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RESEARCH ARTICLE

A comparison of egg yolk lipid constituents between parasitic Common Cuckoos and their hosts

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ABSTRACT

Common Cuckoos (*Cuculus canorus*) are obligate brood parasites that lay their eggs in nests of other species and use these hosts to raise their parasitic offspring. Two key adaptations that increase their reproductive success are (1) the capacity for cuckoos to lay large numbers of eggs and thereby parasitize many nests per year, and (2) the ability of cuckoo eggs to hatch before those of hosts, enabling cuckoo nestlings to evict host eggs and eliminate competition for food. Producing more eggs is generally associated with reduced investment of nutrients and energy reserves per egg, which in turn is associated with shorter incubation periods both within and between species. We hypothesized that Common Cuckoos deposit reduced energy reserves into their eggs than do their hosts to facilitate both (1) and (2). To test these hypotheses, we compared the concentration of yolk lipids (per wet yolk mass) between eggs of 3 cuckoo gentes and their respective host species: Great Reed Warblers (*Acrocephalus arundinaceus*), Common Redstarts (*Phoenicurus phoenicurus*), and Reed Warblers (*A. scirpaceus*). Yolk lipids provide the bulk of energy required for embryonic development and can also serve structural and cell-signalling functions. As a general pattern, cuckoo eggs contained a lower concentration of energy-reserve lipids than eggs of their respective hosts, but not structural or cell-signalling lipids. When controlling for their heavier eggs and yolks, Common Cuckoo eggs had an estimated lower amount of energy reserve lipids for their size than host eggs. Our findings suggest a potential role of yolk lipid composition in facilitating (1) and (2) and advocate the need for further research in this area. We also highlight the potential problems of using either concentration or total yolk mass alone to compare maternal investment across taxa in comparative studies.

Keywords: brood parasitism, coevolution, *Cuculus canorus*, energy reserves, lipids, yolk

Comparación de los constituyentes lípidos de la yema del huevo entre el parásito *Cuculus canorus* y sus hospederos

RESUMEN

Cuculus canorus es un cuco parásito de cría obligado que pone sus huevos en los nidos de otras especies y usa a estos hospederos para criar su descendencia parásita. Dos adaptaciones clave que incrementan su éxito reproductivo son: (1) la capacidad de los cucos de poner grandes números de huevos y así parasitar muchos nidos al año; y (2) la habilidad de los huevos de cuco para eclosionar antes que los de sus hospederos, lo que permite que los polluelos de cuco desalojen los huevos del hospedero y eliminen la competencia por alimento. La producción de más huevos generalmente se asocia con periodos de incubación más cortos dentro y entre especies. Formulamos la hipótesis de que los cucos depositan menos reservas energéticas en sus huevos de lo que depositan sus hospederos para facilitar las adaptaciones (1) y (2). Para poner a prueba esta hipótesis, comparamos la concentración de los lípidos de la yema (por masa de yema líquida) entre huevos de tres grupos especializados de cucos y sus respectivas especies hospederas: *Acrocephalus arundinaceus, Phoenicurus phoenicurus* y *A. scirpaceus*. Los lípidos de la yema proveen la mayoría de la energía requerida para el desarrollo embriónico y también puede tener funciones estructurales y de señalización celular. Como patrón general, los huevos de cuco contienen menor concentración de lípidos para reserva

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de energía que los huevos de sus respectivos hospederos, pero no de lípidos estructurales o de señalización celular. Al controlar por sus huevos y yemas más pesados, los cucos tuvieron un estimado de menor concentración de lípidos energéticos para su tamaño que el de los huevos de los hospederos. Nuestros resultados sugieren un rol potencial de la composición de lípidos en la yema para facilitar las adaptaciones (1) y (2) y resaltan la necesidad de más estudios en esta área. También destacamos los problemas potenciales de usar la concentración o la masa total de yema por sí solos para comparar la inversión materna entre taxones en estudios comparativos.

