic signal across the 1000 resampled phylogenies [median Pagel's Lambda (95th percen-

tiles) = 0.90 (0.88, 0.93)]. In a 1000 phylogenetic generalized least squares models the positive relationship between egg mass and  $G_{\text{H}_2\text{O}}$  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  $G_{\text{H}_2\text{O}}$  [median residual (corrected for phylogeny) =  $-0.57$ ; average rank = 2.01], meaning cuckoo eggs have lower  $G_{\text{H}_2\text{O}}$  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  $G_{\text{H}_2\text{O}}$  than their hosts, or other non-host passerine species with similar sized eggs. Instead, cuckoo eggs exhibited a significantly lower  $G_{\text{H}_2\text{O}}$ . 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  $G_{\text{H}_2\text{O}}$  compared with that of their hosts and  $G_{\text{H}_2\text{O}}$  predicted for their egg mass (Jaekle *et al.*, 2012).

Cuckoo eggshells are thicker and stronger than their hosts (Igcic *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  $G_{H_2O}$ . 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; Jaecle *et al.*, 2012). As such, a thicker eggshell does not necessarily equate with low  $G_{H_2O}$ . 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  $G_{H_2O}$  for faster development (e.g. Carey, 1979; Hanka *et al.*, 1979; Maurer, Portugal & Cassey, 2011b). This increase in  $G_{H_2O}$  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 would also provide the structure and strength required by the cuckoo chick to hatch earlier (Honza *et al.*, 2001; Igc *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). This trait 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  $G_{H_2O}$  between different female cuckoos and their preferred hosts. However, values of cuckoo  $G_{H_2O}$  are (1) lower compared with both host and non-host passerines (Fig. 2); (2) show remarkably small variation (Table 1), suggesting that comparison with appropriate 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  $G_{H_2O}$  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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13	AUTHOR: Cooper, C.E. & Withers P.C. (2006) has not been cited in the text. Please indicate where it should be cited; or delete from the Reference List.	
14	AUTHOR: J. Roy. Soc. Interface has been changed to J. R. Soc. Interface. Please confirm if this OK.	
15	AUTHOR: Kembel et al., (2010) has not been cited in the text. Please indicate where it should be cited; or delete from the Reference List.	
16	AUTHOR: Nybø, S., Staurnes, M. & Jerstad, K. (1997) has not been cited in the text. Please indicate where it should be cited; or delete from the Reference List.	

Query References	Query	Remarks
17	AUTHOR: Oviedo-Rondón et al., (2008) has not been cited in the text. Please indicate where it should be cited; or delete from the Reference List.	
18	AUTHOR: Paradis, E., Claude, E.J. & Strimmer, K. (2004) has not been cited in the text. Please indicate where it should be cited; or delete from the Reference List.	
19	AUTHOR: Please provide volume number and page range for Protugal et al. 2014.	
20	AUTHOR: Revell, L.J. (2010) has not been cited in the text. Please indicate where it should be cited; or delete from the Reference List.	
21	AUTHOR: Morgan <i>et al.</i> (1978) has not been included in the Reference List, please supply full publication details.	
22	AUTHOR: Vleck <i>et al.</i> (1983) has not been included in the Reference List, please supply full publication details.	
23	AUTHOR: Visser <i>et al.</i> (1995) has not been included in the Reference List, please supply full publication details.	
24	*AUTHOR: The page range '208–122' seems to be incorrect; please check.	

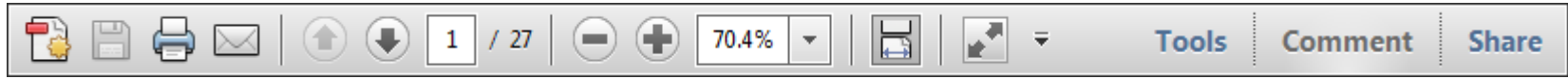
Note: The query which is preceded by \* is added by Toppan Best-set.

USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

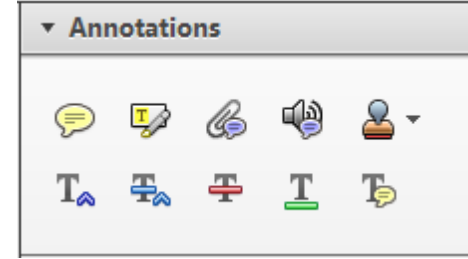
Required software to e-Annotate PDFs: Adobe Acrobat Professional or Adobe Reader (version 8.0 or above). (Note that this document uses screenshots from Adobe Reader X)

The latest version of Acrobat Reader can be downloaded for free at: <http://get.adobe.com/reader/>

Once you have Acrobat Reader open on your computer, click on the [Comment](#) tab at the right of the toolbar:



This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the [Annotations](#) section, pictured opposite. We've picked out some of these tools below:



**1. Replace (Ins) Tool – for replacing text.**

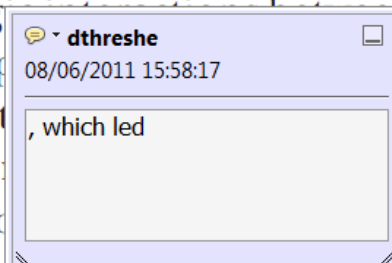


Strikes a line through text and opens up a text box where replacement text can be entered.

**How to use it**

- Highlight a word or sentence.
- Click on the [Replace \(Ins\)](#) icon in the Annotations section.
- Type the replacement text into the blue box that appears.

standard framework for the analysis of microeconomics. Nevertheless, it also led to the emergence of a new paradigm of strategic interaction. The number of competitors in the industry is that the structure of the main components of the model, are exogenous. Important works on this by Shirasaka (henceforth) we open the 'black b



**2. Strikethrough (Del) Tool – for deleting text.**



Strikes a red line through text that is to be deleted.