Palabras clave: coevolución, *Cuculus canorus,* lípidos, parasitismo de cría, reservas energéticas, yema

INTRODUCTION

Obligate brood parasites lay their eggs into nests of other species, usurping parental investment from these hosts to raise parasitic offspring (Payne 1977, Davies 2000). The Common Cuckoo (*Cuculus canorus*) has evolved numerous adaptations to increase the likelihood that its offspring are accepted and successfully raised by other species. Some adaptations include the mimicry of host egg phenotypes, the evolution of host-specific races or gentes (singular: gens; Avilés 2008, Stoddard and Stevens 2010, 2011, Igic et al. 2012), and the eviction of host eggs and nestlings by hatchling Common Cuckoos (Honza et al. 2007, Anderson et al. 2009, Grim et al. 2009).

The eviction of host offspring eliminates the Common Cuckoo nestling's competition for food (Hauber and Moskát 2008, Geltsch et al. 2012) and is facilitated by the ability of Common Cuckoo eggs to hatch before those of their hosts (typically 2 days earlier; Wyllie 1981). Several hypotheses have been proposed to explain the relatively short incubation period of Common Cuckoo eggs: (1) they incubate their eggs internally prior to laying (Payne 1977, Davies 2000, Birkhead et al. 2011); (2) their eggshells are more gas permeable, allowing greater embryonic metabolic rates (Ar and Rahn 1985, Hargitai et al. 2010); and (3) their eggs contain more nutrients or growth factors that hasten their development (e.g., testosterone; Török et al. 2004, Hargitai et al. 2010). To date, only internal incubation of eggs (1) is supported by evidence; Common Cuckoo eggs are laid at 48 hr intervals and contain more developed embryos at laying than the freshly laid eggs of unrelated and nonparasitic birds that lay at 24 hr intervals (Birkhead et al. 2011). In contrast to hypotheses (2) and (3), Common Cuckoo eggs have lower gas permeability (Portugal et al. 2014) and less maternally derived testosterone than eggs of their hosts (Hargitai et al. 2010). Although Common Cuckoo egg yolks contain higher concentrations of carotenoids and vitamin E and are heavier than yolks of a host species with similar-sized eggs (Török et al. 2004, Hargitai et al. 2010), these vitamins are usually involved in reducing oxidative stress rather than increasing developmental rate (Surai et al. 1999, 2001, Hargitai et al. 2010, Royle et al. 2011), and heavier yolks are typically associated with longer, not shorter, incubation periods within and between species (Sinervo 1990, Kattan 1995).

A hypothesis yet to be considered is whether Common Cuckoo eggs hatch earlier because they contain lower energy reserves than host eggs (Sinervo 1990, Kattan 1995). Lower energy reserves may deplete more quickly, forcing Common Cuckoo embryos to hatch earlier (Vleck and Vleck 1986, Sinervo 1990). This hypothesis is contrary to the argument that embryonic development is increased through provisioning of more yolk (Török et al. 2004) but is supported by positive interspecific and intraspecific relationships between the length of incubation and the energy/yolk content of eggs as well as experimental study (Vleck and Vleck 1986, Sinervo 1990). The role of egg yolk composition in generating the short incubation periods of brood parasitic Shiny Cowbird (*Molothrus bonariensis*) eggs is unclear. The incubation period of cowbird eggs is shorter than can be expected from their mass, but similar to that expected from their overall caloric value and size compared to other (nonparasitic) icterid eggs (Kattan 1995, Mermoz and Ornelas 2004). Although the heavier yolk of Common Cuckoo eggs may suggest they contain a greater quantity of energy reserves than host eggs (Török et al. 2004, Hargitai et al. 2010), water is a major component of egg yolk (Carey et al. 1980), and thus a greater water quantity may be responsible for the Common Cuckoo's heavier egg yolk. No study has yet specifically quantified and compared the concentration, or amount, of energy reserves between eggs of parasitic cuckoos and their hosts.

In addition to shortening the incubation period, depositing less energy reserves into eggs may enable Common Cuckoos to lay more eggs per year. For most living organisms, there is a trade-off between the amount of resources invested into producing more offspring and the amount of resources invested per offspring (Roff 1992, Williams 1994). For Common Cuckoos, this is a balance between the number of eggs laid per breeding season and producing offspring that are strong enough to hatch from a thick-shelled egg and evict host young (Honza et al. 2001, 2007, Anderson et al. 2009, Igic et al. 2011). Common Cuckoos are freed from the constraints of having to physically raise their own offspring, and thus their main reproductive investments are the nutrients deposited in their eggs and the number of eggs laid per year (Payne 1974). Common Cuckoos may lay more eggs per season than their hosts (up to 25; Wyllie 1981; although see

Schulze-Hagen et al. 2009), and this could be facilitated by a lower investment of resources per egg. Common Cuckoos lay eggs that are smaller than would be predicted by female body size of nonparasitic birds, which potentially increases their egg-laying capacity (Rahn et al. 1975, Krüger 2007). Whether Common Cuckoos increase their egg-laying capacities further through other mechanisms is unclear.

Lipids provide the bulk of the energy required for embryonic development and are found almost exclusively inside avian egg yolk, where they make up 58% of yolk solids (Sotherland and Rahn 1987). Egg yolk lipids primarily consist of 3 classes: triacylglycerols, phospholipids, and free sterols (Noble 1986). Triacylglycerols are the most common class of lipids present in yolk and provide 90% of the energy required for embryonic development (Christie and Moore 1972). Phospholipids are the second most common and form the main constituent of cell membranes (Romanoff 1967, Noble 1986). Free sterols have a number of different functions, including maintaining cell rigidity (e.g., cholesterol) and mediating cell–cell signalling (e.g., steroids; Romanoff 1967, Noble 1986).

Here, we compared the concentration of energy reserves, in the form of yolk lipids, between eggs of Common Cuckoos and their hosts. We measured the concentrations of the 3 major yolk lipid classes in eggs of 3 Common Cuckoo gentes and their respective host species: Great Reed Warblers (*Acrocephalus arundinaceus*), Common Redstarts (*Phoenicurus phoenicurus*), and Reed Warblers (*A. scirpaceus*). If Common Cuckoo eggs contain lower energy reserves than eggs of their hosts, they should contain lower amounts of triacylglycerols. We compared the concentration of yolk triacylglycerols, both overall and as a proportion relative to concentrations of structural and cell-signalling lipids, and estimated the total quantity of triacylglycerols per egg and in relation to egg mass using published measurements of species-specific yolk and egg mass. Because the energetic costs of embryogenesis are similar for all bird species when controlling for egg mass (Vleck and Vleck 1986), we predicted that Common Cuckoos shorten incubation by depositing less energy reserves into their eggs (in relation to egg mass) than their hosts.

METHODS

Sample Collection

We collected Common Cuckoo and host eggs during egg laying and prior to the onset of incubation at 3 locations across Europe during the northern summers of 2006–2007 (details in Igic et al. 2011, 2012) from nests of 3 different host species (Table 1): Great Reed Warblers in Hungary, Common Redstarts in Finland, and Reed Warblers in the Czech Republic. These passerines are the primary (Great Reed Warblers and Common Redstarts) or secondary

TABLE 1. Egg yolk lipid composition (measured on wet yolk mass), and egg and female mass data for Common Cuckoos and their host species.

Species	Country of origin	Type	*n* [a]	Triacylglycerols per yolk mass (ng g^{-1})	Phospholipids per yolk mass (ng g^{-1})	Free sterols per yolk mass (ng g^{-1})	Total triacylglycerols per egg (ng) [b]	Triacylglycerols per egg mass (ng g^{-1}) [c]	Mean incubation period (d)	Wet yolk mass (g)	Egg mass (g)	Female body mass (g)
Common Cuckoo	Hungary	Parasite	9	86.85	80.62	13.26	68.61	20.18	12 [1]	0.79 [2][3]	3.40 [4]	120 [4]
	Finland	Parasite	7	77.65	74.83	13.67	61.34	18.04	12 [1]	0.79 [2][3]	3.40 [4]	120 [4]
	Czech Republic	Parasite	2	113.66	89.60	12.76	89.79	26.41	12 [1]	0.79 [2][3]	3.40 [4]	120 [4]
Great Reed Warbler	Hungary	Primary host	9	107.83	82.37	10.64	70.09	22.61	14 [5]	0.65 [2][3]	3.10 [6]	29 [7]
Common Redstart	Finland	Primary host	6	141.80	89.33	13.05	68.05	30.39	13 [8]	0.48 [9]	2.24 [9]	15 [5]
Reed Warbler	Czech Republic	Secondary host	3	89.25	71.28	8.33	32.13	18.47	11 [7]	0.36 [10]	1.74 [11]	12 [6]

[a] Number of eggs included in lipid analysis.

[b] Estimated using published species averages of wet yolk mass.

[c] Estimated using published species averages for wet yolk and egg mass.

Sources of data: [1] Snow and Perrins (1998); [2] Hargitai et al. (2010); [3] Török et al. (2004); [4] Wyllie (1981); [5] Gil et al. (2007); [6] Makatsch (1976); [7] Cramp (1992); [8] Garamszegi et al. (2007); [9] Carey et al. (1980); [10] Krištofík et al. (2014); and [11] Lindholm and Thomas (2000)

(Reed Warblers) hosts of Common Cuckoos at these study sites (Igic et al. 2012). Whenever possible, Common Cuckoo and host eggs were collected from the same nest. We could not discern whether all Common Cuckoo eggs were laid by different females because egg phenotype is an unreliable cue to assign particular eggs to particular females (Moksnes et al. 2008), and we could not conduct parentage analysis.

We selected a single host egg per nest, chosen at random, for our analyses. Although eggs within the same clutch can vary in size and composition with respect to laying order, intra-clutch variance is generally lower than inter-clutch variance (Ricklefs 1984, Hübner et al. 2002). Because it is impossible to know the laying order of Common Cuckoo eggs, and because Common Cuckoo females remove 1–3 host eggs during parasitism (Moksnes et al. 2000), we maintained consistency regarding collection of samples by selecting host eggs at random from those available in the nest. Yolk was extracted from intact eggs on the day of collection and immediately stored in air tight Eppendorf tubes and at −20°C for later analyses. The same protocol was used for all eggs. For this study we included 9 Great Reed Warbler and 9 Common Cuckoo eggs from Hungary; 6 Common Redstart and 7 Common Cuckoo eggs from Finland; and 3 Reed Warbler and 2 Common Cuckoo eggs from the Czech Republic. These eggs were collected as collateral data for other studies (Igic et al. 2011, 2012).

We did not measure volume or mass of each egg, and instead used published data on species-specific total wet yolk and whole-egg masses to estimate the average triacylglycerol quantity per egg and per gram of egg mass (Table 1). Egg mass is a good approximation of hatchling mass, and larger eggs require more energy for embryogenesis (Vleck and Vleck 1986, Magrath 1992). It is unclear whether Common Cuckoo gentes differ in egg size because different studies draw different conclusions (Antonov et al. 2010, Spottiswoode 2010). Although intraspecific differences between gentes are possible, egg size is consistently more similar among gentes than between gentes and their hosts (Antonov et al. 2010, Spottiswoode 2010); therefore, using species-specific means is appropriate for estimating interspecific patterns. Because many biotic and abiotic factors can influence egg phenotype within a species (Ricklefs 1984, Hübner et al. 2002), intraspecific comparisons across geographic locations are outside the scope of our study. Our hypotheses thus regard paired differences between Common Cuckoo gentes and their local hosts, not geographical variation.

Lipid Extraction and Quantification

We measured lipid concentrations relative to wet yolk mass following the invertebrate yolk lipid extraction protocol of Sewell (2005) and Cassey et al. (2012a). In short, we extracted 3 samples of ~10 mg from each yolk sample. Lipid extracts were dried in a stream of instrument grade N_2 gas and then resuspended in 500 μL of chloroform. Lipid classes were quantified using an Iatroscan Mark V^{new} TLC/FID system and silica gel S-III Chromarods. The Chromarods were developed for 28 min in 60 mL of hexane, 6 mL of diethyl-ether, and 0.1 mL of formic acid, dried for 5 min in a Rod Dryer TK-8 (Iatron Laboratories) at 60°C, then run in the Iatroscan with a 30 s scan and settings of 2000 mL min^{-1} O_2 and 160 mL min^{-1} H_2. We quantified the concentrations of triacylglycerols, free sterols, and phospholipids based on multilevel calibration curves generated with a 5-component composite (nonadecane, tripalmitin, cholesterol, L-α-phosphoditylcholine, 3- hexadecanone) standard made from highly purified lipid standards (99%) in HPLC-grade chloroform. Measurements of triacylglycerol, free sterol, and phospholipid concentration were highly repeatable among the 3 extracts per egg ($R = 0.94$, 0.76, and 0.83, respectively), and thus we calculated an average per egg for subsequent statistical analyses.

Statistical Analyses

We tested whether Common Cuckoos deposited a lower concentration of lipids in their eggs than their respective hosts. For eggs of each Common Cuckoo gens and its respective host species, we compared (1) the concentration (ng g^{-1}) of triacylglycerols, phospholipids, free sterols, and total lipids (the sum of the 3 lipid classes) and (2) the proportion of total lipids that are triacylglycerols. We compared concentrations using *t*-tests and $\log_{10}$ transformations to improve homoscedasticity. We compared the proportion of total lipids that are triacylglycerols using quasibinomial general linear models with species identity as a predictor and a logit link function to control for over-dispersion (Hinde and Demétrio 1998). We constructed a separate model for each Common Cuckoo/host system; thus, any outcome for one system did not influence the analysis or interpretations of the other. Sample sizes for Common Cuckoo and Reed Warbler eggs from the Czech Republic were too small for robust statistical comparisons; therefore, we simply report patterns. Data from small sample sizes are worth reporting because of their usefulness for future meta-analyses (Nakagawa and Hauber 2011). We estimated the amount of triacylglycerols present per egg (ng) and per egg mass (ng g^{-1}) using our concentration data and published species-specific average yolk and egg masses (Table 1).

All statistical analyses were conducted in R (R Development Core Team 2013). The repeatability of lipid measurements was calculated using the rpt.remlLMM() function of the rptR package (Nakagawa and Schielzeth

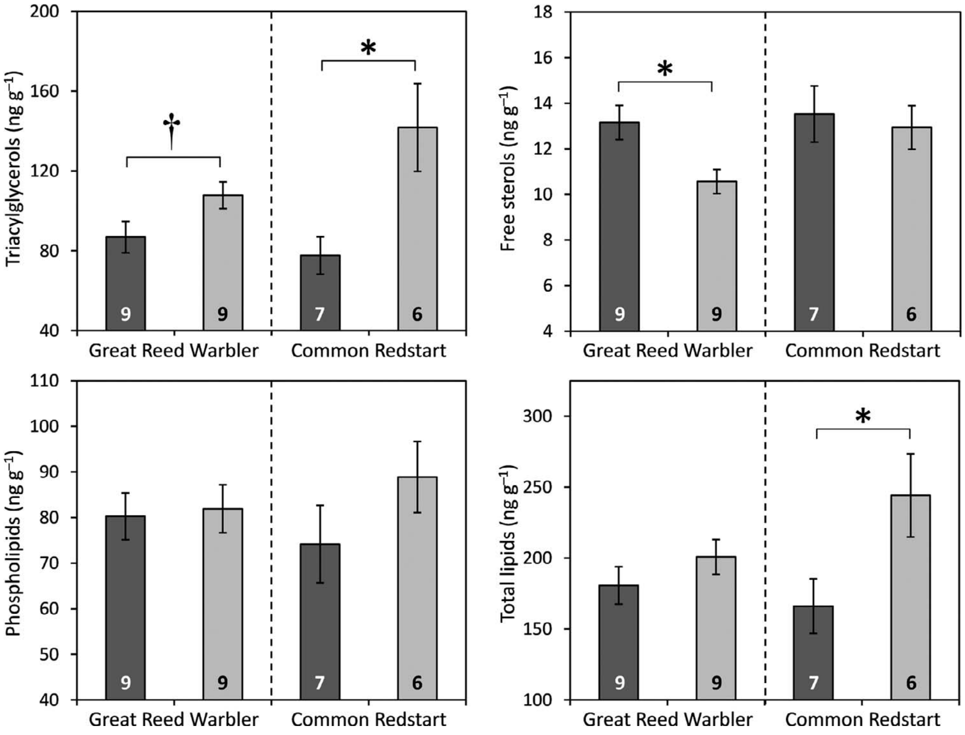

FIGURE 1. Concentration (mean ± SE) of triacylglycerols, free sterols, phospholipids, and total lipids in the egg yolk of Common Cuckoo (dark bars) and host (light bars) eggs. Sample sizes are shown within bars. * $P < 0.05$; † $0.06 > P > 0.05$.

2010). Presented *P*-values were not adjusted for multiple comparisons to avoid reducing statistical power and increasing type II error risk (Nakagawa 2004).

RESULTS

Common Cuckoo egg yolks generally had a lower concentration of triacylglycerols, but not phospholipids and free sterols, than egg yolks of their respective host species (Figure 1, Table 2). The Common Redstart Cuckoo gens had a lower concentration of yolk triacylglycerols than its host, whereas the Great Reed Warbler Cuckoo gens showed a tendency for a lower concentration of yolk triacylglycerols than its host (Figure 1, Table 2). By contrast, the concentrations of yolk free sterols and phospholipids were similar for the Redstart and Great Reed Warbler Cuckoo gentes and their respective hosts, with the exception that the Great Reed Warbler gens had a higher concentration of yolk free sterols than its host (Figure 1, Table 2). When comparing the concentration of triacylglycerols as a proportion of total lipids, both Redstart and Great Reed Warbler gentes' eggs contained a lower proportion of yolk triacylglycerols than eggs of their respective hosts (Figure 2, Table 2). From our few samples, the concentrations of the different egg yolk lipids showed the opposite pattern for eggs of the Reed Warbler Cuckoo gens and its host (Table 1).

When taking into account average species-specific yolk and egg masses, Common Cuckoo eggs were estimated to contain less yolk triacylglycerols per egg. The estimated quantity of yolk triacylglycerols per gram of egg mass is smaller for the Redstart and Great Reed Warbler Cuckoo gentes than their respective hosts, but larger for the Reed Warbler Cuckoo gens (Table 1).

DISCUSSION

As a general pattern, Common Cuckoo eggs contained a lower concentration of energy reserve lipids (per wet yolk mass), but not structural or cell-signalling lipids, than eggs of their respective hosts. Similarly, in proportion to structural and cell-signalling lipids, Common Cuckoos invested reduced amounts of energy reserve lipids per egg than hosts. When controlling for the heavier eggs and yolk of Common Cuckoos, our estimates suggest that Common Cuckoos invest lower energy reserves per gram of egg tissue than hosts, which may enable Common Cuckoos to produce more eggs with shorter incubation periods. Our findings highlight that using total egg yolk mass alone to compare maternal investment between eggs of brood parasites and their hosts is inappropriate (Török et al. 2004, Hargitai et al. 2010).

Although the eggs and egg yolks of Common Cuckoos are heavier than those of hosts, our estimates suggest that

TABLE 2. Tests comparing yolk lipid composition of Common Cuckoo eggs and those of their Great Warbler and Common Redstart hosts.

	Triacylglycerol concentration		**Phospholipid concentration**		**Free sterol concentration**		**Total lipid concentration**		**Proportion Triacylglycerol** [a]	
	t (df)	*P*	*t* (df)	*P*	*t* (df)	*P*	*t* (df)	*P*	*F* (df)	*P*
Great Reed Warbler	2.05 (16)	0.06	0.20 (16)	0.85	2.65 (16)	**0.02**	−1.11 (16)	0.28	26.1 (1, 16)	**<0.001**
Common Redstart	3.02 (11)	**0.01**	1.31 (11)	0.22	0.27 (11)	0.79	−2.39 (11)	**0.04**	12.0 (1, 11)	**<0.01**

[a] Calculated using a quasi-binomial general linear model with a logit link function. *P* values < 0.05 are highlighted in bold.

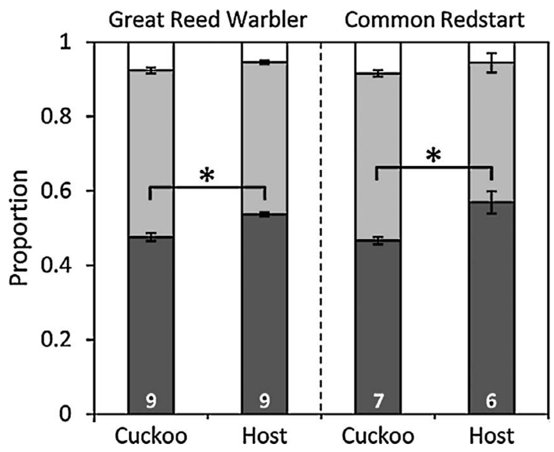

FIGURE 2. Proportion (mean ± SE) of yolk lipids that are triacylglycerols (bottom bars), phospholipids (middle bars), and free sterols (top bars) in Common Cuckoo and host eggs. Sample sizes are shown within bars. * $P < 0.05$.

Common Cuckoo eggs contain lower energy reserves in relation to egg mass. This is contrary to the previous conclusions based on egg yolk mass differences between Common Cuckoos and Great Reed Warblers (Török et al. 2004, Hargitai et al. 2010) and contrary to interspecific patterns that larger eggs contain a greater energetic content (Vleck and Vleck 1986). This finding is especially interesting given the much larger egg size of Common Cuckoos compared to Common Redstarts (Table 1). By contrast, we estimated that the Reed Warbler Cuckoo gens' eggs contain twice the amount of energy reserve lipids as its host, although this is based on small samples of eggs. The lower concentration of energy reserves in Common Cuckoo eggs may reflect their more advanced stage of development at laying compared to hosts (Birkhead et al. 2011); however, lipid absorption is lowest during the initial stages of embryonic development and thus unlikely to be a major contributor to differences found here (Noble 1986).

Our findings suggest that Common Cuckoos may increase their egg laying capacity by investing lower energy reserves into their eggs in relation to egg mass (Payne 1977, Wyllie 1981). Laying eggs that are small in relation to female body mass is likely the primary mechanism enabling Common Cuckoos to lay a large number of eggs per year (Rahn et al. 1975, Krüger 2007). Common Cuckoos may also be able to lay more eggs per year because they spread egg laying over a longer period of time than individual hosts and are freed from parental care (Payne 1974).

In addition to boosting laying capacity, reducing the quantity of energy reserves may facilitate earlier hatching of Common Cuckoo embryos (e.g., Kattan 1995). Common Cuckoo eggs contained lower energy reserves than eggs of their respective hosts, both in relation to egg size and in proportion to structural and cell-signalling lipids. Energy content per egg mass is a reliable predictor of incubation periods across species, in that the energy content of eggs and energy requirements for embryogenesis increase with egg mass (Vleck and Vleck 1986). Therefore, lower energy reserves in relation to egg mass may force Common Cuckoo embryos to hatch earlier. The patterns reported here are correlational, however, and multiple factors can influence the length of incubation in addition to caloric content of eggs, including ambient temperature of the nest, incubation attendance by parents, and egg size (Boersma 1982, Martin 2002, Martin et al. 2007). Comparative and experimental approaches (e.g., Sinervo 1990, Cassey et al. 2012b) are better able to differentiate the influences of these various factors and test whether Common Cuckoos reduce energy content of eggs to shorten incubation. Intriguingly, in addition to having the shortest average incubation period of our 4 study species, our sample of Reed Warbler eggs contained the lowest amount of yolk triacylglycerols (Table 1).

Although depositing lower energy reserves into eggs may increase a female's egg laying capacity and reduce the incubation time of her eggs, these benefits may come at a cost. Reducing the energy reserves of eggs can produce smaller nestlings (Magrath 1992, Roff 1992), which could be disadvantageous if Common Cuckoo hatchlings are too small or not strong enough to evict host offspring from the nest (Grim et al. 2014). The eviction of host eggs and nestlings is likely an energetically demanding task, as evidenced by impaired growth during eviction and increased levels of oxidative stress when evicting more host eggs from deeper host nests (Anderson et al. 2009, Grim et al. 2009, Hargitai et al. 2012). Therefore, Common Cuckoos may trade the benefits of producing larger or fitter hatchlings for the ability to parasitize more nests and produce eggs that hatch sooner. Although selection pressures by hosts may differ across Common Cuckoo gentes (e.g., Spottiswoode 2010), comparing lipid composition across gentes was beyond the scope of our study and is a fruitful area for future research. Reducing investment in eggs could be more beneficial when the probability of rejection by hosts is greater. Interestingly, despite developing from relatively similar-sized eggs with fewer energy reserves, Common Cuckoo hatchlings are larger and heavier than Great Reed Warbler hatchlings (Honza et al. 2001), suggesting that other factors potentially counter the consequences of reducing energy reserves on hatchling size. For example, Common Cuckoo embryos may absorb more calcium from their thicker eggshells during development relative to host embryos (Mänd et al. 2000, Honza et al. 2001, Igic et al. 2011).

Our estimates are based on published data on species-specific yolk and egg masses, which prohibit statistical comparison and highlight the need for further study. Because Common Cuckoo eggs are heavier but contain lower concentrations of yolk triacylglycerols than those of the hosts studied here, any adjustments using yolk or egg mass decreases the potential for detecting differences between Common Cuckoo and host eggs, making our estimates conservative. Our conclusions based on estimation are also supported by the lower concentration of energy lipids relative to structural lipids for Common Cuckoo eggs, which is unaffected by any intraspecific and interspecific differences in egg and yolk mass and confirms that Common Cuckoo and host eggs differ in respect to yolk lipid composition.

In addition to internal incubation of eggs (Birkhead et al. 2011) and reducing energy deposits (this study), Common Cuckoos may facilitate earlier hatching of their eggs through other mechanisms. Many mechanisms are yet to be tested, such as higher embryonic metabolic rates (Noble 1986, Kattan 1995) or the retarding effects of the parasitic egg shape or size on incubation efficiency of host eggs (McMaster and Sealy 1998, Bán et al. 2011), although increasing metabolic rate through increased gas exchange is unlikely (Portugal et al. 2014). Increased embryonic growth and metabolism can trade-off with cellular repair and immune competency (Martin and Schwabl 2008) and may explain why Common Cuckoo eggs contain more antioxidants than eggs of their hosts (Hargitai et al. 2010).

By demonstrating differences between Common Cuckoo and host egg lipid composition, our study highlights a potential role of lipid physiology in facilitating a brood parasitic reproductive strategy. Future studies should investigate the potential physiological and developmental consequences of producing eggs with larger yolks but a lower concentration of macro-nutrients, as well as producing eggs that contain fewer energy reserve lipids in proportion to other types of lipids. Although our findings suggest that Common Cuckoos invest lower energy reserves relative to egg mass, an extensive comparative analysis that includes nonparasitic cuckoo species is still required to test whether this is an adaptation specific to obligate brood parasitism. Larger yolks that contain a lower concentration of nutrients may simply be an artefact of the evolutionary pathway that led to production of smaller eggs relative to body size in Common Cuckoos. Our findings serve as an important starting point and highlight the need for further study in this area.

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