**How to use it**

- Highlight a word or sentence.
- Click on the [Strikethrough \(Del\)](#) icon in the Annotations section.

there is no room for extra profits and the number of firms are zero and the number of firms (net) values are not determined by Blanchard and ~~Kiyotaki~~ (1987), perfect competition in general equilibrium of aggregate demand and supply in the classical framework assuming monopoly. An exogenous number of firms

**3. Add note to text Tool – for highlighting a section to be changed to bold or italic.**



Highlights text in yellow and opens up a text box where comments can be entered.

**How to use it**

- Highlight the relevant section of text.
- Click on the [Add note to text](#) icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.

dynamic responses of mark-ups consistent with the VAR evidence

sation... y Ma... and... on n... to a... on... stent also with the demand-



**4. Add sticky note Tool – for making notes at specific points in the text.**

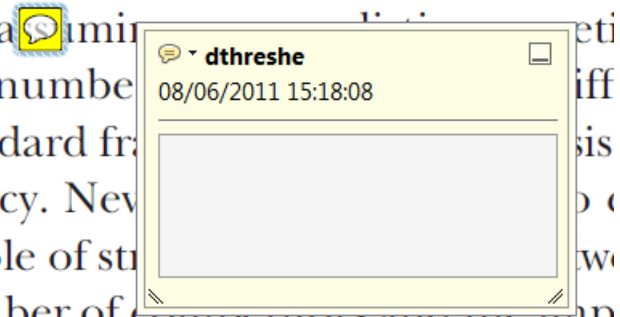


Marks a point in the proof where a comment needs to be highlighted.

**How to use it**

- Click on the [Add sticky note](#) icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.

and supply shocks. Most of the... a... number... dard fr... cy. Nev... ole of st... ber of competitors and the imp... is that the structure of the secto



USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

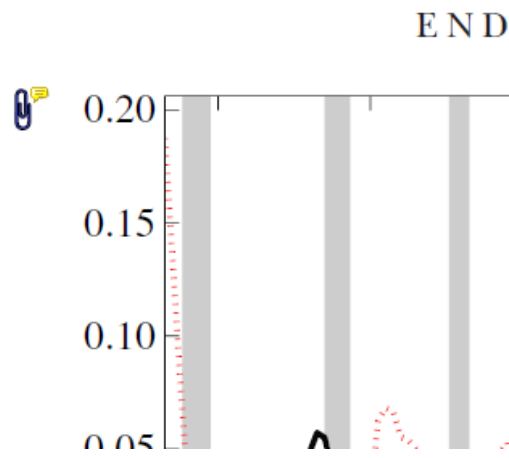
**5. Attach File Tool – for inserting large amounts of text or replacement figures.**



Inserts an icon linking to the attached file in the appropriate place in the text.

**How to use it**

- Click on the [Attach File](#) icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.



**6. Add stamp Tool – for approving a proof if no corrections are required.**

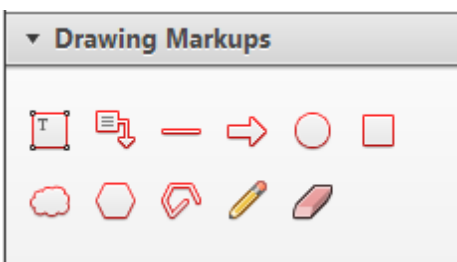


Inserts a selected stamp onto an appropriate place in the proof.

**How to use it**

- Click on the [Add stamp](#) icon in the Annotations section.
- Select the stamp you want to use. (The [Approved](#) stamp is usually available directly in the menu that appears).
- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).

of the business cycle, starting with the  
 on perfect competition, constant ret  
 production. In this environment goods  
 extra profits and the market for marke  
 he market for goods is determined by the model. The New-Key  
 otaki (1987), has introduced produc  
 general equilibrium models with nomin  
 and market-clearing. Most of this literat

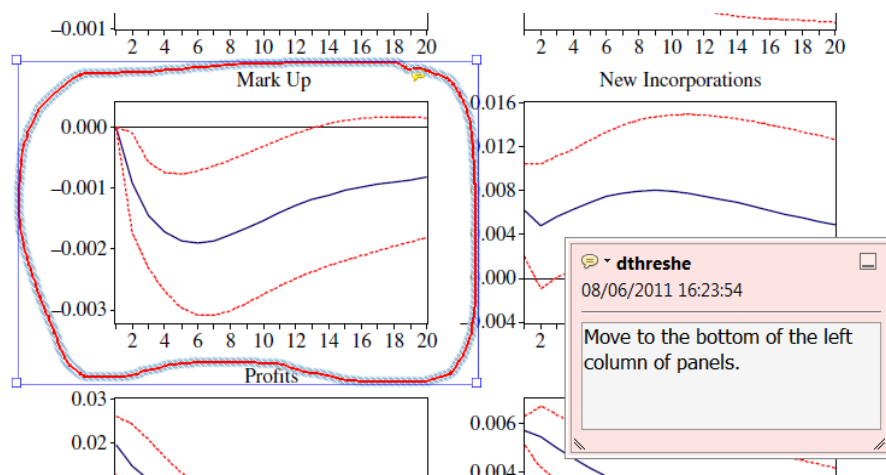


**7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.**

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..

**How to use it**

- Click on one of the shapes in the [Drawing Markups](#) section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



For further information on how to annotate proofs, click on the [Help](#) menu to reveal a list of further options:

